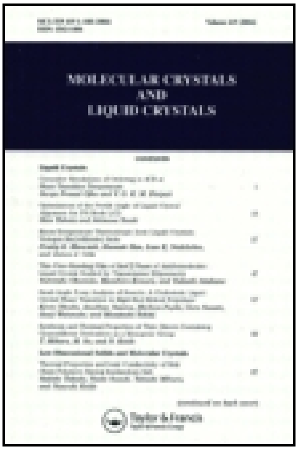

Molecular Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl15

Lyotropic Paracrystalline Phases Obtained With Ternary and Quaternary Systems of Amphiphilic Substances in Water: Studies on Agreous Systems of Lecithin, Bile Salt and Cholesterol

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Version of record first published: 21 Mar 2007.

To cite this article: Donald M. Small & Martine Bourgés (1966): Lyotropic Paracrystalline Phases Obtained With Ternary and Quaternary Systems of Amphiphilic Substances in Water: Studies on Agreous Systems of Lecithin, Bile Salt and Cholesterol, Molecular Crystals, 1:4, 541-561

To link to this article: http://dx.doi.org/10.1080/15421406608083292

Molecular Crystals. 1966. Vol. 1, pp. 541–561

Printed in Great Britain

Lyotropic Paracrystalline Phases Obtained With Ternary and Quaternary Systems of Amphiphilic Substances in Water[†]: Studies on Agreous Systems of Lecithin, Bile Salt and Cholesterol

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Received December 13, 1965

Introduction

Lyotropic liquid crystals given by binary systems have been known for a long time to investigators in the soap and detergent field, mainly by the work of McBain and his school.[1, 2] The first analysis by X-ray diffraction of soap mesophases was done by Stauff[3] and by Kiessig and Philippoff.[4] Liquid crystals obtained with phospholipids in the presence of water were examined by X-ray analysis already in 1941 by Schmitt *et al.*[5] More recently Luzzati *et al.*[6] defined the structure of the different mesophases with more precision.

Lyotropic liquid crystals obtained with ternary systems are much less well known. The texture of different paracrystalline phases given by mixtures of different pairs of amphiphilic substances in the presence of water were described by Dervichian *et al.*[7, 8] and by Lawrence *et al.*[9, 10] Except for the multicomponent mixtures of brain extract, examined by Schmitt *et al.* (loc. cit.), to our knowledge, no crystallographic study of the molecular structure has been carried out on aqueous ternary or quaternary lyotropic mesophases.

[†] This investigation was supported in part by Public Health Service Training Grant 2A 5025-08 and 09.

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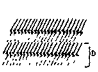
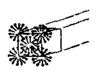
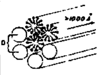
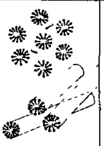
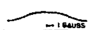
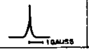
The present work reports the results of the systematic investigation of ternary and quaternary systems obtained with mixtures in all proportions prepared with three or four of the following components: water, lecithin, cholesterol and sodium cholate (bile salt). Their phase diagrams were established by direct observation and microscopic observation utilizing direct light and polarized light and the structure of the different phases was determined by X-ray diffraction. It became clear from the beginning that we were not only dealing with solutions and crystalline solids, but with several different paracrystalline phases. These phases are interesting by themselves and their study may contribute to our knowledge of lyotropic paracrystalline phases.

Before analyzing the different phase equilibria encountered in the mixed systems described in this work, it may be useful to give a brief survey of the features of the different lyotropic paracrystalline phases in general. In Fig. 1 are summarized some of the characteristics of the lyotropic liquid crystalline states compared to those of the crystalline state on one hand and the micellar solution on the other hand. In the top of Fig. 1. are schematic diagrams of the probable molecular arrangement in these states. Below are listed some of the characteristics of these molecular arrangements such as: the long range order, the number of directions in which movement might occur freely, the gross and microscopic aspect, and findings by X-ray analysis. It can be seen in general that, as one adds water to a crystalline mixture, it becomes liquid crystalline. As hydration increases the structure of the liquid crystalline phase transforms from a lamellar type to, in some cases, a face centered cubic type, and finally to a compact hexagonal type before breaking up into a micellar solution. The quantity of water at which these transformations take place is variable. In general for soaps and for our systems the lamellar liquid crystalline state is seen between 5% and 22% water. However, if the hydrophilic-lipophilic balance favors the lipophilic side (as with lecithin) the lamellar paracrystalline phase may be maintained up to 45% or even 50% water, after which it dissociates into two phases: the lamellar paracrystalline phase and water. If a cubic liquid crystalline phase appears, it usually is formed between 23% and 40% water and if the hexagonal paracrystalline phase appears, it is formed between the limits of 34% and 80% water.

