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Structure and Polymorphism of the Hydrocarbon Chains of Lipids: A Study of Lecithin–Water Phases

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This work describes the structure of a variety of lecithin–water phases observed below the "melting" temperature of the hydrocarbon chains, with special emphasis on the conformation of the chains. The lecithins studied in this work are the homologous series dioctanoyl to distearoyl, 2-decanoyl-1-stearoyl, and a preparation from hen eggs. The hydrocarbon chains are found to adopt a variety of conformations in addition to type α, the liquid-like organization observed above the melting temperature. Type β: the chains are stiff and parallel, oriented at right angles to the plane of the lamellae and packed with rotational disorder in a two-dimensional hexagonal lattice ($a \sim 4{\cdot}85$ Å). Type β': similar to β, but with the chains tilted with respect to the normal to the lamellae. Type δ: the chains are probably coiled into helices, whose axes are perpendicular to the plane of the polar groups and are packed with rotational disorder in a two-dimensional square lattice ($a \sim 4{\cdot}80$ Å). α is the predominant conformation, common to most lipids in the presence of water and at sufficiently high temperature, and the one more relevant to membranes; β is observed at lower temperatures in lipids whose chains are heterogeneous and in the presence of very small amounts of water; β' is found in synthetic lecithins with identical chains, in the presence of variable amounts of water; δ is observed in dry lecithins. A highly ordered *crystalline phase*, yet displaying rotational disorder of the chains, is observed in almost dry lecithins. Most of the phases are lamellar, and contain one lipid bilayer per repeat unit. Two phases display two-dimensional lattices: Pδ, formed by ribbon-like elements with the chains in the δ conformation; Pβ', formed by lamellae of type β' distorted by periodic ripples. The results emphasize the clear-cut difference between the liquid-like and the other types of partly ordered conformations, as well as the correlations which exist between the chemical composition and the structure of the lipids below the melting temperature of the chains.

1. Introduction

One of the most remarkable characteristics of lipids is their ability to combine in one phase a periodically ordered long-range organization (in one, two or three dimensions) and a highly disordered short-range conformation; many properties o

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lipids, and probably their function in biological membranes, are closely related to this peculiar mingling of order and disorder. Until the middle fifties, and in spite of clear evidence put forward by Hartley (1936) and Schmitt *et al.* (1941), most of the structure analyses of lipid-containing systems were biased by the *a priori* postulate that the hydrocarbon chains are as stiff as in the fatty acid crystals, and consequently that all lipid aggregates are lamellar. Systematic studies of lipid–water systems, undertaken first on soaps and detergents (Luzzati *et al.*, 1957; review in Luzzati, 1968) and extended later to biological lipids (Luzzati & Husson, 1962; Luzzati *et al.*, 1969) showed that the hydrocarbon chains can "melt" without impairing the long-range order, and that in many of the liquid–crystalline phases the conformation of the chains is in fact liquid-like; in addition lipid aggregates were found of a variety of shapes, not only lamellar. Another result of these studies was to show that the chains undergo a transition between the liquid-like and a partly ordered conformation as a function of temperature; in lipid–water systems the melting temperature decreases as the amount of water increases.

A structural study of several potassium soap–water systems below the melting temperature (Vincent & Skoulios, 1966*a*,*b*,*c*) led to the discovery of a conformation of the hydrocarbon chains similar to one previously observed in paraffins near the melting point (Müller, 1932); the chains in the fully extended form are all parallel, oriented at right angles to the plane of the lamellae, and packed with rotational disorder according to a two-dimensional hexagonal lattice of side 4·85 Å. Some later experiments revealed the same type of conformation in biological lipids (Luzzati & Husson, 1962; Gulik-Krzywicki *et al.*, 1967); this observation led to speculations about the possible biological relevance of order/disorder transitions in membranes (Luzzati & Husson, 1962; Luzzati *et al.*, 1966). The interest in this type of phenomenon was greatly stimulated by the observation of similar and reversible conformational transitions in intact membranes (Engelman, 1970; Esfahani *et al.*, 1971; Shechter *et al.*, 1972).

Our previous work on lipid–water systems (review in Luzzati, 1968) was focused mainly on the phases with highly disordered chains and emphasized the clear-cut distinction between the liquid-like and the more ordered types of conformations of the hydrocarbon chains. The phases below the melting temperature of the chains have never been studied systematically in lipids of biological interest, in spite of the great interest aroused by the discovery of order/disorder transitions in membranes. We describe here the results of an extensive X-ray diffraction study of these phases in a variety of lecithin–water systems; the lecithins used are synthetic compounds and a preparation from hen eggs. The synthetic lecithins used in our studies (a series ranging from di-decanoyl-PC† to distearoyl PC and 1-stearoyl-2-decanoyl PC) were all prepared according to the methods of Baer & Buchnea (1959), van Deenen & De Haas (1964), Cubero Robles & de Jongh (1967) and Singleton *et al.* (1965). Although some of the results have been mentioned elsewhere (Luzzati *et al.*, 1968; Reman, 1971; Luzzati *et al.*, 1972*b*) this is the first extensive description, except for Tardieu's (1972) thesis, of the various types of chain conformations. We should mention that we have observed recently several additional phases in egg lecithin, at the transition between the ordered and the disordered conformations of the hydrocarbon chains, which we shall describe elsewhere.

† Abbreviation used: PC, phosphatidyl choline.

The techniques used in this work are those used previously (Gulik-Krzywicki *et al.*, 1967; Luzzati, 1968), except for the pattern recognition approach to the phase problem (Luzzati *et al.*, 1972*a*).

2. Notation and Partial Specific Volumes

The following chemical notation will be used: PC for phosphatidyl choline, diC10 for didecanoyl, etc., C18-C10 for 1-stearoyl-2-decanoyl, c is the weight concentration (lecithin/(lecithin + water)), d is the lamellar repeat, d_1 is the partial thickness of the lipid layer, S is the average area per polar group at the lipid–water interface (see Luzzati, 1968).

The partial specific volumes of the different lecithins in the phases with chains in the liquid-like conformation can be estimated as follows. The volumes occupied by the CH, CH_2 and CH_3 groups are known (Reiss-Husson & Luzzati, 1964); since the partial specific volume of egg lecithin is 0·987 $cm^3\ g^{-1}$ at 25°C (Reiss-Husson, 1967), the volume occupied by the polar group (glycero-phosphorylcholine plus the carboxylic groups) can be determined: $v_{CH} = 20{\cdot}5$ Å^3, $v_{CH_2} = 27{\cdot}0$ Å^3, $v_{CH_3} = 54{\cdot}0$ Å^3, $v_{pol} = 360$ Å^3 at 25°C. The thermal coefficient is $\partial\bar{v}/\bar{v}\partial T = 7{\cdot}48 \times 10^{-4}$ $(°C)^{-1}$ (Luzzati & Spegt, 1967). In the phases with fully extended chains oriented at right angles to the lamellae (β) the volume occupied by one CH_2 group is the product of the cross-sectional area of one chain ($\Sigma = 20{\cdot}4$ Å^2) and the distance between adjacent CH_2 groups projected on the axis of the chains (1·25 Å): $v_{CH_2} = 25{\cdot}5$ Å^3. Thus, the v_{CH_2} ratio in the β and the α conformations is 0·945; we assume that all the partial volumes are reduced in the same proportion. We obtain $v_{CH_3} = 51{\cdot}0$ Å^3, $v_{pol} = 340$ Å^3. The partial specific volumes of Tables 1, 3, 4 and 7 have been calculated with these values. In the phases of type β and β', which we have studied over a narrow temperature range, we neglect the temperature dependence of the partial specific volumes. In other phases (Lδ, C) which are almost anhydrous, the partial specific volumes can be determined independently from the crystallographic data: the differences with the phases Lβ and Lβ' are found to be small (Table 4 and 7).

It should be noted that the form of the mathematical expressions (see Luzzati, 1968) is such that an error of the partial specific volume would have a large effect on S, and a much smaller one on d_1, θ and θ' (see Tables 1 and 3).

3. Phase Diagrams

The first step in our work was to identify and to locate the different phases in the temperature- and concentration-dependent phase diagrams of the lecithin–water systems. Although our aim was neither to determine the precise position of the phase boundaries nor to explore carefully the regions where more than one phase is present, we noted (in agreement with the phase rule) that the number of phases observed at any given temperature and concentration is never larger than three, except for egg-PC which is chemically heterogeneous. In addition we never met serious difficulties in reaching a state of apparent thermodynamic equilibrium, provided the samples were carefully homogenized and allowed to stand for some time (at maximum a few days).

Two examples of phase diagrams are shown in Figure 1. We use Latin and Greek letters to designate the phases. The capital Latin letter characterizes the type of long-range organization (one-, two- or three-dimensional lattice, space group; review

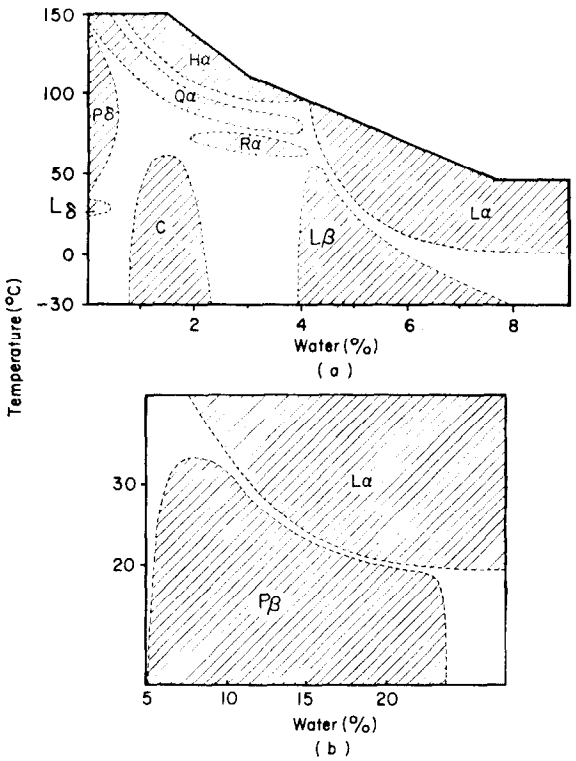

FIG. 1. Phase diagrams. The one-phase regions are hatched. The nomenclature and the structure of the phases are given in the text.

