X-RAY DIFFRACTION STUDY IN WATER OF LIPIDS EXTRACTED FROM HUMAN ERYTHROCYTES

THE POSITION OF CHOLESTEROL IN THE LIPID LAMELLAE

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ABSTRACT Lipids, carefully extracted from fresh human erythrocytes, form liquid-crystalline structures in water. A phase diagram of this system was constructed, characterizing, by X-ray diffraction, the structures which form as a function of concentration of lipid and temperature. One extended range of concentration of the phase diagram, in which a single lamellar phase exists, permitted further analysis of the diffraction data. This phase consists of lipid layers of constant thickness separated by water layers of varying thickness according to the water content of the system. The distribution of the electron density is precisely analyzed and the amplitude of the reflections is, at all concentrations, proportional to the Fourier Transform of an isolated lipid layer. This shows that the lipid layer is filled with the hydrocarbon chains of the phospholipids and is covered on both sides by their hydrophilic groups. Cholesterol, present in high concentration in erythrocyte membranes, is located so that part of its steroid nucleus is between the polar groups of the phospholipid molecules while the rest of the molecule extends into the inner hydrocarbon layer.

INTRODUCTION

It is now well established that amphiphilic molecules form a wide variety of structures in water. This polymorphism has been demonstrated in systems of pure soaps and detergents (Luzzati, Mustacchi, Skoulios, and Husson, 1960; Husson, Mustacchi, and Luzzati, 1960), and in systems of more biological interest, containing either single species of phospholipids–lecithin, phosphatidyl ethanolamine or sphingolipids (Reiss-Husson, 1967), or complex mixtures–brain extract (Luzzati and Husson, 1962), mitochondrial lipids (Gulik-Krzywicki, Rivas, and Luzzati, 1967), lecithin and cholesterol (Bourgès, Small, and Dervichian, 1967). The molecular arrangement in a number of these structures has been determined. The results,

using these model systems, have led to speculation about the physiological role that lipids could play in intact cellular membranous structures (Luzzati and Husson, 1962; Luzzati, Reiss-Husson, Rivas, and Gulik-Krzywicki, 1966; Reiss-Husson and Luzzati, 1966).

The work reported here is a continuation of these studies on model systems and the lipids were extracted from human erythrocytes. In addition to being obtained from the purest source of plasma membranes available at the present time, these lipids are interesting in that they contain a high proportion of cholesterol, a molecule considerably different from the general class of biological phospholipids. The structure of a phospholipid-cholesterol complex has been determined.

STRUCTURAL ANALYSIS

Nomenclature and Formulae

$s = 2 \sin \theta/\lambda$	reciprocal spacing $(A)^{-1}$; 2θ is the angle of diffraction, λ is the wavelength, which throughout this work is Cu $K\alpha_1$ = 1.540 A
N	Avogadro's number
pl, c, w	subscripts which refer to phospholipids, cholesterol, and water, respectively
M	molecular weight or average molecular weight of a mixture
n	number of electrons
c	weight concentration (lipid/lipid + water)
$\bar{v}_{pl}$, v_c , v_w	partial specific volumes of phospholipids, cholesterol, and water
ρ	electron density (electron/A^3)
$\varphi = \left[1 + \frac{(1-c)v_w}{c} \frac{(1+K)}{Kv_c + \bar{v}_{pl}}\right]^{-1}$	volume concentration of lipid, where $K = \frac{M_c}{\bar{M}_{pl}} \cdot f$
f	mole ratio cholesterol/phospholipid

Lamellar Phase:

d	repeat period in one dimension, (A)
$s = l/d$	spacings of the diffracted rays $(A)^{-1}$ l = 1, 2, 3, etc.
$d_l = \varphi d$	thickness of the lipid layer that the lipid molecules would occupy in the repeat period without water, (A)
$d_w = d - d_l$	thickness of water layer, (A)
$N_1 = \frac{d_l \cdot N \cdot 10^{-24}}{\bar{M}_{pl}\bar{v}_{pl} + fM_cv_c}$	number of "molecules" (made up of one phospholipid molecule plus f cholesterol molecules) per unit area $(A)^2$ of the lamellae
$S = 2/N_1$	average area available, at the interface to one phospholipid molecule plus f cholesterol molecules

Procedures used to determine the structures formed in the lipid-water system will be outlined. A comprehensive review of these has recently been made (Luzzati, 1967) and we shall briefly refer only to the general principles pertinent to the results obtained in the present study.

