THE STRUCTURE OF THE LIQUID-CRYSTALLINE PHASES OF LIPID-WATER SYSTEMS

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ABSTRACT

Some simple lipid-water systems have been studied by x-ray scattering techniques, as a function of lipid concentration and temperature. Several liquid-crystalline phases have been found, and their structure has been determined: only one of these is lamellar. In all these phases the hydrocarbon part of the lipid molecules has a disordered, liquid-like structure. One biological phospholipid, a human brain extract, has been studied by the same technique, and two liquid-crystalline phases have been found: a lamellar phase, built up by an ordered sequence of lipid and water planar sheets, and a hexagonal phase, which is a hexagonal array of circular cylinders, each cylinder being a thin water channel covered by the hydrophilic groups of the lipid molecules, the hydrocarbon chains filling the gap between the cylinders. The interpretation of the electron microscope observations of the structure of lipoprotein membranes is discussed, and some possible biological implications are suggested.

INTRODUCTION

It is a matter of general consensus that the most widespread structure of lipoprotein complexes in living cells is lamellar (Sjöstrand, 1959; Fernández-Morán, 1959; Hodge, 1959), as is shown by electron microscope and x-ray diffraction observations.

The lipoproteins, extracted and fixed by different treatments, as well as the lipids alone, have been investigated often by x-ray techniques. In some cases the typical x-ray picture of a lamellar structure has been obtained; in other cases only some of the reflections are consistent with a lamellar structure, and other reflections exist which cannot be indexed on any simple lattice (Finean, 1960*a*, 1960*b*). In spite of their complexity, all these experimental observations seem to have been interpreted only in terms of lamellar structures, no other structure being taken into consideration.

A similar situation existed a few years ago in the field of simple association colloids (soaps and detergents in water), where the structure of all the liquid-crystalline phases was supposed to be lamellar (Stauff, 1939; McBain and Marsden, 1948).[1] The investigations undertaken about 1956 in Strasbourg on some of the simple lipids, either alone (Skoulios and Luzzati, 1961; Spegt and Skoulios, 1960; Gallot and Skoulios, 1961) or in the presence of water (Luzzati *et al.*, 1960; Husson *et al.*, 1960), lipophilic solvents (Skoulios, 1961), or both (Spegt *et al.*, 1961), have shown that many liquid-crystalline structures do in fact exist, the lamellar organization being only one among others. At the time these conclusions were reached it became apparent that the polymorphism observed in the simple lipids might have some bearing on the organization of biological lipids, at least *in vitro*. Furthermore, the lability of the structures with respect to temperature and to lipid concentration casts doubt upon the meaning of electron microscope observations, since the fixation and

[1] McBain and Marsden (1948) mention one example of hexagonal structure as an exceptional case that they find difficult to fit into their classification.

drying procedures seemed likely to disturb the organization.

The cooperation of Dr. W. Stoeckenius, of The Rockefeller Institute, New York, allowed us to extend this work to biological lipids, and to attempt to coordinate x-ray and electron microscope experiments.

We shall first briefly summarize the results of the work on systems containing simple lipids and water; we shall then describe the observations made with aqueous systems containing lipid from human brain, investigated as a function of concentration and temperature.

EXPERIMENTAL TECHNIQUE

The x-ray diffraction experiments have been performed with a Guinier camera, operating *in vacuo:* the x-ray beam is monochromatized and focused by a bent quartz crystal, which isolates the $CuK\alpha_1$ line. The samples are held in a vacuum-tight cylindrical cell, provided with two thin mica windows. An electric furnace raises the temperature, which is controlled by an automatic device.

The samples were prepared at specific concentrations, and were checked by dry weight determination at the end of the x-ray experiments. The concentrations are expressed in grams of lipid per gram of lipid-water mixture.

SYSTEMS CONTAINING WATER AND SIMPLE LIPIDS

The Phase Diagram

The lipid-water systems can be conveniently described by referring to their phase diagram: an example is presented in Fig. 1, the phase diagram of the potassium palmitate and water system (McBain and Lee, 1943). Several regions can be identified:

The *micellar solution* exists above the T_i curve. Samples are transparent and isotropic when observed in the polarizing microscope, and the viscosity is low. At concentrations higher than the "critical micellar concentration" (which generally is lower than 0.01) the lipid molecules are associated as micelles (McBain, 1950).

The *gel or coagel* is found below the T_c curve. In this region the system is not homogeneous, and equilibrium is often difficult to obtain. At equilibrium, soap crystals are found in the presence of water. The samples are gel-like, opaque, and very viscous.

The *liquid-crystalline* region lies between the T_c and T_i lines. The preparations easily reach equilibrium; they are highly viscous, transparent, and optically anisotropic at rest. For a long time two phases have been known to exist in this region: middle and neat. In the intermediate region these two phases have been assumed to coexist in equilibrium. In fact, the situation is much more complicated, and we have shown (see below) that several phases exist in this region.