The crystalline state is characterized by a long-range order in three dimensions and no real freedom of movement of the molecules. The gross aspect is an opaque solid. The microscopic aspect under crossed nicols is a birefringent crystal. By X-ray analysis there is one long spacing (D) and several orders of this spacing. The short spacings are numerous and range between 3 and 6 Å and are quite sharp. The passage to the first liquid crystalline phase, which will be called lamellar, leads to only one long-range order, that order being perpendicular to the planes of the hydrophilic groups. There is relative freedom of movement of the chains in two directions. The gross aspect is a clear moderately viscous fluid and under the polarizing microscope appears the characteristic texture of "neat soap" as systematized by Rosevear.[11] The X-ray analysis on the other hand gives several orders of one long spacing. Short spacings present in the crystal are blended into one diffuse band at about

% Water (General Overall Range)	 0	 5-22---[45] %	 23-40%	 34-80%	 30-99.9%	 > 99 9%
State	crystalline	liquid crystalline, lamellar	liquid crystalline, face centered cubic	liquid crystalline, hexagonical compact	micellar solution	solution (belowCMC)
Long Range Order	3 dimensions	1 dimension	3 dimensions	2 dimensions	none	none
Freedom of Movement	none	2 directions	"none"	1 direction	3 directions, more or less free	3 directions, free
Gross Aspect	opaque, solid	clear, fluid, moderately viscous	clear, very viscous, brittle	clear, viscous	clear, fluid	clear, fluid
Microscopic Aspect (crossed nicols)	crystalline, birefringent	neat soap texture	isotropic with angular bubbles	middle soap texture	isotropic with round bubbles	isotropic
X-ray Analysis Long spacings	D $D/2 : D/3 : D/4$ etc	$D : D/2 : D/3 : D/4$	$1 : \sqrt{3}/2 : \sqrt{3}/\sqrt{8} : \sqrt{3}/\sqrt{11}$ (111) (200) (220) (311)	$D : D/\sqrt{3} : D/\sqrt{4} : D/\sqrt{7}$	diffuse band at 40-80 Å	
Short spacings	several short sharp spacings from 3-6 Å	diffuse band at about 4 5 Å	diffuse band at about 4.5 Å	diffuse band at about 4.5 Å		nothing
NMR*						

* from Lawson & Flautt (14)

Figure 1.

4.5 Å showing that at least part of the chains are liquid. The transition to the cubic liquid crystal phase or what has been called "viscous isotropic" phase, introduces a long-range order in three directions. The gross aspect is clear, very viscous, the material being rather brittle. Under the polarizing microscope this phase appears isotropic but if bubbles are trapped within it they are angularly deformed and do not tend to become spherical with time. X-ray analysis shows long spacings consistent with a face centered cubic lattice as can be seen by the periodical spacings. The corresponding planes are represented beneath the spacings (i.e. plane 111, plane 200, etc.). However, the short spacings are blended again into one single band at 4.5 Å showing that all the chains are liquid inside of each of the elements forming the cubic lattice. These facts and others suggest that this phase is made up of spherical micelles packed in a face centered cubic lattice. When there is very little water, that is 23% or 24%, these spherical micelles would have to be deformed, probably dodecahedronally.

As the mixture is hydrated further, the hexagonal liquid crystalline phase appears, which has long range order in two dimensions and freedom of movement in the third dimension (i.e. along the axis of the cylinders). The gross aspect of this is a very viscous, clear fluid. Under the polarizing microscope it exhibits the "middle soap" texture as systematized by Rosevear.[11] X-ray analysis shows that the long spacings correspond to a compact hexagonal lattice. The short spacings again blend into a band at about 4.5 Å suggesting again that the chains are liquid.

Extensive hydration of the hexagonal liquid crystalline phase leads to the breakup of the cylinders into either rod-shaped or spherical micelles as pictured above.[12] There is of course, no long-range order but only an average distance between these micelles. There is freedom of movement in all three directions more or less hindered depending upon the concentration. The gross aspect of this is of a perfectly clear flowing fluid, and, under the polarizing microscope, it is isotropic with round bubbles. X-ray analysis of these solutions shows a diffuse band with a maximum ranging from 40 to 80 Å as well as marked scattering. Finally, in the case of extreme dilution, below the critical miceller concentration, single molecules and perhaps dimers exist in what might be called a true solution.

Chapman[13] had shown by infrared spectroscopy that the chains of the paracrystalline phases of long chain substances were in a liquid state. Such was also the conclusion of Schmitt *et al.* (loc. cit.) in their study by X-rays of the phospholipids swollen by water and also of Luzzati *et al.*[6] in the case of the aqueous phases of soaps. These results are in agreement with our conclusions drawn from the fact that the different side spacings found in the crystalline state blend together into a more or less diffuse band when the paracrystalline phases of our systems are submitted to X-ray diffraction. At the present conference Lawson and Flautt[14] have shed further light on the structure of the paraffinic chains of the different individual phases by their studies with nuclear magnetic resonance (n.m.r.). From the measurements of line widths and line moments along with a consideration of line shapes they conclude that the chains are in a frozen state in the crystalline state, that the chains are partly frozen and partly liquid in the neat and middle phases and that the chains are completely liquid in the cubic and micellar phases. Curves summarizing the n.m.r. lines have been added for comparison at the bottom of Fig. 1 with the kindest permission of Dr. K. D. Lawson.

The ternary and quaternary systems examined in the present work showed one or all of the three liquid crystalline states, described above, as well as the micellar solution.

In certain detergent systems Luzzati and his coworkers have described other lyotropic liquid crystalline structures such as inverted hexagonal, deformed hexagonal, rectangular, and complex hexagonal.[6, 15, 16] These will not be discussed here.