The Phase Diagram

The first step in a study, by X-ray diffraction, of a multicomponent system is to

identify the different phases present and to find their range of existence as a function of concentration and temperature, i.e., to construct a phase diagram.

Small Scale Organization–Conformation of the Paraffin Chains

The only conformation of the paraffin chains found in this study is the liquid, chaotic, conformation characterized in the X-ray diagram by a diffuse band about $s = (4.5\ \text{A})^{-1}$. This disorder is confirmed by the fact that the dimensions of the structural elements decrease as the temperature is increased, according to a law whose theoretical interpretation is given elsewhere (Luzzati et al., 1960).

Large Scale Organization

The only phase of interest in this study is the lamellar phase, one in which there is a periodic organization in one dimension only. The lipid molecules are associated in parallel equidistant leaflets with no other correlations in position or orientation.

Determination of Structural Parameters

The X-ray data give a series of reflections; from the ratio of their spacings the symmetry of a phase can be determined. For the lamellar phase the spacings are given by $s = l/d$, $l = 1, 2, 3 \cdots$. Having determined the ratio of the spacings and therefore the symmetry, the repeat distance, d, can be determined.

If the concentration, c, the mole ratio of cholesterol in the lipid, f, and the partial specific volumes of the lipids and water are known, further structural parameters can be calculated. First, the assumption is made that the lipid molecules associate so that the hydrocarbon chains of the lipid molecules form a layer that excludes water and the hydrophilic polar groups occupy the interface between the water and paraffin layers. Cholesterol is assumed to be somewhere within the lipid layers as well. Then the thickness of this lipid layer, d_l, can be determined (see formulae above), and the average surface area occupied by the lipid molecules at the water-lipid interface can be calculated. In the present case, this means the area available to one phospholipid molecule plus the mole fraction of a cholesterol molecule (see formulae above).

Electron Density Distribution

The following step is a search for the electron density distribution in the phase which will account for the intensities of the X-ray reflections. A model of the electron density distribution is based on both the lipid composition of the sample and the molecular structure of these lipids. This will later be described in more detail. In accounting for the intensities of the X-ray reflections in all samples, the assumption, made above, about the association of the lipid molecules into leaflets is verified, and the structure of the lipid leaflet is determined.

EXPERIMENTAL PROCEDURE

Preparation and Assay of Lipid

Fresh human blood, collected into acid citrate dextrose, was centrifuged (4000 *g* for 10 min), the serum and buffy coat removed, and the red cells washed three times with an equal volume of 310 milliosmolar sodium phosphate buffer (pH 7.5–8.0). Remnants of the buffy coat were removed after each centrifugation. The erythrocyte lipids were then extracted following a technique modified from that described by Reed, Swisher, Marinetti, and Eden (1960). One volume of a suspension of the washed cells (50–70% hematocrit) was slowly added to four volumes of freshly distilled methanol and agitated by bubbling nitrogen through the mixture for 20 min. Then four volumes of freshly distilled chloroform was added and the agitation continued for another 20 min. The suspension was then filtered, the chloroform-methanol evaporated at a temperature of less than 30°C, and the residue from this dried completely under vacuum for 12 hr at room temperature. This dry residue was then re-extracted three times, 5 min each, with one volume of freshly distilled chloroform. These last extracts were filtered, pooled, and evaporated to complete dryness. These lipids were washed by dissolving them in 100 ml of a mixture of two parts chloroform and one part methanol to which was added 10 ml of distilled water. The mixture was shaken, then separation was speeded up by centrifugation, and the upper layer carefully pipetted off and discarded. This wash was repeated once.

The entire procedure was carried out under nitrogen and the temperature never exceeded 30°C. Three important modifications of the technique as described by Reed et al. (1960) should be noted.

(*a*) Since 0.5–1.0 g of lipid was required, the quantity of solvent used, in relation to the volume of blood, was less than that used by Reed et al. (1960), and, to compensate, the extraction time was increased.

(*b*) It was found that if the first methanol-chloroform extraction was repeated a second and third time on the residual precipitate, the lipids from these extracts were significantly decomposed as shown by thin-layer chromatography. Hence only the lipids which came in the first extraction, and which made up approximately 80% of the weight of the three extractions, were used.

(*c*) The lipids were washed twice as described, and unless this was done, a ninhydrin-positive spot was present at the origin of the thin layer chromatograms. The presence of this component in the lipid greatly affected the phase diagram and probably led to some anomalous results in the original experimentation.