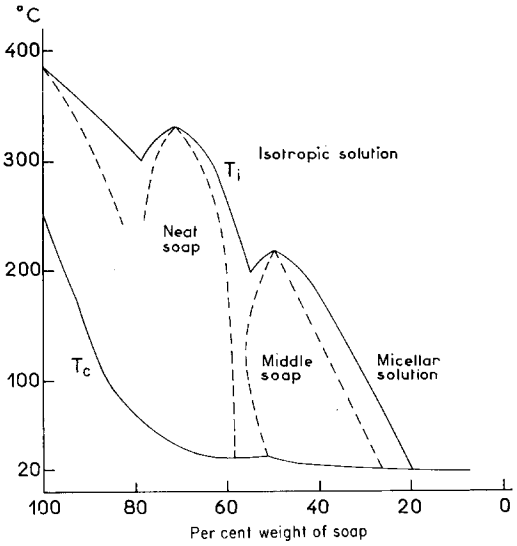


FIGURE 1

The phase diagram of the potassium palmitate-water system. After McBain and Lee (1943).

The x-ray pictures have some characteristic features in each of these three regions. The micellar solutions exhibit a few fairly broad bands at large spacings (20 to 100 A), and a diffuse halo at about 4.5 A. The pictures of gels and coagels contain some sharp lines at large spacings, and a large number of sharp lines, or sometimes narrow bands, at spacings smaller than 5 A. In the liquid-crystalline region the x-ray pictures have a few sharp lines at large spacings, and a broad band at about 4.5 A; the spacings and intensities of the sharp small-angle lines are characteristic of each phase (see below).

The phase diagram of the other lipids is similar to that of potassium palmitate: the positions of the boundary lines depend on the nature of the lipid.

The Liquid-Like Configuration of the Hydrocarbon Chains

For a liquid-crystalline sample of any given composition, the *ratios* of the spacings of the sharp small-angle lines, which are characteristic of each phase, are independent of the temperature, but the *spacings* vary continuously with the temperature. It is quite a remarkable experimental observation that *the Bragg spacings decrease* as the temperature rises. The values of the linear expansion coefficient (d is the Bragg spacing, t the temperature)

$$\alpha = \frac{\partial d}{d\ \partial t}$$

are in the range -0.8×10^{-3} to -1.8×10^{-3} in all the systems we have studied.

The negative sign of α is surprising: in addition, its absolute value is unexpectedly high.[2]

A similar thermal behavior is displayed by rubber-like polymers (see Treloar, 1949): the length of a rubber probe, stretched by two constant and opposite forces, is shorter, the higher the temperature is. This phenomenon is explained by the disordered configuration of the polymer chain. At each temperature the length is defined by the equilibrium of the external forces orienting the polymer, and of the thermal motion, tending to keep the structure chaotic; as the temperature rises, disorder increases, and the probe shortens. The theoretical value of α is $-1/T$ for this simple model: if $T = 300°$, $\alpha \simeq -3 \times 10^{-3}$, which is in close agreement with the experimental values we have observed in lipids.

The formal analogy of the two phenomena can be further justified, if it is assumed that in the lipids one end of the linear molecules (the hydrophilic group) is fastened on the interface between water and hydrocarbon, and that the hydrocarbon chains take up a chaotic configuration, more similar to a liquid than to the ordered structure of a crystal. We have shown that the mechanism of the thermal contraction of this model is similar to that of rubber-like polymers (Luzzati *et al.*, 1960).

A further confirmation of the disordered structure of the hydrocarbon chains is provided by the profile of the broad band at 4.5 A, which is almost identical with the profile of the band of liquid paraffins (Fig. 2).

The liquid-like structure of the hydrocarbon chains is a very essential feature of the liquid-crystalline phases of all lipids and may explain the following properties of these colloids.

a) In the phase diagram, these lipid molecules behave as association colloids only above the T_c line (Fig. 1). The gel and coagel, existing below the T_c line, have quite different properties. *The T_c line is the melting point of the hydrocarbon chains.* This is shown by the sharp change in the thermal expansion and by the appearance of some sharp lines, replacing the broad 4.5 A band in the x-ray pictures (Fig. 2) as a liquid-crystalline sample transforms to a gel.

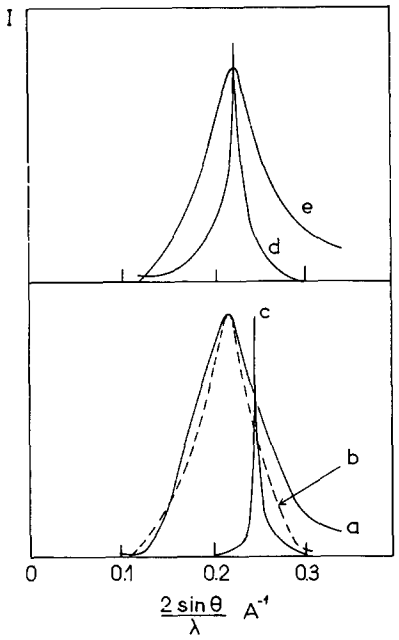


FIGURE 2

Microdensitometer tracing of the 4.5 A region. *a*, potassium palmitate-water ($c = 0.74$), 100°C; *b*, tetradecane, 100°C; *c*, potassium palmitate-water ($c = 0.74$), 22°C; *d*, phospholipid-water ($c = 0.90$), 22°C; *e*, phospholipid-water ($c = 0.90$), 37°C.

b) The highly developed polymorphism of the liquid-crystalline structures (see below) could hardly be compatible with a rigid and ordered structure of the molecules.

[2] It should be noted that the linear thermal expansion coefficient is negative only in one direction: the three-dimensional thermal expansion coefficient is positive.

c) In the liquid-crystalline phases, mixtures of lipids behave as one single component, as far as the phase rule is concerned (Vold, 1941). Indeed, the actual components are water and paraffin, since the liquid hydrocarbon chains of different substances are highly miscible.

d) The liquid-crystalline phases can incorporate large amounts of lipid-soluble substances (McBain, 1950; Klevens, 1950) that are dissolved in the hydrocarbon regions. Such a property is hardly compatible with a crystalline structure.