(a) Egg-PC; (b) diC14-PC; at lower water concentration and at higher temperature the phase diagram of diC14-PC is similar to that of egg-PC. The phase diagrams of all the lecithins of the homologous series diC8 to diC18 are similar, except that the temperatures are shifted, and that $L\beta'$ replaces $P\beta'$ in diC16 and diC18.

in Luzzati, 1968): L, one-dimensional (lamellar); H, two-dimensional hexagonal; P, two-dimensional oblique or centred; R, rhombohedral (space group $R\bar{3}m$); Q, cubic (space group Ia3d); C, three-dimensional crystalline (see below). The Greek letters characterize the short-range conformation of the hydrocarbon chains: α, liquid-like; β, β', δ, partly ordered. The structure of the phases and the conformation of the hydrocarbon chains are described in the next chapter, with reference to previous work when the results are not new.

Figure 1(a) shows one portion of the phase diagram of egg-PC. Several other phases in which some of the chains in the α and some in the β conformation are observed below 0°C and at higher water content (10 to 25%); the structure of these phases will be described in a forthcoming paper devoted to the transitions between the α and the β conformation. The phase diagram of Figure 1(b) is typical for the synthetic lecithins with identical saturated chains although the position of the phase boundaries varies with the length of the chains. The low-water high-temperature region, not shown in Figure 1(b), is similar to egg-PC (Fig. 1(a)) and contains the same phases ($L\delta$, $P\delta$, C, $R\alpha$, $Q\alpha$, $H\alpha$), $L\beta$ being replaced by $L\beta'$ and $P\beta'$ (see Table below). The following Table indicates which phases were observed (+) and which were found to be absent (−) in each system; a blank is left when the experimental data are lacking.

The data on mitochondria lipids are taken from Gulik-Krzywicki *et al.* (1967) and are presented for the sake of comparison.

		Hα	Qα	Rα	Lα	Lβ	Lβ′	Pβ′	Lδ	Pδ	C
Lecithins	diC8				+						+
	diC10				+						+
	diC12	+	+		+	−	−	+	+	+	+
	diC14	+	+		+	−	−	+	+	+	+
	diC16	+	+	+	+	−	+	−		+	+
	diC18	+	+	+	+	−	+	−			+
	diC18:1	+	+	+	+				+	+	+
	C18–C10				+	+	−	−			+
	hen eggs	+	+	+	+	+	−	−	+	+	+
Mitochondria lipids		+	−	−	+	+	−	−	−	−	−

4. Conformation of the Hydrocarbon Chains and Structure of the Phases

(a) α *Conformation: liquid-like disorder*

Although this type of conformation is analysed in several of our previous publications, a survey of the arguments used to establish its liquid-like nature is a proper introduction to a discussion of the more ordered conformations.

The X-ray diagrams of the α conformation are characterized by a broad band around $(4{\cdot}6\ \text{Å})^{-1}$, very similar to a band observed with liquid paraffins (Luzzati *et al.*, 1960). A more detailed study (Levine & Wilkins, 1971) of oriented preparations of the phase Lα of lecithin–water systems has shown that when the amount of water is small the band is anisotropic, the anisotropy decreasing as the hydration increases.

In the non-lamellar phases simple geometrical considerations indicate that the chains must be folded in a fairly regular way in order to fill uniformly the oddly shaped volumes offered to them (review in Luzzati, 1968). The authors (some recent examples are Levine & Wilkins (1971), McFarland & McConnell (1971), Träuble & Haynes (1971)) who lean towards an ordered, or at least a partly ordered conformation of the chains in the phase Lα, do not in our opinion pay adequate regard to the very existence of non-lamellar phases, unless they make the implicit and most unlikely assumption that the conformation of the chains is profoundly different in the lamellar and the non-lamellar phases.

From the chemical standpoint the hydrocarbon chains in the α conformation behave like molecules in miscible liquids. Indeed whenever the conformation of the chains is of type α the properties of the phase are to a large extent insensitive to chemical heterogeneity, even in the presence of large amounts of "impurities" (for example cholesterol); on the contrary the chemical composition plays a critical role in all the phases in which the conformation of the chains is more ordered.

Finally all the phases with chains in the α conformation display a peculiar temperature effect: the short dimension of the structure elements (thickness of the lipid bilayer in the lamellar phases, diameter of the rods in the hexagonal phases, etc.) decreases as the temperature is raised, with an unusually large linear thermal coefficient (of the order of 10^{-3}/°C). This phenomenon, well known in rubber, is typical of a polymer with a highly disordered conformation, moderately stretched by an

external force; a rise in temperature increases disorder and decreases the elongation. A similar mechanism explains the effect observed in lipids; the interfacial tension tends to decrease the area per polar group at the lipid–water interface and thus to elongate the chains, the disordered conformation of the chains on the contrary tends to reduce the elongation; raising the temperature increases disorder and decreases the anisotropy of the chains (see Luzzati, 1968).

In conclusion the α conformation can be visualized as highly disordered, like that of a liquid paraffin, still with the average of the chain orientations perpendicular to the lipid–water interface; this orientation is all the more pronounced as the area per polar group decreases. It is clear that such a "liquid" may well display complex molecular movements (McFarland & McConnell, 1971); the X-ray diffraction data are of little help for this problem.

(b) *β and β' Conformations: phases $L\beta$, $L\beta'$, $P\beta'$*

The β conformation has been observed in a variety of lipids: potassium soaps (Vincent & Skoulios, 1966*a*,*b*,*c*), brain (Luzzati & Husson, 1962) and mitochondria lipids (Gulik-Krzywicki *et al.*, 1967), and more recently in several other lipid preparations. This conformation is characterized by a strong and sharp reflection at $s_1 = (4{\cdot}2\ \text{Å})^{-1}$, followed in some cases by faint and sharp reflections at $\sqrt{3}s_1$ and at $2s_1$; these reflections correspond to a two-dimensional lattice of cylindrical rods, and have been interpreted by the presence of stiff and fully extended chains organized with rotational disorder according to a two-dimensional hexagonal lattice (Müller, 1932; Vincent & Skoulios, 1966*a*,*b*,*c*; Gulik-Krzywicki *et al.*, 1967). The comparison of the cross-sectional area Σ of each chain ($\Sigma = 2{s_1}^{-2}/\sqrt{3} = 20{\cdot}4\ \text{Å}^2$) and of the area $S/2$ per polar group at the interface (see Gulik-Krzywicki *et al.*, 1967, and Table 1)

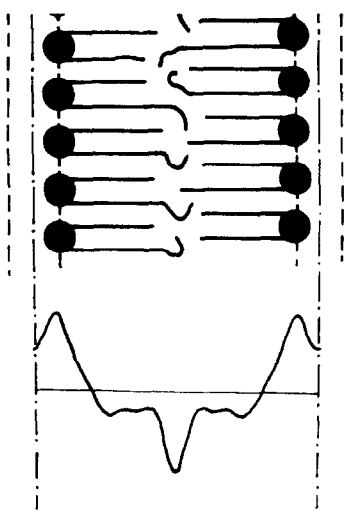

FIG. 2. Structure of the phase $L\beta$. The Figure represents a section through a lamella. Upper frame, schematic representation of the molecular structure. The black circles represent the polar groups, the straight lines represent the fully extended chains, the wriggles represent the chains in the liquid-like conformation. The chains are stiff and parallel, oriented perpendicular to the lamellae; a thin liquid layer is postulated in the centre of the hydrocarbon layer in order to take into account the length heterogeneity. Lower frame, electron density profile (from Luzzati *et al.*, 1972*a*) for mitochondrial lipids.

shows that the chains are oriented at right angles to the plane of the lipid lamellae; this orientation is confirmed by the position of the (4·2 Å)$^{-1}$ reflection in oriented samples and by its sharpness in unoriented preparations (see Appendix).

This type of conformation is observed in a lamellar phase Lβ, which contains a very small amount of water (less than 10%). The dimensions observed with various lipids are reported in Table 1. The structure is sketched in Figure 2. This phase is observed with many lipids, but only if the chains are chemically heterogeneous: brain and mitochondria lipids, egg-PC, C18-C10-PC. In order to take into account the heterogeneity of the chains, particularly conspicuous in the case of natural lipids, we are led to postulate the presence of a disordered layer in the middle of the hydrocarbon leaflet (Fig. 2) and to suppose that a number of double bonds can be accommodated in the ordered regions. The electron density profile is shown in Figure 2.