To assay for purity and decomposition, the individual lipids of the extract were separated by thin-layer chromatography on glass plates spread with Silica Gel G, developed with chloroform:methanol:water, 65:25:4, and detected with iodine vapor. Lecithin from soya bean and crystalline cholesterol were chromatographed simultaneously as standards. The remaining phospholipids were assumed to be the same as those of an identical chromatogram given by Roelofsen, de Gier, and Van Deenen (1964) for lipids extracted from human erythrocytes. The relative amount of cholesterol to phospholipid that is extracted from erythrocyte membranes has been subject to some variation (Bishop and Surgenor, 1964) particularly according to the solvent system used (Rose and Oklander, 1965). Quantitive analysis of cholesterol in our extraction was therefore done.

X-ray samples were prepared immediately after the extraction and the excess lipid was stored dry, under nitrogen, at −20°C. The lipid could be kept under these conditions for at least 1 month with no detectable change in chromatogram or structure as determined in the X-ray diagrams. Sample chromatograms done after the X-ray experiments showed no detectable change.

X-ray Experiments

Samples of lipid were prepared in small weighing bottles and a range of concentrations prepared by adding glass-distilled water. They were then left for approximately 1–2 days at room temperature to come to equilibrium. Finally they were transferred to the X-ray sample holders, which sealed a 1 mm thick sample between thin mica windows. The temperature of the sample could be controlled during the X-ray experiment. Spot checks on the water concentration were made after the X-ray experiments using the analytical technique of Karl Fischer.

The X-ray cameras used were of the Guinier type, operating in vacuum, and fitted with a bent quartz monochromator which isolated the Copper $K\alpha_1$ line, (λ = 1.540 A).

RESULTS

Chemical Makeup of the Samples

The lipid composition of human erythrocytes and the fatty acid composition of the phospholipids have been analyzed by a number of laboratories. A survey of these works is given by Bishop and Surgenor (1964). We have accepted these figures for the phospholipids and have calculated an over-all average of phospholipid content. Cholesterol recovery in our preparation was 26% of the dry weight of lipid giving a mole ratio of cholesterol/phospholipid, f = 0.72. Details of the chemical composition of the total lipid extract, and calculations of the electron content of its various parts, are given in the Appendix.

Phase Diagram

The phase diagram of the lipid-water system was explored, as a function of concentration and temperature, within the limits shown by the experimental points in Fig. 1. The different phases were characterized by their X-ray diffraction pattern; their range of existence is shown. Two major regions can be distinguished in the phase diagram and these are separated by the line *abc* in Fig. 1.

To the left of the line *abc*, i.e. at higher concentrations of lipid, the X-ray diagrams show the existence of both a lamellar phase and lines and spots at high and low angles that can be indexed on a pattern from crystalline cholesterol. Hence there is more than one phase in the sample, and since the composition of these phases is unknown, little useful information can be obtained about the structure of the system at these higher concentrations. Therefore, no further consideration will be made of this region in the phase diagram.

The second region is that to the right of the line *abc*, i.e. at lower lipid concentrations. Here a single lamellar phase exists and we are primarily concerned with the structure of this phase. The conformation of the paraffin chains is chaotic throughout this region. The diffuse band at s = (4.5 A)$^{-1}$, which is seen at 37°C and 20°C, remains diffuse down to −5°C.

Structure of the Lamellar Phase

This phase is characterized by an X-ray diffraction diagram, all of whose lines are

sharp and equidistant, and which contains a diffuse band at $s = (4.5\ A)^{-1}$. The structure of this phase, shown schematically in Fig. 2 *a*, is formed by identical and equidistant leaflets of lipid separated by layers of water. The "liquid" paraffin chains of the phospholipids and the cholesterol molecules fill the interior of the leaflets; the hydrophilic groups lie at the interface between the water and paraffin layers. The position of cholesterol is shown to be well protected from the water layer but oriented with the steroid nucleus toward the polar groups of the phos-