We wish to remark that the liquid-like structure of the hydrocarbon chains in the soap micelles was suggested a number of years ago by Hartley (see Hartley, 1955), and more recently by Palmer and Schmitt (1941) in the liquid-crystalline phases of some phospholipids. McBain and coworkers, and Hess and coworkers (see McBain, 1950), have apparently underestimated, or even disregarded, such disordered configurations; the *a priori* assumption that the hydrocarbon chains were stiff led them to interpret all the structures as lamellar, an assumption that x-ray diffraction experiments show to be incorrect (see below).

The Structure of the Liquid-Crystalline Phases

In all the systems we have studied, we have observed a few types of x-ray diffraction picture,

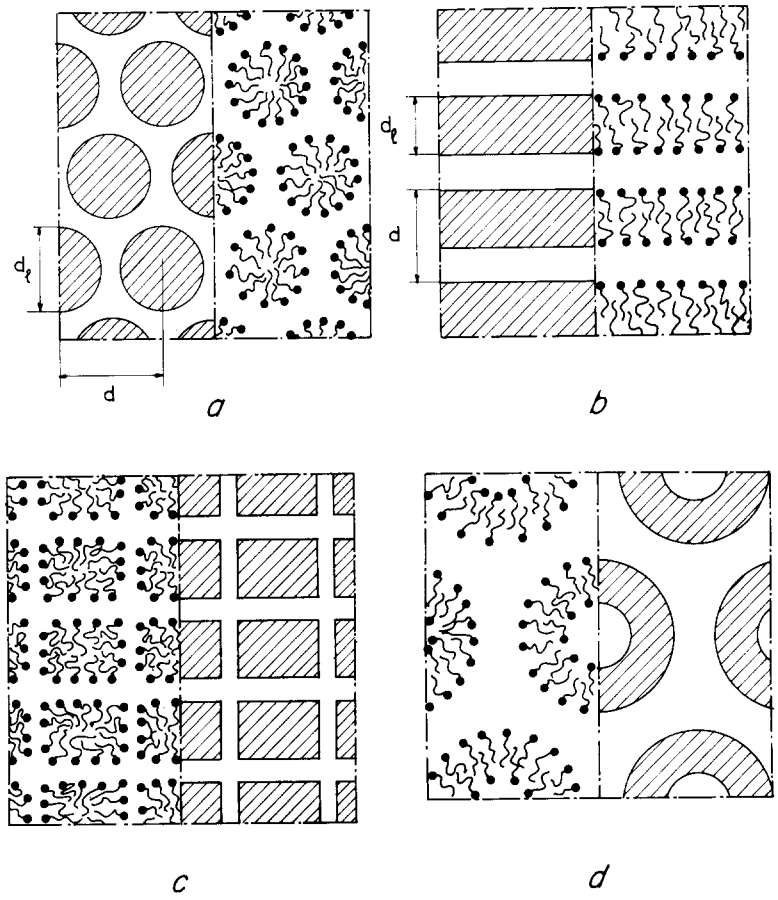


FIGURE 3

The structure of some liquid-crystalline phases of simple lipid-water system.

a. Middle.

b. Neat.

c. Rectangular.

d. Complex hexagonal.

each corresponding to one phase. All the phases are common to several systems.

We describe briefly the structure of the different phases: see Luzzati *et al.* (1960) and Husson *et al.* (1960) for a more detailed account.

Middle

The ratio of the Bragg spacings of the sharp lines is $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}$, typical of the equatorial reflections of a hexagonal lattice.

The structure (Fig. 3*a*) is a hexagonal array of indefinite cylinders: the hydrocarbon chains fill the interior of the cylinders, water is outside, and the hydrophilic groups of the lipid molecules sit on the surface.

The distance between the axes of the cylinders is given by the Bragg spacings of the reflections. If in addition the concentration and the specific volume of the lipid are known, the diameter of the cylinders can be calculated, and the average surface on the cylinder which is available to each hydrophilic group can be determined.

Neat

The Bragg spacings of the reflections are characteristic of a lamellar structure: $1:1/2:1/3:1/4$.

The structure is an alternate sequence of planar layers of lipid and water (Fig. 3*b*). The hydrophilic groups of the lipid molecules lie on the surface separating lipid and water; the liquid hydrocarbon chains fill up the lipid layer.

The thickness of the lipid and water layers can be calculated easily when the repeat distance, concentration, and partial volumes are known.

Intermediate Phases

In the intermediate region between the middle and neat phases it has been assumed that these two phases exist in equilibrium: in fact, the situation is more complicated, and we have discovered several new phases in that portion of the phase diagram.

DEFORMED MIDDLE: This is similar to the middle phase, but the lattice is orthorhombic instead of being hexagonal. The cross-section of the cylinders probably is elliptic.

RECTANGULAR: The x-ray pictures of this phase are formed by two reflections and their higher orders. The structure is an orthorhombic two-dimensional lattice of rectangular prisms (Fig. 3 *c*).

COMPLEX HEXAGONAL: The ratio of the spacings is typical of a hexagonal two-dimensional lattice (see middle phase), but the dimensions of the lattice are much larger than in the middle phase, and the intensities of the reflections are more irregular than in the middle phase, where they regularly decrease with increasing diffraction angle. These observations show that the structure is complex: we have suggested a model (Fig. 3 *d*) in which the lipids form a cylindrical shell, water filling the inner hole and the external gap between the cylinders.