The β' conformation (see Chapman *et al.*, 1967; Levine, 1970) is similar to the β—namely the chains are stiff and parallel, packed with rotational disorder in a two-dimensional quasi-hexagonal lattice—except that the chains are tilted with respect to the normal to the plane of the lamellae. Another difference is the action of water: the amount of water incorporated in the phases of type β' can be larger than in the phases of type β, the angle of tilt increasing with increasing water content (see Table 1); moreover if water is added to a phase of type β often some of the chains take up a disordered conformation and new phases occur which display peculiar segregation of ordered and disordered chains (Sadler & Ranck, unpublished observations). In the X-ray diagrams of unoriented samples the β' conformation is characterized by a sharp reflection at (4·2 Å)$^{-1}$ followed by a more diffuse reflection (Plate I(c)); these can be analysed (see Appendix) in terms of a two-dimensional hexagonal

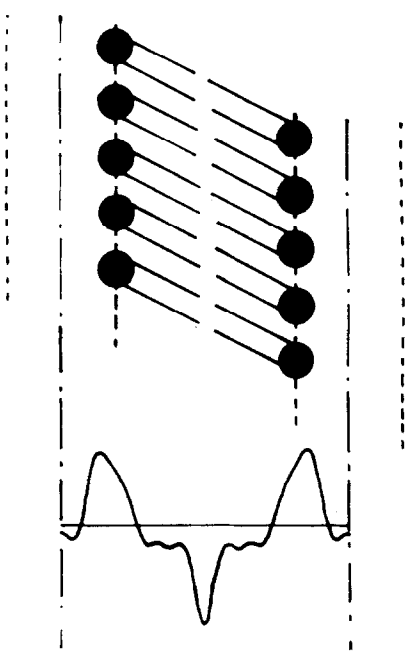

FIG. 3. Structure of the phase Lβ'; see legend of Fig. 2. The chains are fully extended, and tilted with respect to the normal to the lamella; the end CH_3 groups of the two opposite monolayers are in register; the water layer is thicker than in the phase Lβ. The electron density profile is taken from Luzzati *et al.* (1972*a*) for diC16-PC.

lattice of tilted rods. Several observations suggest that this type of conformation requires a chemical homogeneity of the hydrocarbon moiety: the β' conformation is observed in synthetic lecithins with identical saturated chains, apparently not in C18-C10-PC nor in egg-PC; a small amount of hydrocarbon transforms the phase Pβ' or Lβ' into a phase Lβ (experiments made with diC14 and diC16-PC adding 7% decane).

TABLE 1

Dimensions of the phases Lβ and Lβ'

		T' (°C)	$\bar{v}$ (cm^3 g^{-1})	c	d (Å)	d_1 (Å)	S (Å^2)	θ (°)
β	Egg-PC	0	0·932	(0·97)	60·6	(58·8)	(41·4)	0
	C18-C10-PC	0	0·935	0·99	52·0	51·5	40·8	0
	Mitochondria	−20	0·902	0·94	60·6	56·6	40·7	0
β'	diC16-PC	20	0·948	0·94	57·7	54·0	42·7	17
	diC16-PC	20	0·948	0·89	57·0	50·7	45·6	26
	diC16-PC	20	0·948	0·82	60·5	49·2	46·8	30
	diC16-PC	20	0·948	0·80	61·5	48·6	47·4	31
	diC16-PC	20	0·948	(0·75)	64·0	(47·4)	(48·6)	(33)
	diC18-PC	20	0·958	0·90	63·0	56·5	44·6	24
	diC18-PC	20	0·958	0·83	63·0	52·0	48·4	32
	diC18-PC	20	0·958	0·78	63·0	48·5	52·0	38
	diC18-PC	20	0·958	(0·71)	67·0	(47·0)	(53·4)	(40)

θ is the angle of tilt of the chains with respect to the normal to the lamellae. The dimensions in parenthesis are doubtful; egg-PC is hygroscopic and consequently a low water content is difficult to determine with accuracy; the phase Lβ' at high water content might be accompanied by some water in excess.

Two types of phases were observed with the chains in the β' conformation: Lβ' with diC16 and diC18-PC, Pβ' with diC12 and diC14-PC. Lβ' is lamellar, as shown by the small-angle reflections; it is obtained pure over a fairly wide concentration range (see also Levine, 1970). In the presence of water the thickness of the lipid leaflet is smaller than it would be if the chains were fully extended and oriented as in the β conformation (56·6 Å for diC16-PC, 61·6 Å for diC18-PC) and decreases as the amount of water increases (see Table 1). Assuming that the chains are stiff and that their cross-sectional area is $\Sigma = 20{\cdot}4$ Å^2 as in the phase Lβ, the angle of tilt can be estimated using the equation $\cos\theta = 2\Sigma/S$ (see Table 1); note that a small difference of Σ, as discussed in the Appendix, would change $\bar{v}$ and S, but would leave θ almost unchanged (see section 2, above).

The phase Pβ' is characterized by several small-angle sharp reflections which can all be indexed according to a two-dimensional oblique lattice (see Plate I(e) and Table 2). In some of the X-ray diagrams one can clearly distinguish several predominant reflections, all equally spaced (indexed [h0] according to the lattice of Table 3 and Fig. 4), each accompanied by a cluster of weak "satellite" reflections; the spacings of the predominant reflections would be consistent with the lamellar repeat if the same lipid adopted a phase Lβ' at the same concentration (compare Tables 1 and 3). It may thus be inferred that the structure of Pβ' is similar to that of Lβ', namely that Pβ' contains slightly distorted lamellae of the β' type; this type of

PLATE I. X-ray diffraction diagrams of some of the phases. The experiments were carried out on a Guinier camera, sample-film distance 125 mm, operated *in vacuo*, using CuKα_1 radiation (with the exception of (h), in which W Lα_1 radiation was used).

(a) Phase Lα of mitochondria lipids, $c = 0{\cdot}75$, $T = 25°C$. Note the diffuse band around 0·22 Å^{-1}.

(b) Phase Lβ of mitochondria lipids, $c = 0{\cdot}95$, $T = -20°C$. Note the sharp reflection at 0·238 Å^{-1}.

(c) Phase Lβ' of diC16-PC, $c = 0{\cdot}80$, $T = 33°C$. Note the sharp reflection at 0·238 Å^{-1}, surrounded by a more diffuse reflection.

(d) As in (c), $T = 4°C$. Note the difference with (c) (see Appendix).

(e) Phase Pβ' of diC12-PC, $c = 0{\cdot}77$, $T = -7°C$. Note the number of small-angle reflections.

(f) Phase Lδ of egg-PC (plus 6% CTAB) anhydrous, $T = 42°C$. Note the sharp reflections at 0·208 and 0·295 Å^{-1}, and the diffuse bands with sharp edges at 0·147 and 0·208 Å^{-1}.

(g) Phase Pδ of diC12-PC, anhydrous, $T = 121°C$.

(h) Phase C of diC10-PC monohydrate, $T = 25°C$. Note the large number of sharp reflections (see indexing in Fig. 8).

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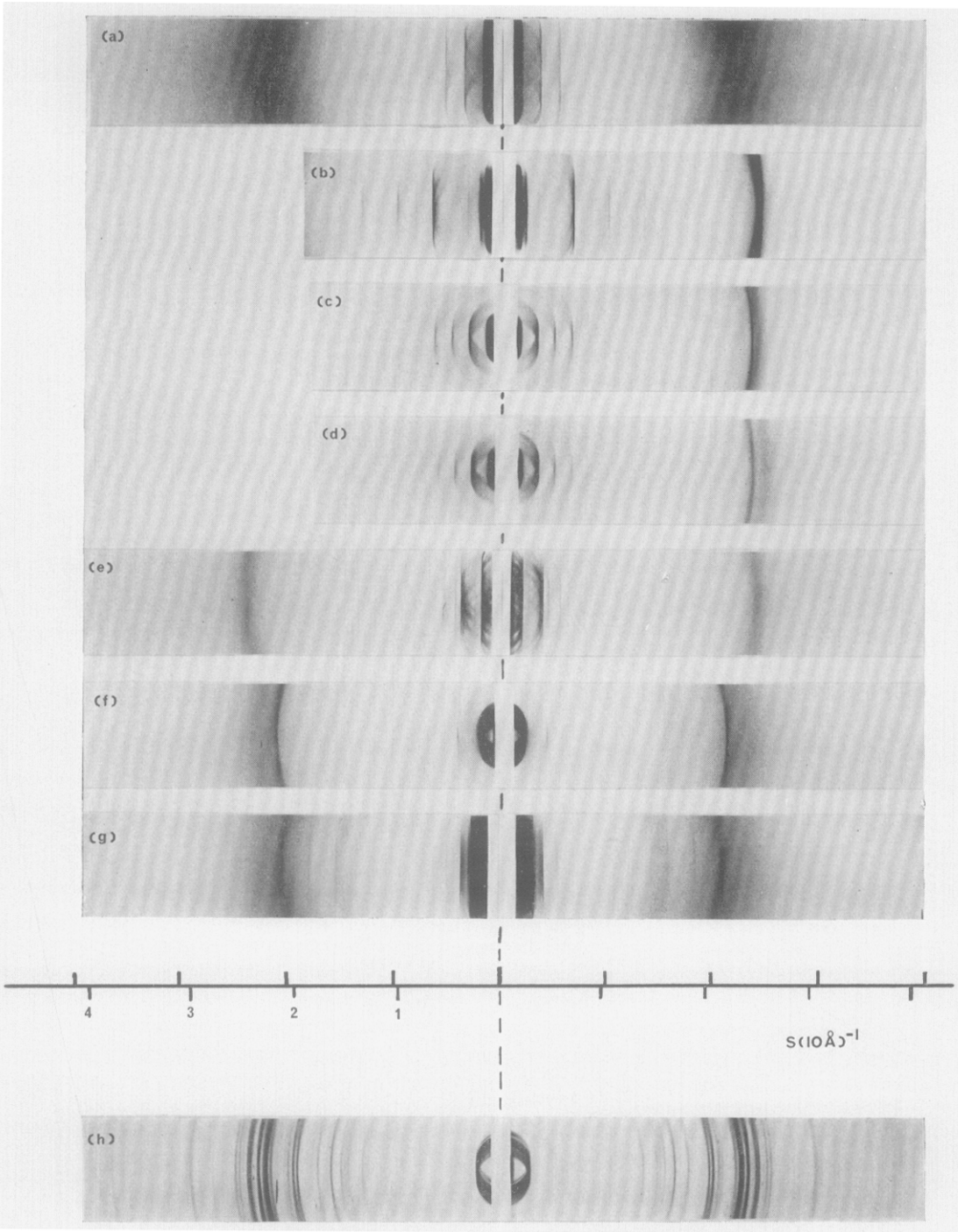