Methods

Over 600 mixtures, representing a sampling of all the possible proportions of bile salt, lecithin, cholesterol and water, were prepared and examined, after equilibration at 25° C, under the polarizing microscope. A selection of some 250 of these were analyzed for both long and short spacings by X-ray diffraction. Two types of X-ray apparatus were used. The first has been described in a previous article.[17] The distance from sample to the film is 23.5 cm. A Debye-Scherrer camera of 48.0 cm circumference was also used to determine the short spacings. The second apparatus is equipped with two Kratky chambers and was used to study the long spacings. The distances from the sample to the film in the two chambers are 22.85 cm and 23.23 cm. Each sample was exposed 24 h at 25° C.

The polarizing microscope enabled to discern in each mixture the presence of one, two or three different phases: crystals of cholesterol, crystals of sodium cholate, isotropic phases or liquid crystalline phases. The liquid crystalline phases could be identified as "neat phase" or "middle phase" by applying Rosevear's[11] classification based on their texture.

The X-ray analysis showed that the so defined "neat phase" had a lamellar structure, and the "middle phase" a hexagonally packed lattice identical to those found respectively with "neat soap" and "middle soap" by Luzzati and coworkers.[6, 15, 16] The isotropic phases were either the micellar solution or the isotropic paracrystalline phase with a face centered cubic lattice similar to that found in binary aqueous systems by Luzzati and coworkers.[6, 15, 16]

After assembling a large number of observations on different mixtures, these were plotted on triangular graph paper and the borders of zones containing one phase or those showing separation of two or three phases could be delineated. This technique permitted the construction of phase diagrams for systems of three and four components.

Results and Discussion

Bile salt (sodium cholate) does not form paracrystalline phases with water as do normal soaps and detergents. It cannot be considered as a typical amphiphilic substance in that respect. On the other hand lecithin alone with water swells to give a paracrystalline lamellar phase of the "neat soap" type.

Three Component Systems

Cholesterol and sodium cholate in water do not form paracrystalline phases. Only a very small amount (less than 2% of the weight of sodium cholate) of cholesterol is solubilized by the bile salt under the form of a micellar solution.

On the contrary cholesterol plus lecithin in water form a paracrystalline phase in which the cholesterol and lecithin molecules probably lie side by side in each of the two layers forming the lipid lamellae.

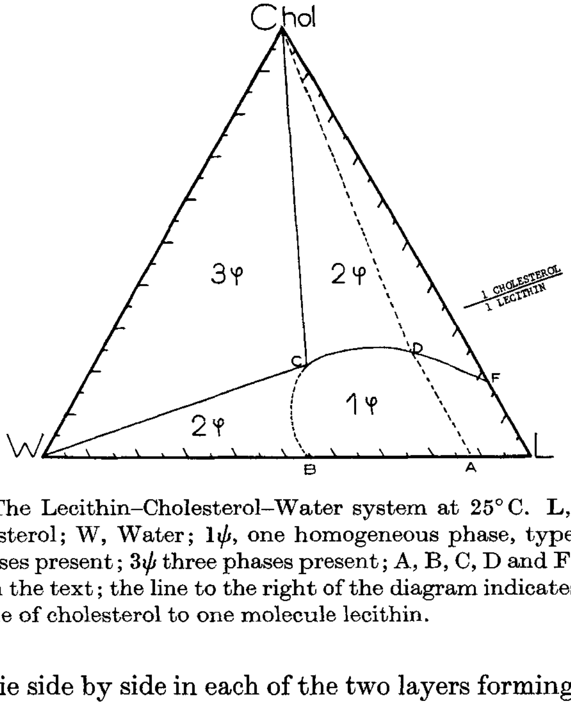


Figure 2. The Lecithin–Cholesterol–Water system at 25° C. L, Lecithin; Chol, Cholesterol; W, Water; 1ψ, one homogeneous phase, type lamellar; 2ψ, two phases present; 3ψ three phases present; A, B, C, D and F are points explained in the text; the line to the right of the diagram indicates a ratio of one molecule of cholesterol to one molecule lecithin.

Figure 2 represents the three component phase diagram Lecithin–Cholesterol–Water. Along the side Water–Cholesterol of the triangle, one notes that the cholesterol is totally insoluble in water at 25° C. However, along the side Lecithin–Water from about 12% to 45% water (A to B), lecithin forms with water a lamellar paracrystalline phase. Mixtures containing more than 45% water separate into this lamellar liquid crystalline phase floating in the excess of water under the form of myelin figures and anisotropic droplets. When the water content in the lecithin increases, the