CUBIC: The ratio of the Bragg spacings of the sharp reflections is $1:\sqrt{3/4}:\sqrt{3/8}:\sqrt{3/11}$, which corresponds to a face-centered cubic lattice. The cubic symmetry is confirmed by the optical properties of this phase, which is isotropic: all the other liquid-crystalline phases are birefringent. The structure is that of close-packed spheres, every sphere is filled by the hydrocarbon chains, water is outside.

Description of the Phase Diagram of Simple Lipids

We have investigated several lipids, chosen among three main families:

Anionic

a) Saturated sodium and potassium soaps (abbreviation $C_{12}Na$, etc.):

$$[CH_3—(CH_2)_{n-2}—COO]Na \text{ or } K$$

b) Sodium and potassium oleates (ONa, OK):

$$[CH_3—(CH_2)_7—CH{=}CH—(CH_2)_7—COO]Na \text{ or } K$$

c) Sodium-lauryl-sulfate (SLS):

$$CH_3—(CH_2)_{11}—O—SO_3Na$$

d) Aerosol MA:

$$\begin{array}{l} CH_3—(CH_2)_5—COO—CH_2 \\ \qquad\qquad\qquad\quad | \\ CH_3—(CH_2)_5—COO—CH—SO_3Na \end{array}$$

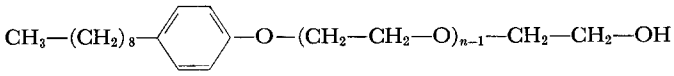
Cationic

e) Cetyl - trimethyl - ammonium bromide (CTAB):

$$CH_3—(CH_2)_{15}—N(CH_3)_3—Br$$

NON-IONIC

f) Arkopal *n*:



molecule is relatively short, and the neat is the only liquid-crystalline phase; for $n \geq 10$ the hydrophilic part becomes large and only the middle phase is present; for $n = 9$ both middle and neat phases exist. Another example is Aerosol MA, where two hydrocarbon chains join to one hydrophilic group: the only liquid-crystalline phase is the

A schematic representation of the phase diagram of all these compounds is given in Table I. In Fig. 4 we have plotted, as a function of the concentration, the Bragg spacings and the dimensions of the structural elements for a few examples.

TABLE I

Schematic Representation of the Phase Diagrams of Simple Lipid-Water Systems

The concentrations of the phase boundaries are given. The phases existing in each system are shown by full lines.

Lipid	*t* (°C)	Neat	Cubic	Complex hexagonal	Rectangular	Deformed middle	Isotropic	Middle
$C_{12}Na$	100	—— 0.59					0.59 ——	
$C_{14}Na$	100	—— 0.59	0.59 ——	0.55	0.55 ——	0.54	0.54 ——	
$C_{16}Na$	100	—— 0.56	0.56 ——	0.52	0.52 ——	0.51	0.51 ——	
$C_{18}Na$	100	—— 0.54	0.54 ——	0.51	0.51 ——	0.50	0.50 ——	
$C_{12}K$	100	—— 0.69 ——	0.61				0.61 ——	
$C_{14}K$	100	—— 0.66 ——	0.59				0.59 ——	
$C_{16}K$	100	—— 0.65 ——	0.59 ——	0.55 ——	0.55 ——	0.54	0.54 ——	
$C_{18}K$	100	—— 0.65	0.65 ——	0.59	0.59 ——	0.58	0.58 ——	
ONa	65	—— 0.69	0.69 ——	0.59 ——	0.52		0.52 ——	0.28
OK	20	—— 0.72	0.72 ——	0.68 ——	0.60		0.60 ——	0.21
SLS	75	—— 0.69	0.69 ——	0.62			0.62 ——	0.38
MA	20	——						
CTAB	70	—— 0.84 ——	0.78				0.78 ——	0.38
Arkopal 9	20	—— 0.61					0.61 ——	0.48 —— 0.45
Arkopal 13	20						0.63 ——	0.43

Several remarks can be made:

a) As the concentration increases, the different phases follow each other in the following order: micellar, middle, deformed middle, rectangular, complex hexagonal, cubic, neat. This sequence shows many gaps (Table I), but no example of inversion is observed.

b) Some features of the phase diagram bear an obvious relation to the chemical structure of the lipid. The bulkier the hydrophilic end of the molecule, the more extended is the middle phase range; and, reciprocally, the bulkier the hydrocarbon chains, the more extended is the neat phase range. The Arkopals clearly illustrate this rule. From $n = 6$ to $n = 8$ the hydrophilic part of the neat. In CTAB, where the hydrophilic end is fairly bulky, the middle phase extends over a larger range than in soaps.

Other chemical factors play a role: for instance, the presence of double bonds in the paraffin chains increases the surface available to each hydrophilic group (compare oleates and stearates in Table II): the polarity of the double bond is probably responsible for this phenomenon.