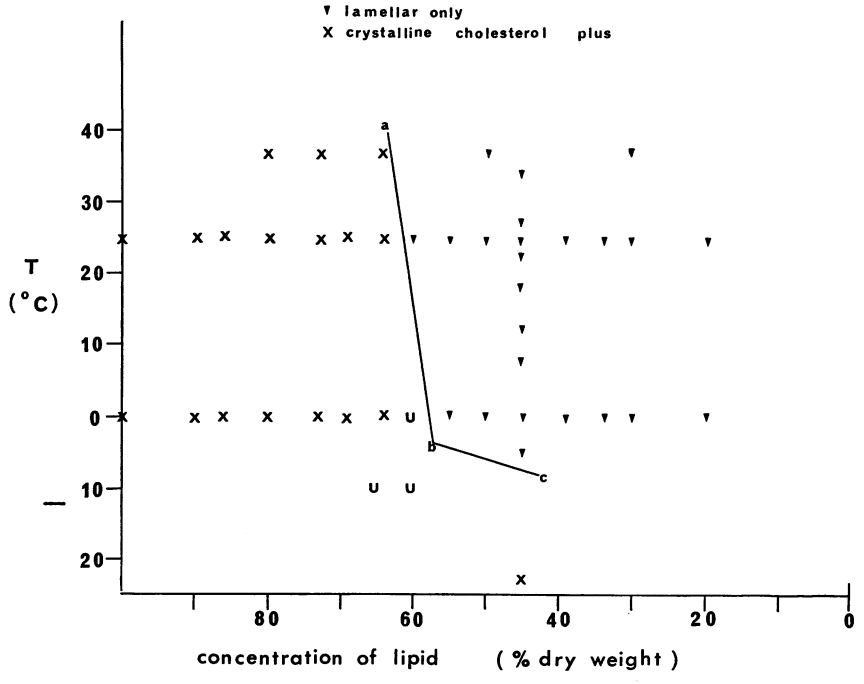

FIGURE 1 Phase Diagram. The marked points indicate region explored. X indicates crystalline cholesterol plus another phase. U indicates an unstable sample. ▼ indicates a single lamellar phase. The line *abc* depicts the boundary between the two major regions of the phase diagram.

pholipid molecules. This structure and molecular arrangement will be justified in the following.

For each sample the repeat period is measured and the thickness of the lipid leaflet (d_l) and the water (d_w) layers are determined (see above). These parameters are shown in Fig. 3. At constant temperature the thickness of the lipid leaflet is independent of concentration. This suggests that the structure of the lipid leaflet is the same at all concentrations and that, consequently, a precise study of the electron density distribution through it can be made because the structure factors, or am-

plitudes of the X-ray intensities, are a function of s and independent of the concentration of the sample:

$$F(s) = \int_{\text{leaflet}} [\rho(z) - \rho_w] \cos 2\pi sz \, dz \qquad (1)$$

z is the direction perpendicular to the plane of the leaflet.
$\rho(z)$ is the electron density distribution through the leaflet.
ρ_w is the electron density of water, assumed constant.

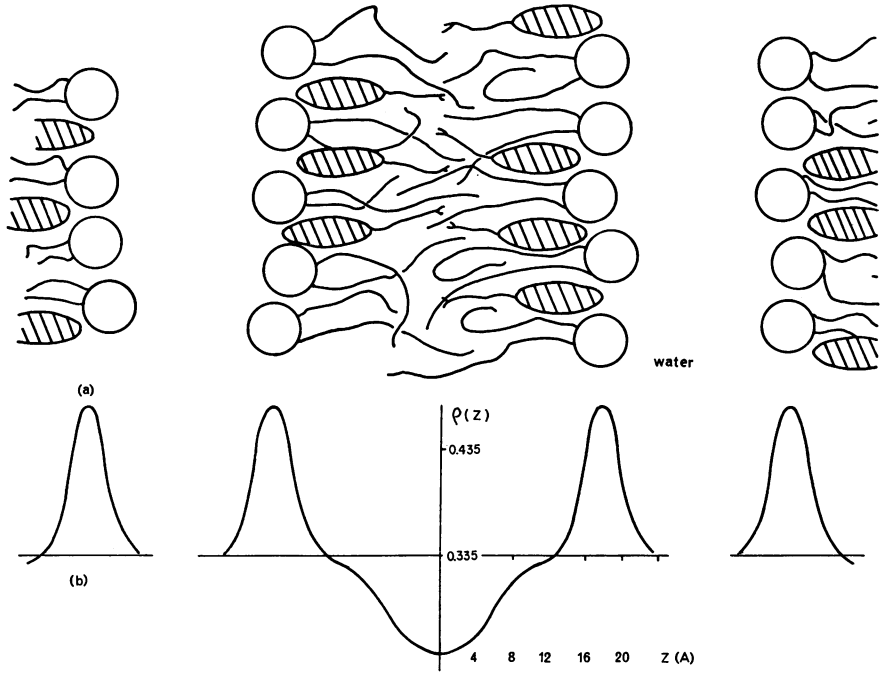

FIGURE 2 (*a*) Schematic representation of the structure of the lamellar phase. The circles represent the polar groups of the phospholipid molecules. The curved lines represent the paraffin chains. The hatched group represents the steroid nucleus of the cholesterol molecule. (*b*) The electron density distribution through the lipid leaflet, indicating the absolute levels of electron densities and the dimensions, z, from the center of the leaflet.