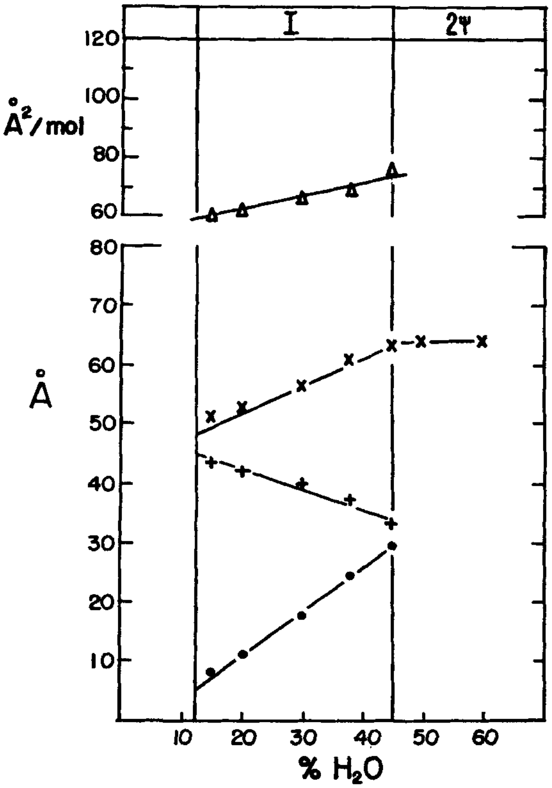


Figure 3. Dimensions of the structural elements of lamellar phase of the Lecithin–Water system. △, average surface area per molecule; ×, D, the repeat distance; + D_L, the calculated thickness of the lipid layer; ●, D_W., the calculated thickness of the water layer; I, one homogeneous phase, type lamellar; 2ψ, two phases.

long X-ray spacing, starting from about 50 Å, increases gradually to reach 65 Å at 45% water, after which it remains constant (Fig. 3). At this concentration of 45% water, the sample appears slightly turbid. Microscopic examination confirms the presence of two phases. The thickness of the lipid layer can be calculated from the long spacings and the quantity of water present, assuming that all of the water is between the polar heads of the lipids and that both the lipid layer and the water layer have a density of 1.0. The thickness of the lipid layer calculated in such a way decreases as the lecithin becomes more hydrated. As a consequence of this the average surface area per molecule at the water interface increases as the quantity of water is increased.

All mixtures containing the three components appear as points within the inside of the triangular diagram. It should be stated that between 12% water and 5% water the microscopic appearance of lecithin or of the lecithin–cholesterol mixtures is that of "neat soap", but by X-ray examination one sole, sharp, long spacing at about 48 Å is noted. There is no second or third order to substantiate that it is in fact truly lamellar. Below 5% water one notes several spacings and the anisotropy is no longer that of neat soap. These systems have a "waxy" appearance. Therefore, the zone marked DFLA in Fig. 2 will be omitted from discussion because of the lack of information on the number of phases present (one or more). It appears from the diagram that cholesterol can be added to lecithin up to a molecular ratio of 1:1 to give the same lamellar paracrystalline phase, already given by lecithin and water. Any excess above this proportion separates as cholesterol crystals.

The X-ray analysis shows that in general the addition of cholesterol to lecithin, at a constant water concentration, tends to make the lipid layer slightly thicker. But this slight increase does not seem sufficient to account for the cholesterol being between the lipid tails of the lecithin in the lamellar layer.[18] It is, therefore, more probable that the cholesterol is interdigitated between the lecithin molecules with its hydroxyl group lying in the water layer, as has been shown to be the case with mixtures of lecithin and cholesterol spread on the surface of water.[19]

Sodium cholate and lecithin in water form a variety of paracrystalline phases: lamellar, cubic and hexagonal. Figure 4 represents the three component phase diagram of lecithin, sodium cholate and water. Although this diagram appears very complex, it really exhibits only four areas each of one homogeneous phase. These four areas are marked in Roman numerals, I, II, III, and on IV on the diagram. Phase I is a lamellar paracrystalline phase analogous to that formed by cholesterol and lecithin or by lecithin alone with water. Again area AGQL where the amount of water is less than 12% and where the mixtures may consist of more than

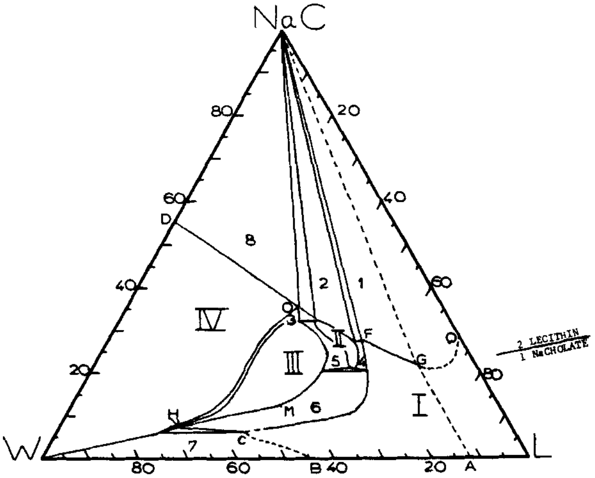


Figure 4. The Lecithin–Sodium Cholate–Water system at 25° C. L, Lecithin; NaC, Sodium Cholate; W, Water; alongside W–NaC numbers 20, 40, 60, etc. indicate the percentage by weight of sodium cholate; alongside L–W numbers 20, 40, 60 etc. indicate the percentage by weight of water; alongside NaC–L numbers 20, 40, 60 etc. indicate the percentage by weight of lecithin; I, one homogeneous phase, lamellar; II, one homogeneous phase, cubic; III, one homogeneous phase, hexagonal; IV, one homogeneous phase, micellar; numbers 1–8 represent zones of separation of two phases (see text): A, B, C. D, F, G, H, M, O and Q are points explained in the text; the line to the right of the diagram indicates a ratio of two molecules of lecithin to one molecule of sodium cholate.