The factors that determine the existence of the intermediate phases are more obscure and do not seem to bear an obvious relation to the chemical structure of the lipid.

c) As long as the thickness of the water layer is great enough (the borderline thickness is at

abuot 15 A), the dimensions of the lipid-containing structural elements are independent of the concentration: some examples are given in Fig. 4. The diameter of the cylinders, the thickness of the lipid layers, and the average surface available to each hydrophilic group are given in Table II for all the cases where these dimensions are fairly constant over a large concentration range. It

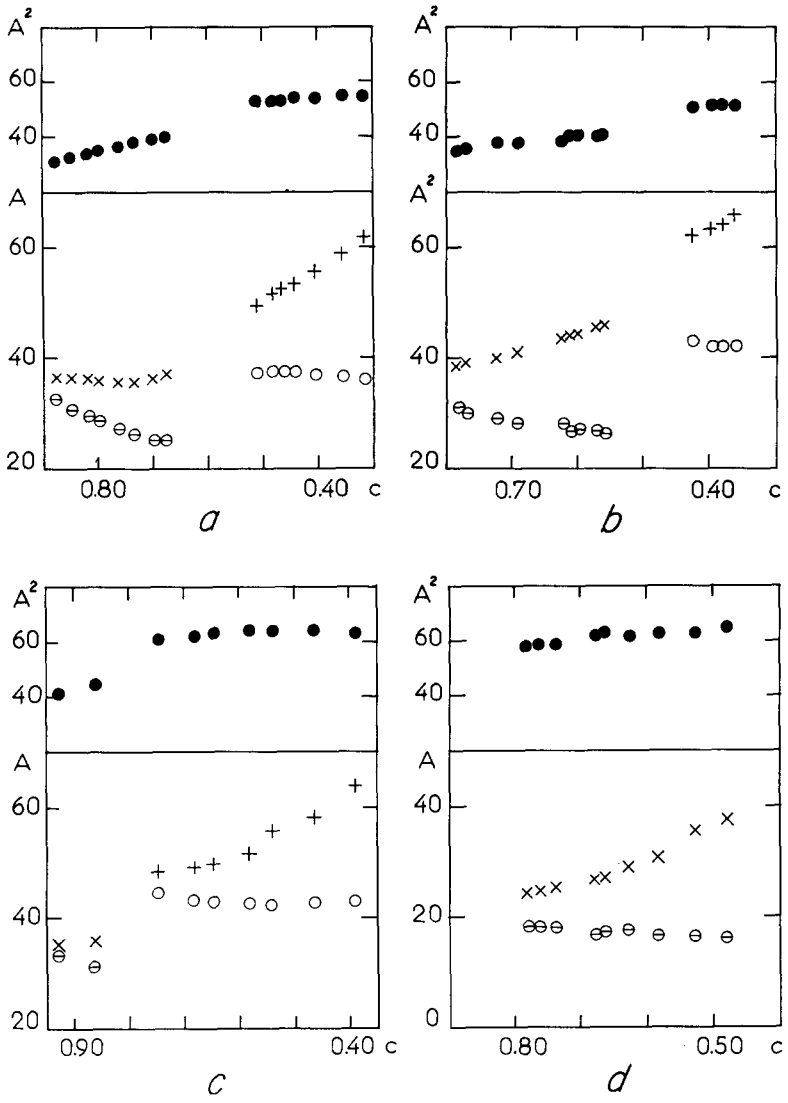


FIGURE 4

Dimensions of the structural elements of the liquid-crystalline phases of some lipid-water systems (see Fig. 3).

a. Potassium palmitate, 100°C.
b. Sodium stearate, 100°C.
c. Cetyltrimethylammonium bromide, 70°C.
d. Aerosol MA, 20°C.

● average surface area per hydrophilic group
\+ distance between the cylinder axes, d } Middle phase (see Fig. 3)
○ diameter of the lipid cylinders, d_l }
× repeat distance, d } Neat phase (see Fig. 3)
⊖ thickness of the lipid leaflet, d_l }

should be noted that the surface per hydrophilic group mainly depends on its chemical structure: in all the sodium and potassium saturated soaps the surface is the same in the middle phase (Table II).

When the thickness of the water layer becomes too small, all the dimensions vary as a function of concentration. For instance, in the neat phase of the saturated soaps, the thickness of the water layer decreases, and the thickness of the lipid layer increases, as the concentration rises (Fig. 4).

TABLE II

Diameter of the Cylinders of the Middle Phase (d_l) and Average Surface Available to Every Hydrophilic Group (S) in the Different Lipids

Lipid	t	d_l	S	d_l(100 °C)	S(100 °C)
	°C	A	A^2	A	A^2
$C_{12}Na$	100			29.0	51
$C_{14}Na$	100			33.5	52
$C_{16}Na$	100			37.8	52
$C_{18}Na$	100			42.6	52
$C_{12}K$	100			29.9	52
$C_{14}K$	100			32.7	54
$C_{16}K$	100			37.1	54
$C_{18}K$	100			41.6	54
ONa	65	39.2	52	37.4	55
OK	20	39.4	52	35.7	58
SLS	75	34.3	57	33.2	59
CTAB	70	43.0	63	41.3	65
Arkopal 13	20	14.0	55	—	—
MA (neat)	20	17.1	62	15.3	69
Arkopal 9 (neat)	20	13.5	58	—	—

The dimensions at 100°C have been calculated by the formula:

$$(d_l)_{100^\circ} = (d_l)_t\,[1 - 1.3 \times 10^{-3}\,(100 - t)].$$

The over-all effect on the lattice dimension becomes complex: the lattice may shrink or swell, a phenomenon that is not so puzzling as it was thought to be (McBain and Marsden, 1948).