The assumption is made that the leaflet contains a plane of symmetry.

Fig. 4 *a* shows the relative structure factors observed at different concentrations. The variation in structure factors is the same for all concentrations and shows three values of s where the structure factor goes to zero. The envelope forming the variation in structure factor as a function of s follows the Fourier Transform of the electron density distribution through one isolated lipid leaflet (Equation 1).

A model for the electron density distribution through the lipid leaflet has been constructed on the basis of the structural parameters given in Fig. 3 and the chemical

makeup of the total lipid. The construction of the model which is outlined in detail in the Appendix was based on the following principles:

(*a*) The phospholipid molecules are associated into a bimolecular layer forming an internal paraffin layer lined on both sides with the polar groups (Fig. 2 *a*).

(*b*) Into the phospholipid layer must be placed cholesterol molecules in the stoichiometric ratio found in our preparation. The position of cholesterol can be determined by trial and error; it turns out that a satisfactory agreement of the ob-

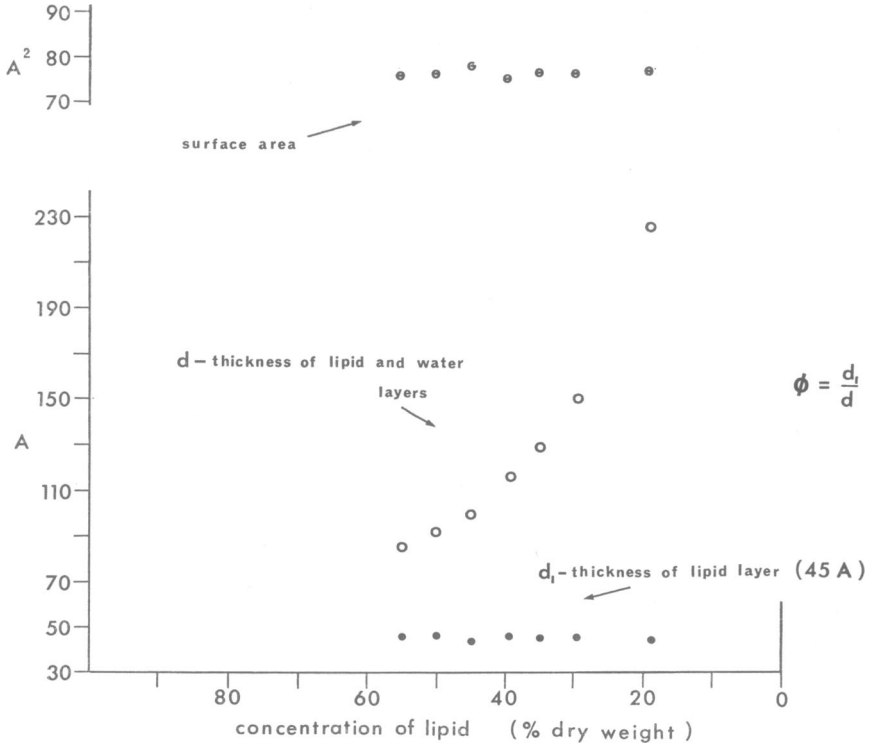

FIGURE 3 Dimensions of the lamellar phase at 0°C. The surface area represented is that occupied by one phospholipid molecule plus 0.72 of a cholesterol molecule. This is equivalent to one of each of these molecules occupying a total of 86 A². (see text)

served and calculated structure factors can only be reached by locating the steroid nucleus near the interface, thus giving an electron density close to that of water in the region of 9–14 A (Fig. 2 *b*).

(*c*) The precise model was built assuming that one-half of the steroid nucleus belongs to the polar layer, the rest, with its paraffin chain, to the hydrocarbon layer (Fig. 2 *a*). The number of electrons in these two layers is determined in the Appendix.