one phase, will be disregarded in this discussion. Paracrystalline phase II is confined to a small area representing systems containing between 22% and 27% water. This phase appears isotropic under the microscope but exhibits angular squarish bubbles and is quite rigid. X-ray analysis gives long spacings corresponding to a face centered cubic lattice. The calculated dimensions suggest that the mixed micelles composed of sodium cholate and lecithin and surrounded by a thin layer of water. This structure is compatible with the fact that this phase is isotropic. It is analogous to the cubic phase described by Luzzati *et al.*[15] for certain soap and detergent systems as was mentioned before. Phase III is identified with what is usually called "middle soap" phase. Both the "cloudy middle soap" texture and the "angular middle soap" texture according to Rosevear's classification[11] were seen. All compositions in this zone gave long spacings corresponding to the hexagonal type. Area IV represents mixtures which gave an isotropic micellar solution. The areas between each of these zones of one homogeneous phase evidently represent compositions which separate into two or three phases. For instance, the zone marked 1 represents the separation of sodium cholate crystals and the lamellar phase having a composition falling along FG. Zone 2 marks the separation of sodium cholate crystals and of the cubic phase. Zone 3 marks the coexistence of the isotropic micellar phase and of the middle phase. It is very interesting that in this zone one finds what Rosevear[11] has called "fanlike units" floating in the isotropic fluid. Long X-ray spacings in Zone 2 correspond to a structure of the hexagonal type. Zone 4 represents the separation of cubic and lamellar phases. Here islands of the isotropic phase are observed encircled by neat soap. When one presses on the cover-slip the neat soap flows between the rigid islands of the isotropic cubic phase. Zone 5 represents the separation of cubic and middle phases. Here are found islands of middle phase trapped in areas of isotropic paracrystalline phase. X-ray diagrams in this area often show lines corresponding both to the cubic and to the hexagonal lattice. Zone 6 represents a large zone of separation of middle and lamellar phases. Here are observed islands of "angular middle soap" floating in a field of neat soap texture. Zone 7 represents compositions separating into the lamellar phase and the isotropic micellar phase. In this zone myelin figures and anisotropic droplets float in the isotropic fluid.

It appears that to incorporate the largest quantity of water in the lamellar lattice (point C, Fig. 4) the proportions of the components

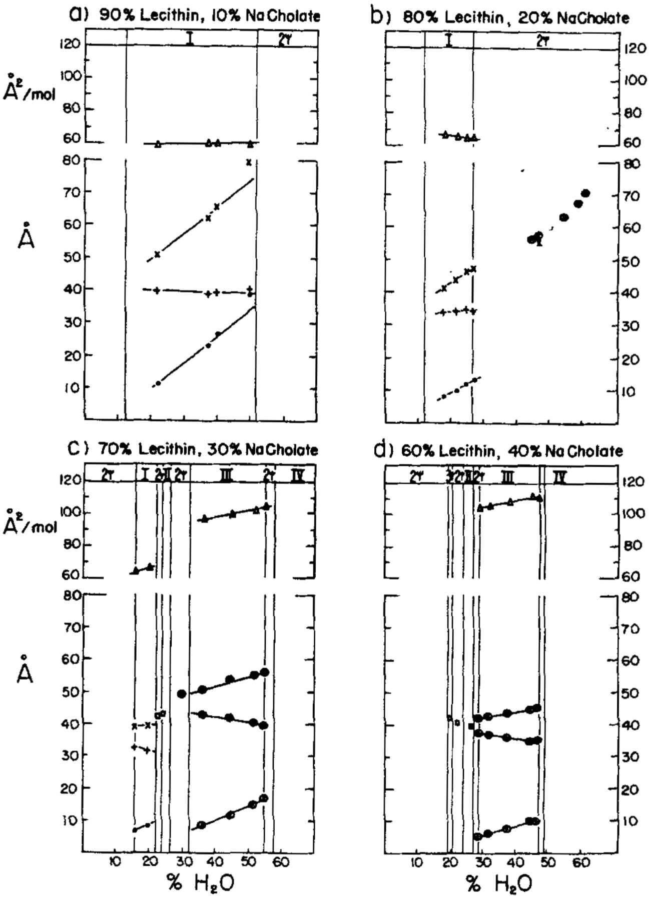


Figure 5. Dimensions of the structural elements of the liquid crystalline phases of the Lecithin–NaCholate system. Effects of increasing hydration. (a) 90% Lecithin 10% NaCholate; (b) 80% Lecithin 20% NaCholate; (c) 70% Lecithin 30% NaCholate; (d) 60% Lecithin 40% NaCholate.