The explanation of the different properties of the water layer probably depends on the ordered structure of water and on the solvation shell of ions, which become important factors when the water layer is thin; in contrast, water in thick layers behaves as a continuum.

PHOSPHOLIPID-WATER SYSTEM

A preliminary report on this system has been published recently (Husson, 1961).

The lipid is an ether extract of human brain which contains approximately 52 per cent cephalin, 35 per cent lecithin, and 13 per cent phosphoinositides (Stoeckenius, 1959): all the samples were kindly provided us by Dr. W. Stoeckenius, The Rockefeller Institute, New York. The extract was preserved in acetone as a powder precipitate. The samples for the x-ray experiments were made by evaporating acetone *in vacuo* and adding definite amounts of water. Exposures of the lipid to air were kept as short as possible, in order to prevent oxidation.

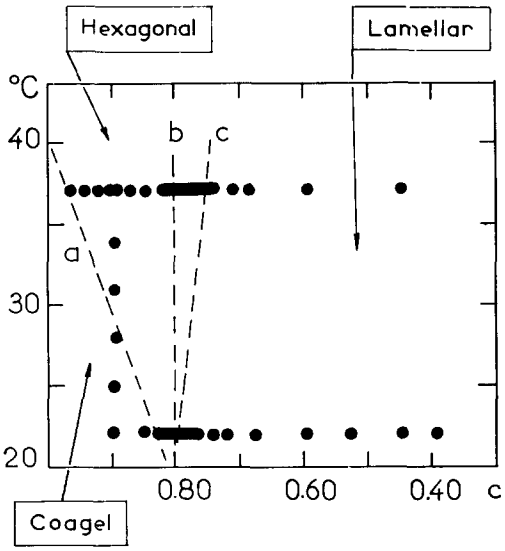


FIGURE 5

Phase diagram of phospholipid--water system, and position of the experimental points.

The phase diagram was explored as a function of concentration, at two temperatures, 22° and 37°C; in addition, several x-ray pictures were taken with one sample (c = 0.90), as a function of the temperature. The distribution of the experimental points in the phase diagram is shown in Fig. 5. No systematic investigation has been carried out in the concentration range below 0.40.

Three main phases can be identified; the approximate position of the phase boundaries is drawn in Fig. 5. On the left and lower side of the a line the properties of the samples are typical of a coagel (see below): the a line is equivalent to the T_c line of the soap-water systems (Fig. 1). On the right and upper side of the a line the behavior is characteristic of liquid-crystalline structures:

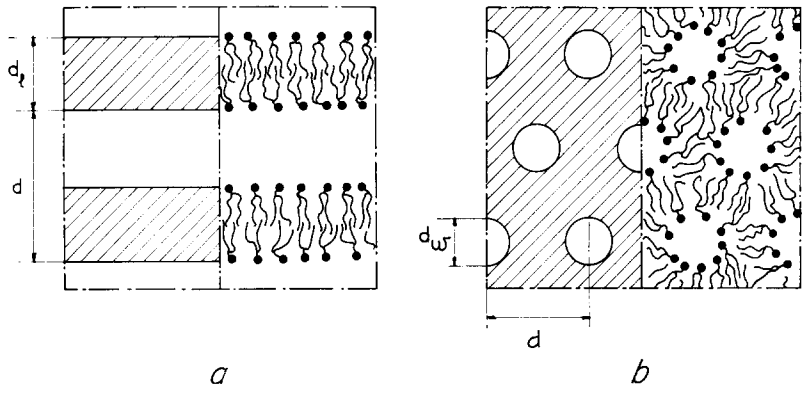


FIGURE 6

The structure of the liquid-crystalline phases of the phospholipid-water system.
a. Lamellar.
b. Hexagonal.

equilibrium is easily obtained, the samples are transparent and spontaneously birefringent, viscosity is high. On the x-ray pictures a few sharp lines are found in the small-angle region, and a broad band at about 4.5 A (Fig. 2).

Two liquid-crystalline phases exist in this system, each characterized by its x-ray diffraction pattern: we call them *lamellar* and *hexagonal*. Their regions in the phase diagram are shown in Fig. 5. In the intermediate region (between the *b* and *c* lines, Fig. 5) both phases exist in equilibrium.

Description of the Phases

LAMELLAR

The Bragg spacings of all the sharp lines are the integral orders (up to the 3rd) of one fundamental spacing, showing that the structure is lamellar. As in the neat phase of the previous examples, the structure is built up by the alternate sequence of lipid and water layers (Fig. 6 *a*).

Assuming that the specific volumes of lipid and water are equal,[3] the thicknesses of the lipid and water layers (d_l and d_w, Fig. 6) can be calculated:

$$d_l = cd \qquad d_w = (1 - c)d$$

The average surface available to one hydrophilic group is

$$S = \frac{2\bar{M}10^{24}}{Nd_l}$$

where N is Avogadro's number, $\bar{M}$ the mean molecular weight of the phospholipid.

The numerical values of these parameters are given in Table III and plotted in Fig. 7. At each temperature the thickness of the lipid layer and the surface S are approximately independent of the concentration, in agreement with the observations made with the simple lipids under conditions where the water layer is thick enough (see above). In this case the thickness of the water layer decreases with increasing lipid concentration, from 60 A at $c = 0.40$ to 13 A at $c = 0.77$. Furthermore, the thickness of the lipid layer decreases as the temperature increases: the linear expansion coefficient is approximately

$$\alpha = \frac{\partial d_l}{d_l \partial t} = -3 \times 10^{-3}$$

The sign and the magnitude of α, and the profile of the 4.5 A band (Fig. 2), show that the structure of the hydrocarbon chains is liquid-like, as it is in all the liquid-crystalline phases of the previous examples.