PLATE I

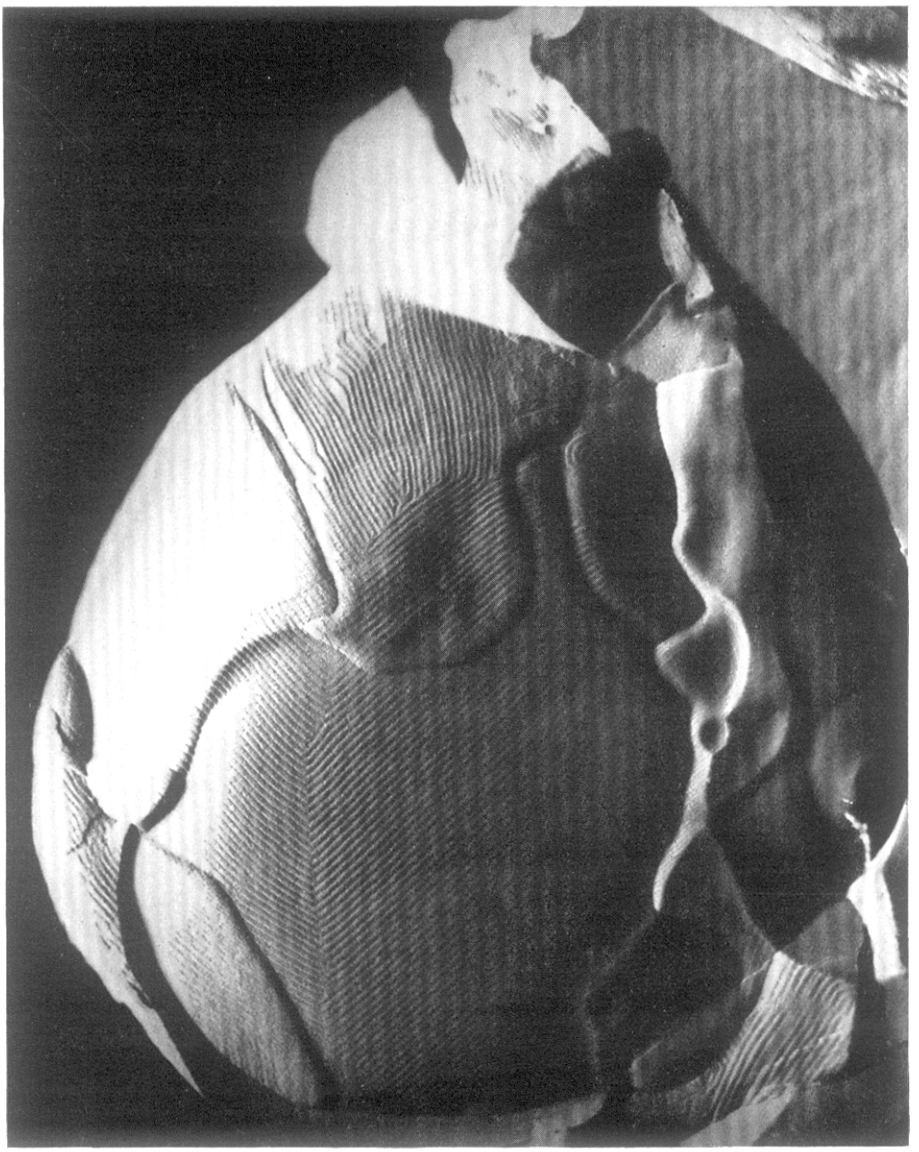

PLATE II. Freeze-etching electron micrograph showing a diC14-PC multilayered vesicle. The specimen was fractured and Pt shadowed at −150°C. Magnification 42,000. By courtesy of Ververgaert & Elbers (see also Ververgaert *et al.*, 1972; Verkleij *et al.*, 1972).

structure is sketched in Figure 4. In one case the reflections were phased (see the legend to Table 2) and the electron density distribution was calculated; the result is shown in Figure 4. As an additional verification the intensities of the reflections were calculated assuming that the amplitude of the ripples is 15 Å; the agreement of the observed and calculated intensities is quite satisfactory (see Tardieu, 1972). An independent verification of the structure is provided by the electron microscope study

TABLE 2

Crystallographic data of the phase Pβ′

h	k	s_{obs} (10^{-4} Å^{-1})	s_{calc} (10^{-4} Å^{-1})	I	F
0	1	125	127	30	+ 5·5
1	0	192	193	1426	−36·4
1	$\bar{1}$		193		−10·0
0	2	252	255	10	+ 3·2
1	1	262	264	45	+ 5·5
1	$\bar{2}$		264		− 3·9
2	$\bar{1}$	366	364	510	−22·4
1	2		366		+ 3·0
2	0	385	387	1340	−30·0
2	$\bar{2}$		387		−21·0
2	1	445	445	365	+19·1
2	2	525	527	48	+ 6·9
3	$\bar{1}$	554	550	365	+16·3
3	$\bar{2}$		550		+10·0
3	0	578	578	33	+ 5·7
3	1	630	630	94	− 9·7
3	2	704	704	70	+ 8·3
4	$\bar{1}$	740	737	92	− 9·6
4	0	775	775	58	− 7·6

The data are relevant to diC12-PC, $c = 0{\cdot}77$, $T = -7$°C; two-dimensional space group $P2$; $a = 55{\cdot}3$ Å, $b = 85{\cdot}3$ Å, $\gamma = 110$°. s_{obs} and s_{calc} are the observed and the calculated spacings of the reflections. I are the observed intensities. The determination of the phases is complicated here by the fact that several pairs of reflections overlap in the powder diagram, and that the distribution of the observed intensity between the two reflections is not known. A variety of procedures were used for phasing: trial, pattern recognition approach (Luzzati *et al.*, 1972*a*), comparison with Lβ′ (see text). The structure factors (F) and the signs chosen are given in the Table. The Fourier transform is shown in Fig. 4.

TABLE 3

Dimensions of the phase Pβ′

	T (°C)	$\bar{v}$ (cm^3 g^{-1})	c	a (Å)	b (Å)	γ (°)	θ' (°)
diC12-PC	−7	0·922	0·82	55·3	113·0	110	23
diC12-PC	−7	0·922	0·77	55·3	85·3	110	30
diC14-PC	19	0·935	0·83	59·3	175·0	102	21
diC14-PC	19	0·935	0·79	57·0	150·0	99	30
diC14-PC	19	0·935	0·75	58·6	144·0	94	32

a, b, γ are the lattice parameters. θ' is the angle of tilt of the chains with respect to the normal to b (see Fig. 4).

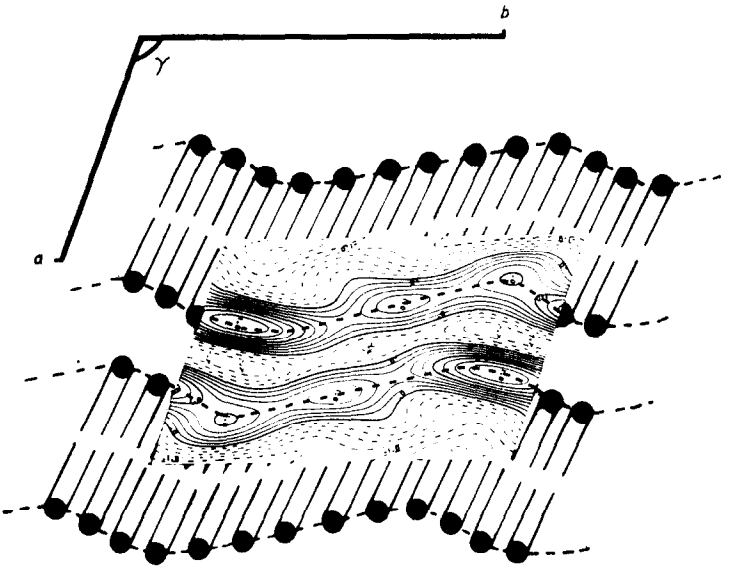

FIG. 4. Structure of the phase Pβ'. The Figure represents a section of the two-dimensional periodic structure, and shows the rippled lamellae. Symbols as in Figs 2 and 3. The insert is the electron density distribution, calculated with the data of Table 2; the negative contours are dotted.

of freeze-etched preparations of diC14-PC (Ververgaert *et al.*, 1972; Verkleij *et al.*, 1972), which reveals the presence of periodically rippled lamellae (Plate II); the period of the ripples is 200 Å (we observe 150 Å at $c = 0{\cdot}75$).

The precise structure of the corrugated lamellae is more difficult to ascertain. If it is assumed, as suggested by the similarity of the phases Pβ' and Lβ', that all the chains are in the β' conformation, namely that in each lamella all the chains are stiff and parallel, the distortions of the planar lamellae are bound to involve gliding movements of the stiff, close-packed chains past each other; moreover the displacements of neighbouring molecules must be of small amplitude if the hydrocarbons are to be secluded from water. The type of structure sketched in Figure 4 satisfies these criteria. Nevertheless other types of structures, involving perhaps a small fraction of the chains in a disordered conformation, cannot be ruled out with certainty; we shall discuss this problem elsewhere, in the more general context of the order–disorder transitions of the chain conformation.