△	Average surface area per molecule	
×	D, The repeat distance	lamellar phase
+	D_L, the calculated thickness of the lipid layer	
●	D_W, the calculated thickness of the water layer	
⊗	D, the distance between the lipid cylinders	hexagonal phase
⊕	D_L, the calculated diameter of the lipid cylinder	
⊙	D_W, the calculated minimal thickness of water between the cylinders (D–D_L)	
□	The spacing of the cubic phase (distance between the 111 planes)	

should be about one molecule of sodium cholate for three molecules of lecithin. It is interesting to note that the minimum quantity of sodium cholate necessary to form the middle phase (frontier HM) is one molecule of sodium cholate for two molecules of lecithin. This is the same quantity of sodium cholate necessary to transform a turbid two phase system, made up of myelin forms, into a micellar solution (frontier WH). Therefore, one molecule of sodium cholate per two molecules of lecithin is the minimum quantity of sodium cholate needed to transform the lamellar neat phase into either the middle phase (III) (the cylindrical phase), or the micellar phase (IV). This fact strongly suggests that the micellar phase is formed by the breaking up of the long cylinders of the middle phase into either rods or spheres. Preliminary light scattering and viscosity studies on micellar phase IV, in the zone which contains a high proportion of lecithin, suggests in fact that the micelles in this area are long rods of diameter roughly the same as the cylinders from which they are probably derived.[20]

Further, the passage of the middle phase into the micellar phase is markedly affected by the quantity of bile salt in the middle phase. Those mixtures containing relatively little bile salt remain in the middle phase up until nearly 70% water, while those containing more bile salt dissolve at about 34% water.

At concentrations of sodium cholate higher than one per two molecules of lecithin, if insufficient water is present to form the hexagonal phase (II), either the cubic phase (III) or the lamellar phase (I) will be formed.

Having established the limits of composition giving the three liquid crystalline phases, we now can examine the fine structure and the changes in fine structure produced by varying the composition of one component or another within the limits of the phase. Figure 5 represents the principal long X-ray diffraction spacing,

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I	One homogeneous phase type I (lamellar)
II	One homogeneous phase type II (cubic)
III	One homogeneous phase type III (hexagonal)
IV	One homogeneous phase type IV (micellar)
2ψ	Two phases present
3ψ	Three phases present

the calculated thickness of the lipid layer or of the cylinder, the calculated thickness of the water layer, and the calculated average area per molecule at the water interface, obtained with mixtures having a fixed ratio of sodium cholate to lecithin and an increasing quantity of water. In Fig. 5a (90% Lecithin–10% NaCholate), it can be seen that as the percentage of water increases the spacings also increase. The thickness of the lipid tends to fall very slightly or stays practically the same and the overall increase in thickness is almost entirely due to the water layer. The vertical line at 52% water represents the appearance under the microscope of two phases. Figure 5b at 80% lecithin-20% sodium cholate is similar to Fig. 5a but shows clearly that the long spacings have decreased. Here again the thickness of the lipid remains relatively constant and most of the augmentation of the long spacings is due to the thickness of the water layer. In the zone of two phases at least one X-ray film showed clear evidence of both the hexagonal and lamellar structures. However, by polarizing microscope all mixtures having more than 27% water and the same fixed ratio of lecithin to sodium cholate (80%:20%) gave two phases. Figure 5c (70% Lecithin–30% NaCholate) shows that the zone of lamellar phase has been reduced. There is a very small zone of the isotropic paracrystalline phase (II). Long spacings of the cubic lattice are noted in this zone. In the middle phase (III) long spacings range between 50 and 58 Å. The diameter of the cylinders decreases gently as the content of water increases. As a consequence the average surface area per molecule in the cylinders increases as the diameter of the cylinder falls. Finally, in Figure 5d, one can see that the region which has been occupied by the lamellar phase corresponds now to two phases, the lamellar phase and the crystalline sodium cholate. Again, spacings belonging to the cubic phase and the hexagonal phase are seen. Again when water is added, the diameter of the cylinders decreases and consequently the surface area occupied by each molecule increases. It can be seen that the calculated thickness of the water layer between each cylinder is quite small, averaging from 6 to 10 Å.

Therefore, in general, it can said that the addition of water to a constant proportion of sodium cholate and lecithin causes an increase in the long spacing, a slight increase or decrease in the thickness of the lipid layer in the lamellar phase, and a more marked decrease in the diameter of the cylinders in the middle phase.

Figure 6 shows what happens to the structural dimensions of the various phases as the proportion of the hydrophilic sodium cholate to the lipophilic lecithin is increased while keeping the overall quantity of water constant. Figure 6a exhibits the changes in dimensions of the structures with increasing sodium cholate concentration at a constant 45% water. As one moves along the line at 45% water (parallel to the L–NaC side of the triangle, Fig. 4) the long spacings increase from about 63 to 70 Å and the thickness of the lipid layer also increases, while the water layer remains constant. The average surface area per molecule decreases from about 71 to 60 Å^2 per molecule. Passing from the lamellar phase (I) into the hexagonal phase (III), one notes that the long spacings fall rather abruptly as the proportion of sodium cholate is increased from 23 to 43%. Further, the diameter of the lipid cylinders decreases, and the average area occupied by each molecule at the surface of the cylinder increases from 110 to 100 Å^2 per molecule. It can therefore be said that at 45% water, the thickness of the lipid layer in the lamellar phase increases with increasing sodium cholate while the thickness of the cylinders decreases with increasing cholate. In Fig. 6b, at a constant 32% water, the findings are similar except that the thickness of the lipid layer in the lamellar phase remains nearly the same. It increases only slightly. The thickness of the cylinders in the hexagonal phase tends to fall. However, in Fig. 6d, at a constant 21% water, one can see that not only do the long spacings decrease, but also the thickness of the lipid layer decreases. That is to say, above 30% water, the thickness of the lipid layer of the lamellar phase increases with increasing cholate concentration, whereas at 21% water the thickness decreases. The explanation of this phenomena is not yet clear. As can be seen on Fig. 4, the frontier HO represents the most hydrated mixtures giving the middle phase for different proportions of sodium cholate and lecithin. In Fig. 6d are examined the structural