HEXAGONAL

The ratio of the Bragg spacings of the sharp small-angle reflections is $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}$, as in the middle phase: the structure is a hexagonal array of indefinitely long cylinders. The cross-sectional area of the unit cell, and the areas σ_l and

[3] This approximation can be justified by the remark that the values of d_w, d_l and S are only slightly dependent on the specific volumes (see Husson *et al.*, 1960).

σ_w that lipid and water occupy, can be calculated (Fig. 6 *b*):

$$\sigma = \frac{d^2\sqrt{3}}{2}$$

$$\sigma_l = c\sigma$$

$$\sigma_w = (1 - c)\sigma$$

The dimensions of the structural elements can be determined easily when these areas are known.

Two models can be taken into consideration: one, similar to the middle phase (Fig. 3 *a*), where the hydrocarbon chains of the lipid molecules are located inside the cylinders; and another, where the water is in the interior of the cylinders, and the lipid molecules fill the gap between the cylinders (Fig. 6 *b*). The second structure (Fig. 6 *b*) is in fact the correct one, as can be shown in several ways:

a) In all the previous examples the hexagonal (middle) phase exists at low lipid concentrations with respect to lamellar (neat) phase. The opposite occurs in this phospholipid.

b) The chemical structure of the phospholipid molecules is not likely to favor the middle phase, since the lipophilic part of the molecules is rather bulky. In Aerosol MA (see above), which has a similar chemical structure, the middle phase is absent.

c) In the structure depicted in Fig. 6 *b*, the average surface S available to every hydrophilic group at the interface is smaller than in the lamellar phase, and decreases as the lipid concentration increases (Table IV). This variation of S as a function of the concentration agrees with all the previous examples. On the contrary, if the structure were that of the middle phase (Fig. 3 *a*), S would be larger than in the lamellar phase, and would increase as the lipid concentration increases (Table IV).

d) An independent confirmation has been provided by electron microscope observations performed by Stoeckenius (1961). Stoeckenius has

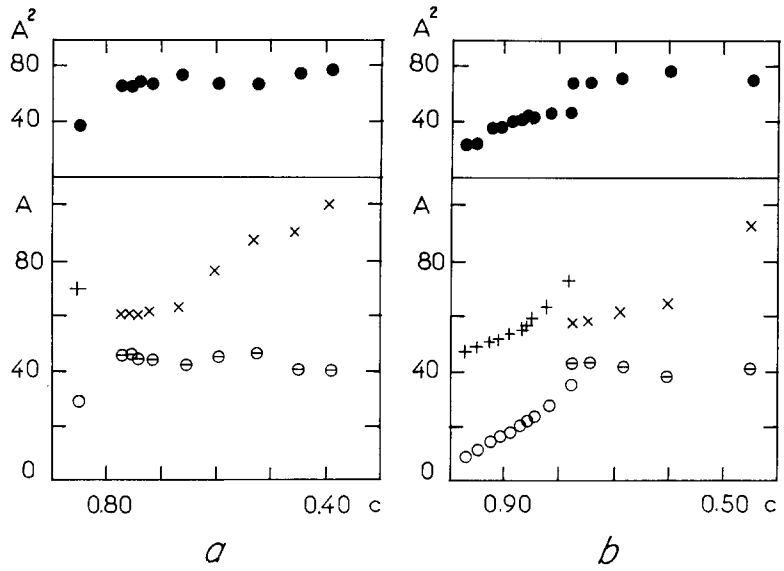


FIGURE 7

Dimensions of the structural elements of the liquid-crystalline phases of the phospholipid-water system (see Fig. 6).

a. 22°C.

b. 37°C.

● average surface area per hydrophilic group

\+ distance between the cylinder axes, d } Hexagonal phase

○ diameter of the water cylinders, d_w }

× repeat distance, d } Lamellar phase

⊖ thickness of the lipid leaflet, d_l }

succeeded in fixing two hexagonal liquid-crystalline structures, one of the Na linolenate-water system, and one of the phospholipid-water system. In the first case the electron microscope pictures show a honeycomb-like distribution of black lines, in the second a hexagonal array of black spots: the two structures look like "negatives" of each other. We have confirmed by x-ray observations that in the Na linolenate-water system the hexagonal phase is of the middle soap type (Fig. 3 *a*).

All these considerations indicate that the structure of the hexagonal phase is as shown in Fig. 6 *b*. The dimensions of the structural elements are given in Table IV and Fig. 7. It should be pointed out that the thickness of the lipid layer ($d - d_w$, in Fig. 6 *b*) is independent of the concentration in the hexagonal phase (Table IV and Fig. 7 *b*).

The presence of a broad band at 4.5 A is consistent with the liquid-like structure of the hydrocarbon chains.