(d) In the absence of cholesterol the electron density distribution was previously assumed to take the form of the sum of step functions (Gulik-Krzywicki, Rivas,

and Luzzati, 1967). As the presence of cholesterol at the interface smooths out the sharp boundaries, we prefer to approximate here the electron density distribution by a sum of Gaussian functions (Fig. 2 *b*).

$$\rho(z) - \rho_w = A \exp [-\alpha(z - a)^2] + A \exp [-\alpha(z + a)^2] + B \exp [-\beta z^2] \quad (2)$$

The first two terms of the right-hand side of Equation 2 correspond to the polar

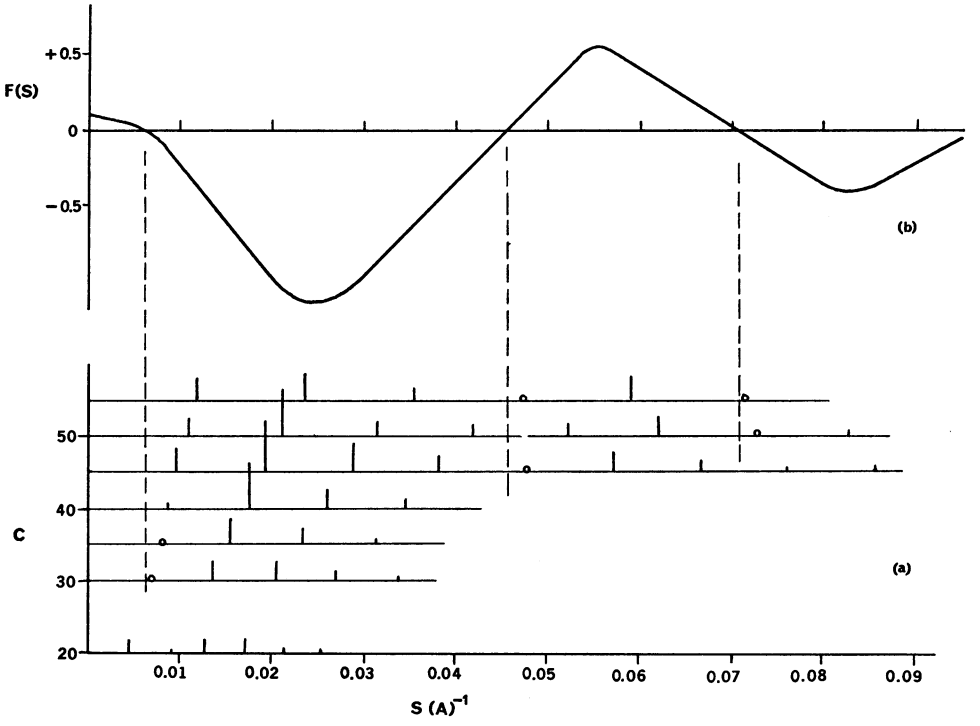

FIGURE 4 Crystallographic verification of the model shown in Fig. 2.

(*a*) The ordinate represents the concentration of the sample and the abscissa represents the reciprocal space parameter, *s*. The vertical lines give the intensity (estimated visually) of the X-ray reflections. Therefore each horizontal line represents the X-ray diagram for that concentration.

(*b*) $F(s)$ is the Fourier Transform of the electron density distribution of Fig. 2 *b*.

layers, the third to the paraffin core. As the electron content of these layers is known (Appendix, III), the following equations are obtained:

$$2A \int_{-\infty}^{\infty} \exp [-\alpha(z - a)^2] \, dz = \frac{2\sqrt{\pi}}{\sqrt{\alpha}} A = +1.490 \text{ e/A}^2$$

$$B \int_{-\infty}^{\infty} \exp [-\beta z^2] \, dz = \frac{\sqrt{\pi}}{\sqrt{\beta}} B = -1.285 \text{ e/A}^2$$

The model thus depends on the choice of three independent parameters: the width,

α and β of the Gaussian functions, and the position a of the center of the polar layer. These three parameters were adjusted by comparing the observed (Fig. 4 *a*) and calculated (Equation 1 and Appendix, IV) structure factors; the most sensitive test is in fact the position of the zeros and the best agreement (Fig. 4) was obtained for the parameters $\alpha = 0.120$, $\beta = 0.0156$, $a = 17.8$ A, $A = 0.145$ and $B = -0.091$. The electron density distribution is shown in Fig. 2 *b*.