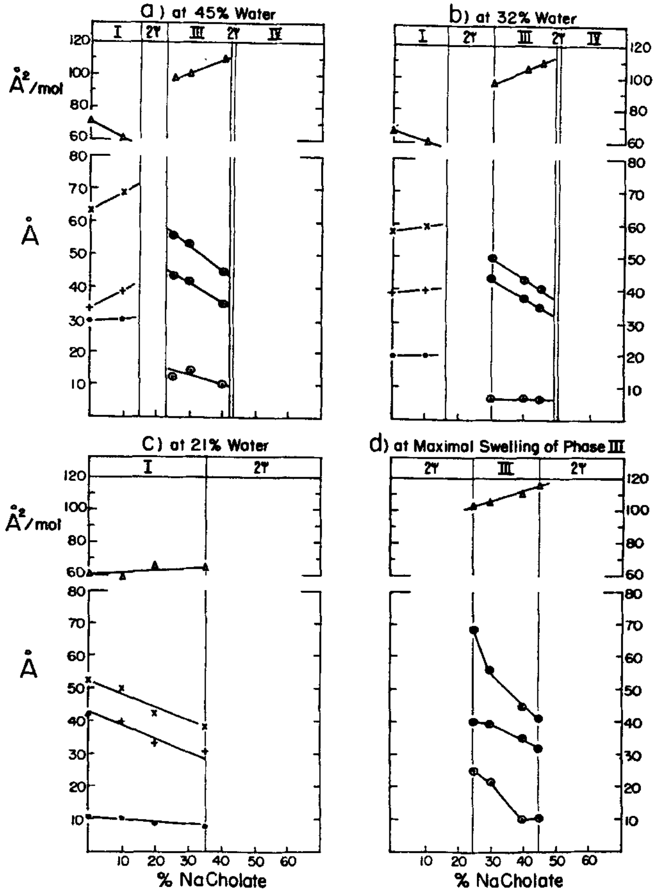

Figure 6. Dimensions of structural elements of the liquid crystalline phases of Lecithin–NaCholate system. Effects of increasing NaCholate concentration at constant water concentration (a, b and c) and at the maximum swelling of phase III (d). (a) at 45% water; (b) at 32% water; (c) at 21% water; (d) at the maximal swelling of the hexagonal phase III (i.e. along HO in Fig. 4).

△	Average surface area per molecule	
×	D, the repeat distance	lamellar phase
+	D_L, the calculated thickness of the lipid layer	
●	D_W, the calculated thickness of the water layer	
⊗	D, the distance between the lipid cylinders	hexagonal phase
⊕	D_L, the calculated diameter of the lipid cylinder	
○	D_W, the calculated minimal thickness of water between the cylinders (D–D_L)	

I One homogeneous phase type I (lamellar). III One homogeneous phase type III (hexagonal). IV One homogeneous phase type IV (micellar). 2ψ Two phases present dimensions of the hexagonal (middle soap) phase along HO. It can be seen that there is an abrupt fall in the long spacing, along this frontier from 71 Å down to about 45 Å. This is accompanied by a moderate fall in the thickness of the cylinders and by a decrease of the thickness of the water layer between the cylinders. It can be seen in mixtures rich in lecithin, that the water thickness between the cylinders is of the order of 27 or 28 Å while in mixtures rich in NaCholate the thickness is only 10 Å.

The Four Component System

The next part of the study deals with the modification of the three component system Lecithin–NaCholate–Water by the addition of cholesterol. Indeed, the addition of cholesterol to the different phases of this ternary system does not change their general structure but simply modifies the frontiers of their corresponding areas on the diagram. Figure 7 represents twelve sections of the quaternary phase diagram represented as a prism. The base of the prism (Fig. 7a) is the original lecithin–sodium cholate–water diagram simplified to show only the areas of one phase. The three liquid crystalline phases and the micellar phase are numbered I, II, III and IV respectively. Each diagram beneath diagram a represents the base diagram with a given overall per cent of cholesterol added to the compositions of the base diagram, that is, Fig. 7b corresponds to 0.5% cholesterol, Fig. 7c 1% cholesterol, etc. The maximum quantity of cholesterol which can be contained in a single phase can be deduced from the level at which this phase disappears on the diagram of Fig. 7. Thus the maximum solubility of cholesterol in the micellar solution of any composition is less than 5%. The middle phase and the cubic phase can hold cholesterol up to 6% but not 7%. The lamellar phase on the other hand is able to hold cholesterol up to at least 25%.