TABLE III

Dimensions of the Structural Elements of the Lamellar Phase of the Phospholipid-Water System (See Figs. 6 and 7)

c	t	d	d_l	d_w	S
	°C	A	A	A	A²
0.39	22	99.7	39.3	60.4	77
0.45	"	89.0	40.2	48.8	75
0.53	"	86.8	46.0	40.8	66
0.60	"	75.5	45.4	30.1	67
0.66	"	62.2	41.4	20.8	73
0.72	"	61.6	44.6	17.0	68
0.74	"	59.7	44.5	15.2	68
0.75	"	60.8	45.7	15.1	66
0.77	"	58.8	45.6	13.3	66
0.85	"	54.9	46.7	8.2	65
0.45	37	92.4	41.7	50.7	72
0.60	"	64.3	38.7	25.6	78
0.69	"	60.8	41.8	19.0	72
0.74	"	58.9	43.8	15.1	69
0.77	"	56.6	43.8	12.8	69
0.80	"	56.2	44.9	11.3	67

TABLE IV

Dimensions of the Structural Elements of the Hexagonal Phase of the Phospholipid-Water System

			Correct structure (Fig. 6)		Middle phase structure (Fig. 3)	
c	t	d	d_w	S	d_l	S
	°C	A	A	A²	A	A²
0.78	37	72.8	35.2	46	67.4	90
0.82	"	63.0	28.1	47	59.6	102
0.85	"	58.9	24.0	44	57.0	106
0.86	"	56.6	21.8	45	58.0	104
0.87	"	54.9	20.8	43	55.4	110
0.89	"	53.2	18.6	40	52.6	115
0.91	"	51.4	16.2	36	51.4	118
0.92	"	50.3	14.5	34	50.8	119
0.95	"	48.5	11.4	23	49.6	122
0.96	"	48.3	9.4	22	49.8	122
0.97	"	48.0	8.8	21	49.6	122

Coagel

In all the x-ray pictures taken below the *a* line (Fig. 5), one fairly sharp reflection is found in the 4.5 A region (Fig. 2), in addition to several sharp small-angle lines. The spacings of these lines do not fit with any simple lattice. In some cases the reflections appear to belong to two lamellar structures, in others to a lamellar and a hexagonal structure. Furthermore, the x-ray patterns depend on the thermal and mechanical treatments of the samples and are not easily reproducible.

Remarks and Conclusion

It may seem surprising to notice that practically all the non-lamellar structures that we describe here have not been found by the numerous investigators who have studied the liquid-crystalline phases of association colloids in the past. One reason for this has to be found in the *a priori* assumption that all the structures are lamellar. A second explanation is a technical one: an improved x-ray apparatus, provided with a bent quartz monochromator, and operating *in vacuo*, proved to be essential for the study of the small-angle diffraction region, where sharp lines have to be looked for. Finally, no systematic exploration of the whole phase diagram, as a function of concentration and temperature, seems to have been performed, and unless this is done some important characteristic of the system may be entirely missed.

Some recent electron microscope observations have confirmed the existence of non-lamellar structures. Stoeckenius (1961) has studied some lipid-water systems; under certain conditions, he has found hexagonal arrays of cylinders whose structure and dimensions are in excellent agreement with the x-ray observations. Miller (1961) has observed hexagonal structures in the lipopro-

tein granules of the cortical collecting tubules of mouse kidneys.

The highly developed polymorphism is a common feature of all the lipid-water systems. Small variations in temperature and concentration may induce drastic changes in the liquid-crystalline regions. The only structure common to all systems is to be found in the coagels, where lamellar structures exist in the large majority of the lipids we have investigated. These remarks may have some bearing on the interpretation of electron microscope observations of lipids, since the liquid-crystalline structures that may exist *in situ* prior to fixation, drying, and embedding are not likely to withstand all these operations. The observed lamellar structures might be coagel-like artifacts, at least in some cases. Non-lamellar liquid-crystalline structures similar to that of the hexagonal phase of phospholipids (Fig. 6 *b*), if they existed *in vivo*, would have remarkable permeability properties, related to the long and narrow water channels, which are covered by the polar groups of the lipid molecules (Mullins, 1956).

We wish to emphasize the significance of the liquid-like structure of the hydrocarbon chains, in all the liquid-crystalline phases. The same kind of short range disorder is likely to exist in lipoprotein complexes *in vivo*, since such a structure appears to be more suitable to physiological activity than a rigid crystalline configuration. It might be tempting to speculate on one possible mechanism whereby lipids could cooperate in the regulation of the physicochemical conditions in the cell. It might be assumed, for example, that for some special lipoprotein complex (say a cell membrane) the ordinary physiological conditions are not far from the border line of a phase transition from a liquid-crystalline structure to a coagel. When one of the parameters (concentration, temperature, electric potential) is altered, the hydrocarbon chains crystallize, blocking some physiological activity of the lipid (say permeability of the membrane) and providing a feedback mechanism for the restoration of the normal conditions. In connection with these speculations, it may be noticed that in the human brain phospholipids the transition from the coagel to the liquid-crystalline region occurs at a temperature that is close to the body temperature, at least when small amounts of water are present. One may wonder if this fact is only a coincidence.

Furthermore, one remark can be made which may have some bearing on the specific and selective properties of certain lipoprotein membranes with respect to Na and K (see for example Nachmansohn, 1959). The properties of the saturated soap-water systems are differently dependent on the nature of the cation on both sides of the T_c line (Fig. 1): the structure, as well as other properties, of the Na and K soaps are quite different from each other in the coagel region of the diagram (work in progress in our laboratory), whereas they are almost identical in the liquid-crystalline region (Husson *et al.*, 1960). If the lipoprotein-water systems displayed similar properties, the phase transitions of the lipid (melting and crystallization of the paraffin chains) would cause drastic changes in the selective permeability to cations, and could play a role in the biological mechanisms where selective permeability is involved.

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