We may discuss these observations, and try to rationalize the correlations between the structure of the phases and the chemical composition of the chains. We can first distinguish three effects of tilting the chains away from the normal to the lamellae: the organization of the terminal CH_3 groups is modified, the area S is increased, the packing of the chains is altered (since the distances between CH_2 groups of neighbouring chains are changed, see Appendix). Second, we remark that the area S and the water content are interdependent parameters, which vary together and in the same direction (see Table 1).

It is now clear that if the chains are heterogeneous the organization of the terminal CH_3 groups is disordered and is presumably insensitive to the angle of tilt; since in this case (phase Lβ) the water content is small and the chains are perpendicular to the interface, it may be inferred that in lecithins increasing the hydration of the

polar layer and/or tilting the chains requires energy. On the contrary when the chains are homogeneous the organization of the CH_3 groups may be well ordered and could be quite sensitive to the angle of tilt; it appears in this case that the packing of the CH_3 groups becomes more satisfactory as the chains are tilted, and that the gain of energy is sufficient to overcome the losses due to the increase of the area S (and thus of hydration of the polar layer) and/or the increase of the angle of tilt. The interactions of the different parts of the structure (terminal CH_3 groups, chain CH_2 groups, polar layer) may well lead to a more complex situation, in which "strains" are alternately cumulated and released in different parts of the structure: this is the case of phase Pβ' (see Fig. 4). We have previously evoked similar cumulative strain effects in connection with other phases containing ribbon-like (Skoulios & Luzzati, 1961) and rod-like elements (Luzzati *et al.*, 1968).

Lamellae with ripples of fairly long period (approx. 400 Å) have in fact been observed in freeze-fractured electron microscope preparations of diC16-PC (Pinto da Silva, 1971), a lipid in which we have observed the phase Lβ', not the phase Pβ' (see above). Although this discrepancy can be a consequence of the experimental conditions (purity of the lipid, amount of water, etc.), it may suggest that Pβ' is the phase common to all the diC2n-PC with chains in the β' conformation; the absence of non-lamellar reflections in diC16-PC and diC18-PC could indeed be explained by small fluctuations of the period of the ripples over the volume of the sample.

(c) δ *Conformation: phases Lδ and Pδ*

This type of conformation, mentioned in one of our previous publications (Luzzati *et al.*, 1968), is characterized by two sharp reflections, one strong at $(4{\cdot}8\ \text{Å})^{-1}$ and one weak at $(3{\cdot}4\ \text{Å})^{-1}$, and by a few diffuse bands with a sharp inner edge, typical of the powder diagram of two-dimensional point lattices (see Appendix); the inner edge of the bands is at $(6{\cdot}8\ \text{Å})^{-1}$, $(4{\cdot}8\ \text{Å})^{-1}$, $(3{\cdot}4\ \text{Å})^{-1}$ and $(3{\cdot}05\ \text{Å})^{-1}$ (see Plate I(f) and Fig. 5). The ratio of the spacings $(1:\sqrt{2}:\sqrt{4}:\sqrt{5})$ is that of a two-dimensional square lattice. By analogy with the β conformation we ascribe the sharp reflections to stiff

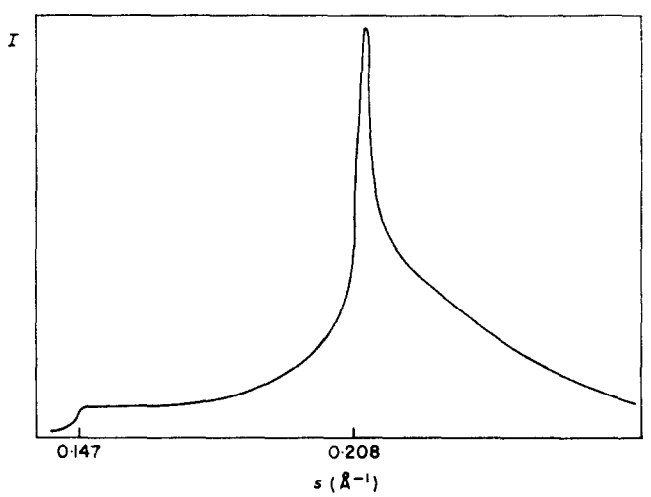

FIG. 5. Microdensitometer tracing of an X-ray diagram of the phase Lδ. Note the presence of diffuse bands with sharp inner edges at 0·147 and 0·208 Å^{-1}, and of a sharp reflection at 0·208 Å^{-1}. The signals at $(3{\cdot}4\ \text{Å})^{-1}$ and at $(3{\cdot}05\ \text{Å})^{-1}$ are much weaker (see text and Plate I(f)).

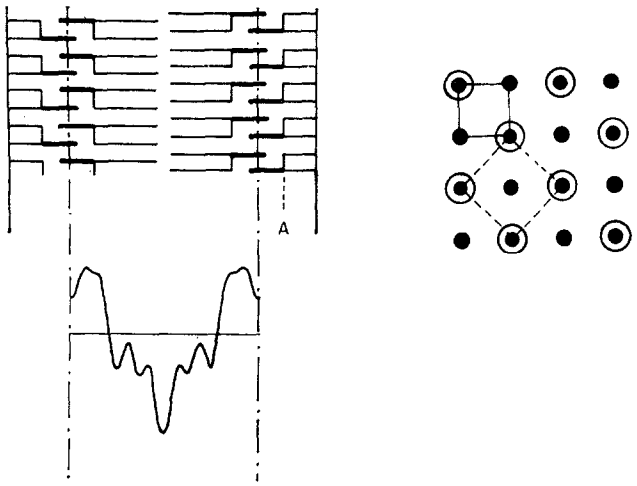

FIG. 6. Structure of the phase Lδ. The straight lines represent the paraffin chains coiled into helices (see text); the thick bars represent the polar groups. The right side shows schematically an enlargement of the section A, with the lattice of the chains (black dots) and the lattice of the polar groups (open circles). The electron density profile corresponds to anhydrous diC12-PC, at 90°C; the phases were chosen as in the case of Lβ and Lβ' (Luzzati *et al.*, 1972*a*). $F_1 = +50$, $F_4 = -18$; $F_7 = +6$; $F_8 = -10$; $F_2 = F_3 = F_5 = F_6 = 0$.

rods organized with rotational disorder in a two-dimensional square lattice, and we assume that each rod contains one hydrocarbon chain. The diffuse bands can be ascribed to the polar groups (one for two chains) organized according to the square two-dimensional lattice whose side is the diagonal of the lattice of the chains (Fig. 6). The relation of the two lattices shows that the rods are oriented at right angles to the plane of the lamellae; this orientation is confirmed by the sharpness of the reflection at $(4{\cdot}8\ \text{Å})^{-1}$ (see Appendix) and by the very fact that the lattice of the phase Pδ is rectangular (see below). We discuss in the Appendix the "high"-angle region of the powder diagram of this type of structure, and we show that it displays sharp reflections and diffuse bands as observed experimentally.

The conformation of the hydrocarbon chains appears to be helical, with 3·4 monomers (the CH_2 groups) per turn. This is suggested by the conformational analysis of polyethylene (De Santis *et al.*, 1963) which leads to three stable conformations (the fully elongated *trans* and two helical forms with, respectively, 2, 3·4, 4·0 residues per turn and axial residues distances 1·25, 1·09, 0·89 Å) and by the fact that the ratio of the axial residue distances between the extended (*trans*) form and the helix with 3·4 residues per turn ($1{\cdot}25/1{\cdot}09 = 1{\cdot}145$) is in close agreement with the contraction of the hydrocarbon leaflet of the phase Lδ with respect to the phase Lβ ($d = 41{\cdot}2$ Å for the phase Lδ of diC12-PC, see Table 4; the thickness of the lipid layer of the same lecithin in phase Lβ would be 46·6 Å, see Table 1).

As a confirmation it may be noted that the volume per CH_2 group, equal to the product of the cross-sectional area per chain and the axial monomer distance, is very similar in the two cases: $v_{CH_2} = 23{\cdot}0 \times 1{\cdot}09 = 25{\cdot}1$ Å^3 for the δ conformation, $v_{CH_2} = 25{\cdot}6$ Å for the β conformation.

The lamellar phase Lδ is observed at the very anhydrous end of the phase diagram, and over a narrow temperature range (see Fig. 1). We have observed this phase pure

TABLE 4

Dimensions of the phase Lδ

	T (°C)	$\bar{v}$ (cm^3 g^{-1})	d (Å)	Σ (Å^2)	$\bar{v}'$ (cm^3 g^{-1})
diC12-PC	90	0·922	41·1	46·0	0·920
egg-PC	42		47·2	46·0	0·953

$\bar{v}$ is the partial specific volume of the phases Lβ and Lβ′, $\bar{v}'$ is the partial specific volume determined from the crystallographic data, on the assumption that the cross-sectional area per chain is $\Sigma = 23$ Å^2 (see text). A small amount of cetyltrimethylammonium bromide was added to egg-PC; as a consequence $\bar{v}$ is unknown.

only in diC12-PC and in egg-PC with 6% CTAB; in all the other lecithins Lδ was found accompanied by some other phase. The spacings of the small-angle reflections are typically lamellar: the dimensions are given in Table 4. The structure of the phase, and the electron density distribution, are shown in Figure 6. In the case of egg-PC the heterogeneity of chain length suggests, as in phase Lβ (see above), the presence of a disordered layer in the centre of the hydrocarbon leaflet; furthermore it appears that the helical regions can accommodate a number of double bonds without severe distortions.