It should be noted that the discrimination of the models is quite severe, and that it is difficult to reach a satisfactory agreement of the structure factors. This fact gives us confidence that the model of Fig. 2 is not too far from reality.

Another important parameter that can be calculated is the surface area, S, occupied by the molecules at the water interface. In the present case the area occupied by one phospholipid molecule plus 0.72 of a cholesterol molecule is 76.5 A^2 (see above formulae).

DISCUSSION

The behavior of this lipid system at low concentrations is similar to two others which have now been studied in detail (Luzzati and Husson (1962); Gulik-Krzywicki et al., 1967). The remarkable fact is that well developed order on the large scale occurs in a system where order on a smaller atomic scale is chaotic or liquid-like. In these systems of mixed lipids, as opposed to others containing one particular species (Reiss-Husson, 1967), a large amount of water can be incorporated in the lamellar phase and is intercalated between lipid leaflets of constant thickness.

The most novel result of this work is perhaps the establishment of the position that cholesterol takes in a bimolecular layer of phospholipids. The molecular arrangement in Fig. 2 shows that cholesterol takes a position where its paraffin side chain extends toward the center of the leaflet and the steroid nucleus is interdigitated between the polar groups of the phospholipid molecules, with a considerable part of it, however, overlapping with the paraffin chains of the phospholipid molecules. The model suggests that the single hydrophilic hydroxyl group of the cholesterol molecule is weakly exposed to the water layer or may even interact with the polar groups of the phospholipid molecules, but our data do not provide resolution of this order. A similar model has been postulated recently by Bourgès et al. (1967) and some very specific configurations of cholesterol-phospholipid interactions have been suggested (Finean, 1953; Vandenheuvel, 1963). However, considering the liquid chaotic order on the atomic scale, as well as the size and species distribution of the polar groups and fatty acid chains, it is unreasonable to picture the lipid interactions as specific and immobile. The data provide proof of the position that cholesterol does take up in a fully hydrated bimolecular layer of phospholipids and suggests that it is in a "liquid" environment.

Consider the surface area, on the leaflet, occupied by a phospholipid molecule and 0.72 of a cholesterol molecule: 76.5 A^2. Cholesterol is considered to occupy a minimum of 35 A^2 in a monolayer at an air-water interface (van Deenen et al.,

1962). Assuming that this is the area occupied by cholesterol in our model, then the phospholipid molecule would occupy a maximum area of 51 A^2, or about 25 A^2 per fatty acid chain. The area occupied by similar phospholipid molecules without cholesterol (Luzzati and Husson, 1962; Gulik-Krzywicki et al., 1967) is about 60 A^2 or about 30 A^2 per hydrocarbon chain at 0°C. The reduction in area occupied by the phospholipid molecules, caused by the presence of cholesterol, is identical, quantitatively, to the area changes observed in mixed monolayers of these two types of lipids (van Deenen et al., 1962). This observation suggests that the condensing effect of cholesterol in a monolayer of unsaturated phospholipids at an air-water interface occurs in the fully hydrated bimolecular layer as well. Although the cholesterol increases the packing of the paraffin chains, presumably by restricting their thermal movement somewhat, the cholesterol prevents the ordering and crystallization of the hydrocarbon chains that has been observed at low temperatures in the cholesterol-free phospholipid systems of mitochondrial lipids (Gulik-Krzywicki et al., 1967) and lipids of brain extract (Luzzati and Husson, 1962).

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APPENDIX

The chemical makeup of the X-ray samples is given in the table below. The values were obtained in the following manner.

(*a*) Taking into account the composition of the phospholipids present in erythrocytes (Bishop and Surgenor, 1964), an average molecular weight and electron content per phospholipid molecule was calculated. ($\bar{M}$ = 794, $\bar{n}$ = 432e). Then using the value of the partial specific volume of lecithin (Reiss-Husson, 1967), the average volume occupied by a phospholipid molecule was calculated.