TABLE 5

Crystallographic data of the phase Pδ

h	k	s_{obs} (10^4 Å^{-1})	s_{calc} (10^4 Å^{-1})	I	F
2	0	172	172	158·0	+12·6
1	1	273	274	1370·0	+37·0
4	0	345	344	43·5	− 6·6
3	1	366	365	121·0	−11·0
5	1	504	504	8·4	+ 2·9
6	0	519	516	8·8	+ 2·1
0	2		520		+ 2·1
2	2	548	547	2·9	− 1·7
4	2	622	623	1·2	+ 1·1
7	1	655	654	0·6	− 0·8

The data are relevant to diC12-PC, $c = 1{\cdot}00$, $T = 121$°C; two-dimensional space group *cmm*; $a = 116{\cdot}5$ Å, $b = 38{\cdot}5$ Å. s_{obs}, s_{calc}, I and F as in Table 2. The phases were determined by the pattern recognition technique, assuming three levels; the signs are those corresponding to the best mathematical merit (see Luzzati *et al.*, 1972*a*).

The phase Pδ was observed pure with diC12-, diC14-, diC16- and egg-PC, at the anhydrous end of the phase diagram, at a higher temperature than phase Lδ. We present in Table 5 the crystallographic data relevant to diC12-PC; those relevant to diC16- and egg-PC were presented previously (Luzzati *et al.*, 1968). The high-angle region of the X-ray diagrams is typical of the δ conformation, with sharp and diffuse reflections (see above and Plate I(g)); at small angles several sharp reflections are

TABLE 6

Lattice dimensions of the phase Pδ

	T (°C)	a (Å)	b (Å)
diC12-PC	114	115·0	38·5
diC12-PC	121	116·5	38·5
diC12-PC	136	108·5	38·5
diC14-PC	118	126·0	42·3
diC14-PC	129	116·5	41·4
diC14-PC	140	108·0	39·7
diC16-PC	120	117·6	44·4
diC16-PC	130	114·4	44·1
diC16-PC	137	109·2	43·5
diC16-PC	145	105·0	43·2
egg-PC	60	118·4	45·5
egg-PC	90	101·0	43·2
egg-PC	112	94·4	42·4

See Luzzati *et al.* (1968) and Fig. 7.

observed which can all be indexed according to a two-dimensional centred rectangular lattice, space group *cmm*. The dimensions of the lattice in the various lecithins and at different temperatures are given in Table 6; it may be noted that in each lecithin the parameter b is barely dependent on temperature and is very close to the repeat distance of the phase Lδ, and that the parameter a is almost independent of the length of the hydrocarbon chains, at least for the synthetic lecithins and at the same temperature. This type of two-dimensional lattice is commonly observed in anhydrous soaps (Skoulios & Luzzati, 1961; and review in Luzzati, 1968), and has been interpreted by the presence of indefinitely long ribbons packed in a two-dimensional centred rectangular lattice. In the case of lecithin the ribbons can be visualized as strips of the lamellae of Lδ (see Fig. 7). This type of structure is confirmed by the

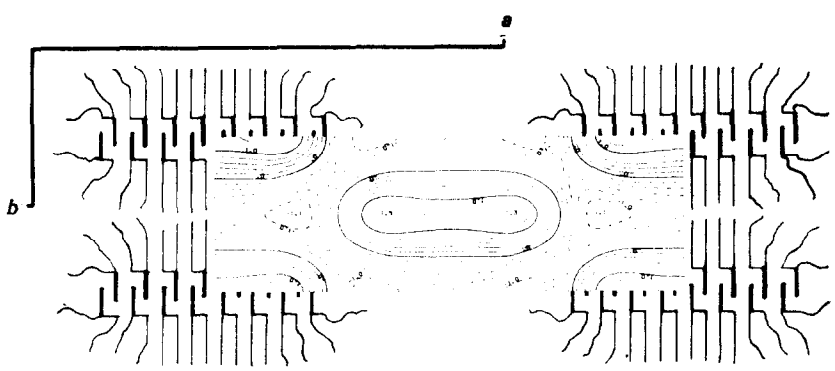

FIG. 7. Structure of the phase Pδ. The Figure represents a section of a two-dimensional periodic structure. The ribbons are similar in structure to the lamellae of the phase Lδ (see Fig. 6), except for the fact that the chains are somewhat disordered, the disorder propagating from the end CH_3 group towards the polar end, and increasing near the edge of the ribbons (see text). The electron density distribution was calculated with the data of Table 5.

electron density distribution shown in Figure 7 and by the agreement of the observed structure factors with those calculated for a physical model derived from Figure 7 (see Tardieu, 1972). It is possible to rationalize the transition from the lamellae to the ribbons by a "cumulative strain" effect, as we have done above in the discussion of phase Pβ'. More precisely in the case of the δ conformation we can assume that as the temperature is raised the chains melt progressively beginning from the CH_3 end, the rest of the chain remaining in the δ conformation. Under these conditions the average area per chain remains constant; if the area is too small to accommodate the disordered portions, the chains are subjected to a repulsion which increases as the distance from the centre of the ribbon increases. This strain is finally released in the disordered regions near the edge of the ribbons. This explanation is consistent with the observation that the width of the ribbons (and to a less extent the distance between the ribbons) decreases as the temperature is raised (see parameter a in Table 6).

The very anhydrous state of the phases Lδ and Pδ suggests that the two monolayers apposed by their polar faces are in register; under these conditions the absence of a periodic three-dimensional organization requires the presence of rotational and translational disorder in the middle of the hydrocarbon layer.

(d) *Phase C, crystalline*

This phase is observed in all the lecithins, anhydrous in diC18-PC, slightly hydrated (2 to 4%) in the other compounds; the temperature range is approximately −30 to 90°C for the synthetic compounds, −30 to 50°C for egg-PC. The properties of diC18-PC are somewhat different from those of the other lecithins, and will be discussed separately. The "anhydrous crystals" mentioned by Chapman *et al.* (1967) probably belong to this phase. The X-ray powder diagrams display sharp reflections at all spacings from $(40\ \text{Å})^{-1}$ to atomic resolution (see Plate I(h)), and thus indicate the presence of long- and short-range three-dimensional periodic order, as in an ordinary crystal.

The small-angle reflections are all integral orders of one large repeat (38 to 57 Å); this indicates that the structure contains lipid lamellae similar to those of other phases, and that the sides of the unit cell projected on the plane of the lamellae are much shorter than the lamellar repeat. Beyond $s \sim (10\ \text{Å})^{-1}$ (see Plate I(h)) the number of reflections increases sharply and indexing becomes uncertain. Nevertheless the comparison of the different compounds allows us to remove the ambiguities, since a family of unit cells is found, all primitive tri-rectangular with the same dimensions a and b, differing by the length of c (Table 7 and Fig. 8). The estimated density (see Table 7) indicates that the number of molecules per unit cell is four. The occurrence of tri-rectangular cells in such a large family of compounds rules out an accidental coincidence: the symmetry is thus orthorhombic. The presence of an asymmetric carbon in the lecithins used in this work, the lack of systematic absences in the reflections [00l] and the absence in all the cases of the reflection [100] restrict the choice of the space group to $P2_122$ and $P2_12_12$, both with one lecithin molecule per asymmetric unit. The unit cells of diC18-PC is twice as large ($a' = a$, $b' = 2b$, see Table 7) and probably face-centred on C.

Although the size of the unit cell and the lack of single-crystal data rule out a precise structure analysis, some information can be gathered from the powder data.

The presence in all the X-ray diagrams of a few strong reflections near $s = (4{\cdot}2\ \text{Å})^{-1}$

TABLE 7

Dimensions of the phase C

	T (°C)	$\bar{v}$ (cm^3 g^{-1})	a (Å)	b (Å)	c (Å)	$a \cdot b$ (Å^2)	$\bar{v}'$ (cm^3 g^{-1})
diC10-PC	25	0·905	10·7	8·60	38·4	92·0	$0{\cdot}91_1$
diC12-PC	25	0·922	10·7	8·65	43·0	92·5	$0{\cdot}93_2$
diC14-PC	25	0·935	10·7	8·65	47·6	92·5	$0{\cdot}95_0$
C18-C10-PC	25	0·935	10·7	8·65	47·6	92·5	$0{\cdot}95_0$
diC18-PC	25	0·958	10·7	17·30	56·8	185	$1{\cdot}00_0$
egg-PC	25	0·932	10·7	8·70	54·4	93·0	$0{\cdot}96_8$

$\bar{v}'$ is determined from the crystallographic data on the assumption that the unit cell contains one molecule of water per molecule of lecithin, except for diC18-PC which is anhydrous (see Fig. 10); $\bar{v}$ of the phases Lβ and Lβ' (see Tables 1 and 3) is given for the sake of comparison.

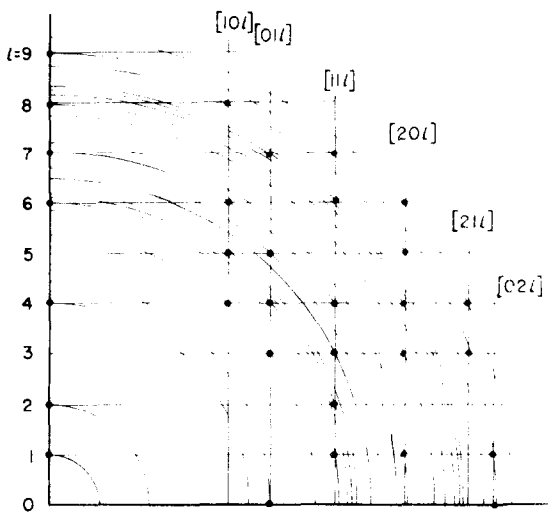

FIG. 8. The reciprocal lattice of the crystalline phase of diC10-PC (see Table 7 and Plate I(h)). The radii of the circles are the spacings of the observed reflections for $s < 0{\cdot}250$ Å^{-1}; the black dots indicate the indexing corresponding to the lattice of Table 7.

(see Plate I(h)) suggests that the chains are fully extended as in the β and β' conformations; besides, the close similarity of the unit cells indicates that the angle of tilt of the chains with respect to c is very nearly the same in all the compounds. These notions agree with the fact that c is a linear function of the length of the chains; the observed increment of c (2·3 Å per pair of CH_2, Table 7) compared to the increment of a fully extended chain (2·5 Å per pair of CH_2 in the β conformation) allows to estimate the angle of tilt: $\cos\theta = 2{\cdot}3/2{\cdot}5$, $\theta = 23°$. Packing long stiff chains, tilted with respect to c, in accordance with the symmetry operations of space group $P2_122$ or $P2_12_12$, and with the known dimensions of the unit cells, is sterically feasible only if the chains are clustered in planar sheets normal to b (the alternative orientation normal to a would entail parallel chains 3·97 Å apart, a very

short distance indeed). In each sheet the chains of one side of the bilayer are all parallel and equally spaced; the orientation with respect to c is reversed from one sheet to the next. The 2-fold axis parallel to c restricts the position of the sheets to $y = 1/4$ and $y = 3/4$; as a consequence the chains of the two sides of the bilayer are coplanar (see Fig. 9).

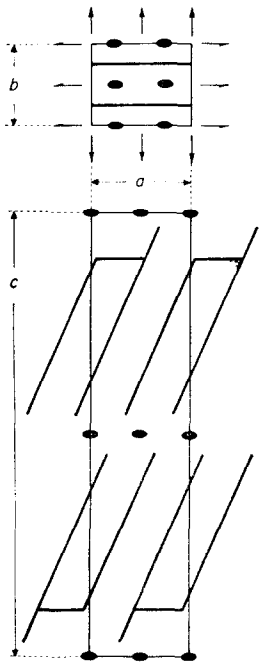

FIG. 9. Structure of the phase C of diC14-PC. The *ab* projection of the unit cell, with the position of the symmetry elements, is shown in the upper frame. The lower frame shows the schematic position of the hydrocarbon chains and of the polar groups. The molecules are contained in the planes $y = 1/4$ and $y = 3/4$ (see text).

The observation that the unit cells of the two compounds C18-C10- and diC14-PC are identical (see Table 7) suggests that each chain of one side of the bilayer is precisely on the prolongation of one chain of the other side (see Fig. 9); indeed with this arrangement C18-C10 and diC14 are easily interchangeable. In this case the space group is $P2_122$; this space group and the orientation of the chains are probably valid for all the compounds. On the contrary the following arguments suggest that the position of the chains precisely in each other's prolongation may well be a special property of that pair of compounds. First, it would be difficult to justify the small water content of the phase C, its three-dimensional crystalline structure, and the similarity of all the unit cells, if the polar groups were not ordered and the structure of the polar layer were not precisely the same in all the compounds; second, we have concluded that the angle of tilt is the same for all the compounds, a result consistent with a precisely defined structure at and near the polar groups. It is now clear that these three conditions, positioning the chains in each other's prolongation, preserving the organization of the polar layer, keeping constant the angle of tilt, cannot all be satisfied in all the compounds, given the observed dimensions of the unit cells (see Tardieu,

1972). Of these three conditions the first is probably the weakest; its general validity could be assessed by a study of other pairs of compounds analogous to C18-C10/diC14.

The result of this discussion is illustrated in Figure 9. Structure factor calculations not reported here (see Tardieu, 1972) show that this organization of the chains takes into account the intensity of the reflections in the range $(4{\cdot}5\ \text{Å})^{-1}$–$(4{\cdot}1\ \text{Å})^{-1}$. Finally the observations of the phase C both in egg-PC and in the synthetic compounds suggests that the organization of the chains displays some degree of disorder, rotational or of a more general nature.

5. Discussion

We have emphasized the liquid-like nature of the α conformation, which is the most widespread of all the conformations and probably the most relevant to biological membranes. We should stress the sharp distinction between the α and the other partly ordered conformations: the presently available X-ray diffraction evidence does not support the existence of any intermediate conformation. Some authors who have recently attempted to analyse the conformation of the chains in the phase Lα appear to shift the emphasis from disorder to order. In most cases the conflict with our views is more apparent than real (see for example Levine & Wilkins, 1971; McFarland & McConnell, 1971) in the sense that our work denies a periodic order of the chains qualitatively different from that of a liquid, not some kind of anisotropic organization (see Luzzati *et al.*, 1960). In some cases nevertheless (for example Träuble & Haynes, 1971) the conclusions are based upon an incorrect interpretation of the X-ray data, often caused by the authors overlooking the structural differences between synthetic lecithins and other lipids.

The other conformations are at least partly ordered, namely the chain axes are organized according to two- or three-dimensional lattices; the chains are fully elongated in the β and β' conformations as well as in phase C, and coiled into helices in the δ conformation. Nevertheless none of the phases (with the exception perhaps of phase C) displays crystalline order, with each atom precisely located in a three-dimensional cell. Two types of disorder should be distinguished. One is typically mesomorphic, according to Friedel's definition (Friedel, 1922): the structure is periodically ordered only in one (phases L) or two (phases P) dimensions, not in three as in an ordinary crystal. The lack of periodic order in one or two dimensions bears closely upon translational and rotational disorder in the polar or the hydrocarbon layers; the only cases in which some evidence is observed of correlations between adjacent monolayers are the δ conformation (correlations across the polar layer) and the β' conformation (interlocking of the CH_3 groups). The other type of disorder concerns the orientation of the chains around their axes; we have pointed out that the rotational disorder appears to be complete in the phases Lβ, Lδ, Pδ, perhaps less extensive in the phases Lβ' and Pβ' (see Appendix). Phase C is unique in displaying a three-dimensional crystalline lattice; even in this case, nevertheless, the organization of the chains probably involves some degree of disorder (see above).

In many of the phases (type L) the structure elements are planar lamellae; in one phase (Pβ') the lamellae are distorted by periodic ripples, in another (Pδ) the structure elements are indefinitely long ribbons of finite width. We have explained these deformations of the lamellae by a "cumulative strain" effect, previously evoked in the discussion of other phases of lipid-containing systems (see Luzzati, 1968).

One of the main conclusions of our work is the striking correlation between chemical composition and physical structure below the "melting" temperature. In the phases with fully extended chains the orientation is restricted to the normal to the lamellae (β conformation) if the chains are chemically heterogeneous. If the chains are homogeneous the orientation becomes tilted (β' conformation) and the lamellae are distorted by periodic ripples (phase Pβ'); as an explanation of these phenomena we have proposed a precise interlocking of the CH_3 groups. In addition we should stress the fact that some of the partly ordered conformations (β and δ, as well as phase C) seem to accommodate a large number of unsaturated bonds.

From the biological standpoint our work suggests that the "ordered" conformation of the chains observed in some membranes at low temperature (Engelman, 1970; Esfahani *et al.*, 1971; Shechter *et al.*, 1972) is of type β, on account of the sharpness of the $(4{\cdot}2\ \text{Å})^{-1}$ reflection and of the chemical heterogeneity of the chains. In addition a recent analysis of the order $\rightleftharpoons$ disorder transitions in model lipid-containing systems and in *E. coli* membranes has called attention to the segregation of the lipid molecules into separate domains with chains respectively in the α and in the β conformations, and to the diffusion phenomena involved in this segregation (Dupont *et al.*, 1972; Luzzati *et al.*, 1972*b*, and unpublished results).

This work brings once more to the fore the extraordinary polymorphism of lipids (see Luzzati, 1968). The various phases described here belong to the extended region of the phase diagrams often and loosely called the "coagel"; in fact this description of the lecithin–water systems is not exhaustive (unpublished observations). In the case of other lipids similar studies would probably uncover other phases and other conformations of the chains. A word of caution is in order here on the widespread and uncritical use of synthetic lecithins as model compounds for the study of structure and conformational transitions in membranes; for example dipalmitoyllecithin, so widely used for that purpose, has its chains in the β' conformation at room temperature, and displays structural properties in many respects different from those of membrane lipids.

As a final comment of technical interest, we wish to stress once more that the analysis of the type of structure described in this work relies much more heavily upon the chemical, physical and geometrical parameters (namely composition, partial volumes, cell dimensions, space groups) than on the electron density profiles. These profiles are indeed critically dependent upon a choice of phases which most often is to some extent arbitrary; the pattern recognition technique (Luzzati *et al.*, 1972*a*) is intended to provide a more systematic and objective approach to the phase problem, but it could hardly be expected to remove all ambiguity.

APPENDIX

Crystallographic Analyses of the β, β' and δ Conformations

Let us analyse first the intensity distribution in the X-ray powder diagram of an object formed by identical planar lamellae, each of which is a two-dimensional crystal, all parallel and equally spaced without other correlations in relative position and orientation. $\boldsymbol{a}$ and $\boldsymbol{b}$ are the unit vectors of the two-dimensional lattice, $\boldsymbol{c}$ is the stacking repeat of the lamellae; $\{\boldsymbol{a}^*, \boldsymbol{b}^*, \boldsymbol{c}^*\}$ are the reciprocal vectors of $\{\boldsymbol{a}, \boldsymbol{b}, \boldsymbol{c}\}$;

h, k, l are integers, ζ is a continuous real number. The Fourier transform of this structure is non-zero only along the straight lines $\boldsymbol{s} = h\boldsymbol{a}^* + k\boldsymbol{b}^* + \zeta\boldsymbol{c}$, normal to the plane of the lamellae; the axis $\boldsymbol{c}^*$ contains sharp reflections at $\boldsymbol{s} = l\boldsymbol{c}^*$; the intensity along the other lines is proportional to the square of the intensity scattered by one unit cell. The intensity distribution in the powder diagram is obtained by spherical integration (note that we deal here with a special case of a well known problem in X-ray diffraction; see for example Guinier, 1964, pp. 541–548).