TABLE I

CHEMICAL COMPOSITION OF LIPID EXTRACT FROM ERYTHROCYTES

Phospholipid Content (wt. %)	Lysolecithin-2: lecithin-36: phosphatidyl serine-10: phosphatidyl ethanolamine-29: sphingomyelin-23.			
Average Phospholipid Molecule	$\bar{M}$ = 794 $\bar{n}$ = 432e $\bar{v}$ = 0.98 cm³/g (lecithin) ∴ Volume 1305 A³			
	Group	CH	CH_2	CH_3
Average fatty acid content of phospholipid molecules	No. of groups	6	28	2
	M of groups	13	14	15
	Electrons per group	7	8	9
	Volume per group (at 25° C) (A³) (Reiss-Husson and Luzzati, 1964).	20.5	27.0	54.0
Hydrocarbon part of phospholipid molecule	$\bar{M}$ = 500 $\bar{n}$ = 284e		Volume = 987 A³	
Polar Group of Phospholipid (by difference)	$\bar{M}$ = 294 $\bar{n}$ = 148e		Volume = 318 A³	
Cholesterol content of lipid	26% of total lipid ∴ f = 0.72			
Cholesterol	M_c = 387 n_c = 216e v_c = 0.938 cm³/g ∴ Volume 600 A³			
Hydrocarbon side chain	Group	CH	CH_2	CH_3
	No. of groups	2	3	3
	M = 113 n = 65e		Volume = 284 A³	
Steroid nucleus (by difference)	M = 274 n = 151e		Volume = 316 A³	

(*b*) A division between the paraffin chains and the polar parts of the phospholipid molecule is made between the first CH_2 group and the carboxyl group. The average fatty acid composition was determined from Bishop and Surgenor (1964). Given the volumes occupied by CH, CH_2, and CH_3 groups (Reiss-Husson and Luzzati, 1964), the molecular weight, number of electrons, and total volume of the paraffin part of the phospholipid molecule was calculated.

(*c*) By difference, between the whole phospholipid molecule and the paraffin part of the molecule, the same parameters were determined for the polar part of the phospholipid molecule.

(*d*) A similar division of the cholesterol molecule into two parts was made between the

steroid nucleus and its paraffin side chain, i.e. between the C_{17} and C_{20} carbons. Using the over-all partial specific volume of cholesterol and the same data for CH, CH_2, and CH_3 groups as above, the molecular weight, number of electrons, and volume of these two parts of cholesterol were calculated. Figures for cholesterol in the table are for the whole molecule: in ous sample for every phospholipid molecule there was only 0.72 cholesterol molecules.

Construction of the Model of Electron Density Distribution through the Lipid Leaflet

I. $N_1 = 0.02605$ "molecules"/A² (see *Nomenclature and Formulae*). Here one "molecule" refers to one phospholipid molecule and 0.72 cholesterol molecule.

II. Divide the lipid leaflet into two layers, a polar (pol) layer and a hydrocarbon (HC) layer. (All figures from previous table.)

Into the polar layer put:	n (electrons)	V(A³)
(*a*) The polar group of one phospholipid molecule	148	318
(*b*) ½ the steroid nucleus of 0.72 cholesterol molecule	54	113
Totals in polar layer	$n_{pol} = 202$ electrons	$V_{pol} = 431$ A³
Into the hydrocarbon layer put:		
(*a*) Hydrocarbon chains of one phospholipid molecule	284	987
(*b*) ½ steroid nucleus of 0.72 cholesterol molecule	54	113
(*c*) hydrocarbon sidechain of 0.72 cholesterol molecule	47	204
Totals in Hydrocarbon Layer	$n_{HC} = 385$	$V_{HC} = 1297$

III. The total content of the electron density contrast between the polar (and paraffin) leaflet and water is: ($\rho_w = 0.335$ e/A³):

$$\int_{\text{polar layer}} [\rho(z) - \rho_w]\, dz = N_1(n_{pol} - V_{pol}\rho_w) = +1.490 \text{ e/A}^2$$

$$\int_{\text{paraffin layers}} [\rho(z) - \rho_w]\, dz = N_1(n_{HC} - V_{HC}\rho_w) = -1.285 \text{ e/A}^2$$

IV. Let the electron density distribution through the lipid layers take the form of Gaussian functions. The distribution through the whole leaflet is given by

$$\Delta\rho(z) = \rho(z) - \rho_w = A \exp[-\alpha(z - a)^2] + A \exp[-\alpha(z + a)^2] - B \exp[-\beta z^2]$$

and its Fourier Transform (see Equation 1) is

$$F(s) = \frac{A\sqrt{\pi}}{\sqrt{\alpha}} \cdot \cos 2\pi as \cdot \exp\left[-\frac{\pi^2 s^2}{\alpha}\right] - \frac{B\sqrt{\pi}}{2\sqrt{\beta}} \cdot \exp\left[-\frac{\pi^2 s^2}{\beta}\right]$$