The geometric construction is sketched in Figure 10. On the left side of the Figure a lamella is shown in projection; the right side represents the corresponding section of the reciprocal lattice and its contribution to the powder diagram. Three cases are considered. In the upper frame the structure of the lamellae consists of an array of delta functions: in this case the intensity is uniform along the line $\boldsymbol{s} = \boldsymbol{a}^* + \zeta\boldsymbol{c}^*$, and the powder diagram contains a diffuse band with a sharp inner edge at $\boldsymbol{s} = h\boldsymbol{a}^*$,

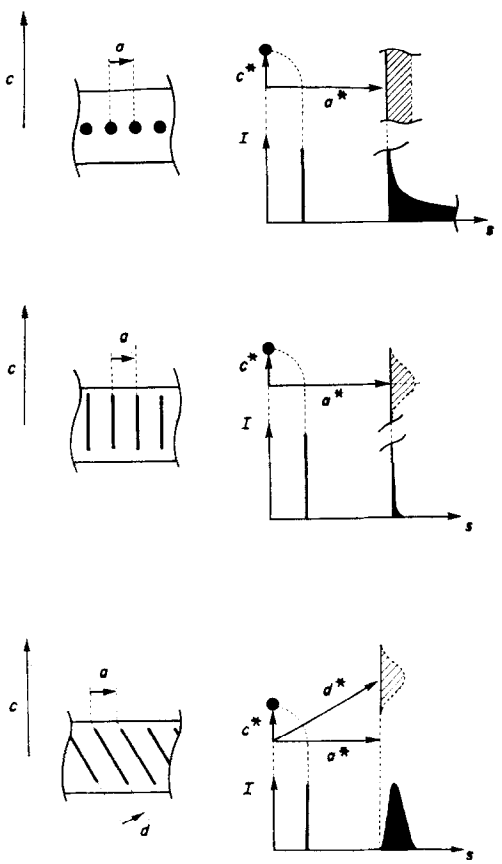

FIG. 10. Generation of the powder diagram of a system of planar lamellae, all parallel and equally spaced without other correlation in position and orientation, each lamella with an ordered structure according to a two-dimensional lattice. Left side, projection of the structure of one lamella. Right side, above, corresponding section of the reciprocal space, with the intensity localized in a delta function at $\boldsymbol{c}^*$ and (hatched surface) along the line $\boldsymbol{a}^* + \zeta\boldsymbol{c}^*$; below, contribution of that section to the powder diagram. Upper frame, the lamella contains a two-dimensional array of delta functions; middle frame, the lamella contains identical thin rods, all parallel and oriented at right angle to the plane of the lamella; lower frame, the rods are tilted, without changing the perpendicular distance from one rod to another.

in addition to the sharp reflections [00l]. In the middle frame the structure consists of identical thin rods, all parallel and oriented at right angles to the plane of the lamellae: in this case the intensity along the line $\boldsymbol{s} = \boldsymbol{a}^* + \zeta \boldsymbol{c}^*$ is the square of the Fourier transform of one rod; this is a bell-shaped curve with its maximum at $\zeta = 0$, which in the powder diagram gives a band, all the sharper as the rods are the longer. The structure of the lower frame is similar to that of the middle frame, with the difference that the rods are tilted; the intensity distribution along the line $\boldsymbol{s} = \boldsymbol{a}^* + \zeta \boldsymbol{c}^*$ is similar to that of the middle frame, but the bell-shaped curve is shifted, with the result that band in the powder diagram becomes more diffuse. We have drawn in Figure 11 the shape of the band for different angles of tilt; it may be noted that the bands are centred approximately at the spacing corresponding to the perpendicular distance from one rod to another.

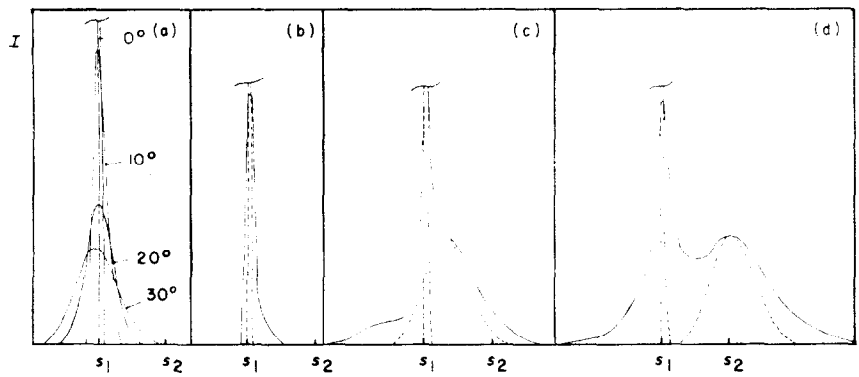

FIG. 11. Observed and calculated intensity distribution in the $(4{\cdot}2\ \text{Å})^{-1}$ region. $s_1 = 0{\cdot}238$. $s_2 = 0{\cdot}250\ \text{Å}^{-1}$. (a) Calculated profiles in the case of Fig. 10, lower frame, for rods 36 Å long, with a central gap 4 Å long (the CH_3 groups); the different curves correspond to different values of the angle of tilt θ. (b) (————) microdensitometer tracing for a phase Lβ (Plate I(b)). (– – – – –) calculated profile for a hexagonal array of rods (as in (a) above) 4·85 Å apart, perpendicular to the lamella. (c) (————) microdensitometer tracing for a phase Lβ'; diC16-PC, $c = 0{\cdot}20$, $T = 33°C$ (see Plate I(c)). (– – – – –) calculated profile for a quasi-hexagonal array of rods, each rod surrounded by 4 rods at 4·82 Å and by 2 rods at 4·75 Å, tilted by 30°; the direction of tilting with respect to the quasi-hexagonal lattice is taken from Levine (1970). (d) (————) as in (c), $T = 4°C$ (see Plate (d)). (– – – – –) as in (c), with each rod surrounded by 4 rods at 4·78 Å and by 2 rods at 4·55 Å.

Let us now consider the phases Lβ and Lβ'. We have discussed above the evidence in favour of fully extended chains organized with rotational disorder according to a two-dimensional lattice; we may thus identify the chains with the rods. Furthermore we have shown that the area of the unit cell of the two-dimensional lattice is equal to the cross-sectional area of one chain; since two chains are attached to each polar group, the lack of "high"-angle bands or reflections at $s < (4{\cdot}2\ \text{Å})^{-1}$ indicates that the position of the polar groups is not ordered (see below the discussion of the phase Lδ). In the phase Lβ the rods are oriented at right angles to the plane of the lamellae; in this case (see Fig. 11 and Plate I(b)) the bands at high angles may be expected to be quite sharp. In the phase Lβ' the chains are tilted; it should be clear in this case that the apparent angle of tilt (see Fig. 10, lower frame) will depend upon the orientation with respect to the lattice of the projection of one rod on the plane of the lamella, and upon the indices of the reflection. We have constructed the powder

diagrams represented in Figure 11 for the orientation determined by Levine (1970), and on the assumption that at low temperature the packing of the chains becomes slightly anisotropic and thus that the hexagonal symmetry breaks down; the cross-sectional areas per chain (see dimensions of the two-dimensional cells in the legend of Fig. 11) are 20·4 Å^2 in the β conformation, 20·0 and 19·2 Å^2 in the β' conformation at 33 and 4°C, respectively, well within the variations observed in saturated paraffins near the transition orthorhombic-hexagonal (Vand, 1953). It must be noted that the calculations are made for a perfect two-dimensional lattice, and for a unit cell containing one rod with a gap (see legend of Fig. 11), on the assumption that in Lβ' pairs of lipid monolayers are coherently positioned across the CH_3 groups and that the rotational and translational disorder is located in the polar layer. It is clear that the bands tend to be broadened by lattice imperfections, and that a more realistic distribution of matter in the unit cell may well distort the profiles of the bands. Taking into account these approximations the agreement of the observed and calculated curves of Figure 11 is quite satisfactory.

We may now consider the phase Lδ. We have discussed above the evidence in favour of the presence of stiff rods (which we have assimilated to helically coiled chains) all parallel and normal to the plane of the lamellae, organized with rotational disorder according to a two-dimensional square lattice of side 4·8 Å. This lattice of rods is responsible for the sharp reflections at (4·8 Å)$^{-1}$ and (3·4 Å)$^{-1}$ (see Plate I(f) and Fig. 5). In addition the presence of diffuse bands with a sharp inner edge at (6·8 Å)$^{-1}$, (4·8 Å)$^{-1}$, (3·4 Å)$^{-1}$ and (3·05 Å)$^{-1}$ suggests a two-dimensional lattice of elements of small size, analogous to the case of the upper frame of Figure 10; the lattice is square, its side is the diagonal of the lattice of the chains, and thus it contains two chains (Fig. 6). It is clear that these elements of small size can be identified with the polar groups of the lipid molecules; in addition we have suggested above that the two monolayers opposed by their polar faces are in register, and that some rotational and translational disorder is present in the middle of the hydrocarbon layer. We may note again that the shape and the intensity of the sharp and diffuse bands depend upon the precise position of the polar groups and of the hydrocarbon chains, and on the imperfections of the lattice.

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