The X-ray analysis of a large number of mixtures falling within the liquid crystalline zones shows that increasing cholesterol produces no structural change (e.g. from hexagonal to lamellar). However, for a given mixture of sodium cholate, lecithin and water, the addition of cholesterol increases the long spacings slightly,

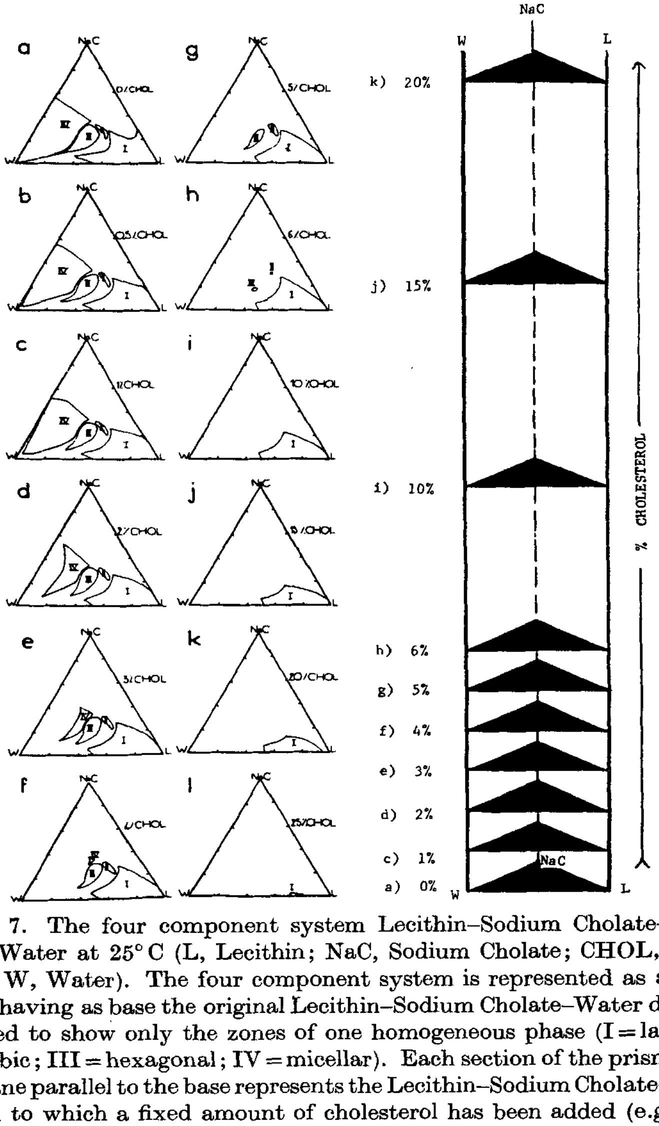

Figure 7. The four component system Lecithin–Sodium Cholate–Cholesterol–Water at 25° C (L, Lecithin; NaC, Sodium Cholate; CHOL, Cholesterol; W, Water). The four component system is represented as a prism (right) having as base the original Lecithin–Sodium Cholate–Water diagram modified to show only the zones of one homogeneous phase (I = lamellar; II = cubic; III = hexagonal; IV = micellar). Each section of the prism taken in a plane parallel to the base represents the Lecithin–Sodium Cholate–Water system to which a fixed amount of cholesterol has been added (e.g. 0.5% cholesterol, 1.0% cholesterol; 2.0% cholesterol, etc.). The height (proportional to the amount of cholesterol added) at which the sections were taken is diagrammed on the right and the actual sections (i.e. the modification of the base diagram (Fig. 7a) as more and more cholesterol is added), are shown on the left (Fig. 7b–c).

that is 1–3 Å. Further, cholesterol increases the thickness of the lipid layer of the cylinders slightly. Thus, although it cannot be stated with certainty, it would appear that the cholesterol is interdigitated alongside the lecithin in the lamellar phase. If the cholesterol was all in between the tails of the lecithin molecules, a much greater increase would be expected than was found in the long spacings and in the calculated thickness of the lipid layers. It seems more reasonable to suggest that the increase in thickness, as cholesterol is added to these mixtures, is probably due to the condensing effect of cholesterol on lecithin as was demonstrated by de Barnard in mixed monolayers.[19]

How the sodium cholate fits into the lamellar and the cylindrical structures is not at all clear. Sodium cholate is a peculiar molecule: it possesses a hydrophilic tail like soap, but, on one side of the molecule for nearly its entire length, there are three hydroxyl groups. On the other side, three methyl groups protrude. This molecule can be considered as being fairly rigid having one side which is hydrophilic, one side which is lipophilic and a tail which is hydrophilic. It is possible that two of these molecules lying side by side may bind together by H-bonds forming a dimer, thus hiding the hydroxyl groups and exposing the lipophilic parts. These dimers may be surrounded by the paraffinic chains of the lecithin molecules in the lamellar phase. However, in the phase of hexagonally packed cylinders, it is hard to see just exactly what the molecular arrangement might be.

The influence of increasing water in a mixture of any given proportion of lecithin and sodium cholate has been summarized in Fig. 5. At a fixed water concentration, in the lamellar phase, the addition of sodium cholate to a quantity of lecithin causes an increase in the calculated thickness of the lipid layer if the percentage of water is above 25–30%, and a decrease if below that percentage. It always causes a decrease in the diameter of the lipid cylinders in the middle phase.

From the study of these various systems and of the paracrystalline phases obtained, one can conclude that cholesterol, with its amphiphilic but insoluble molecule, gives no paracrystalline phase with bile salt in water but associates with lecithin in the presence of water to give one paracrystalline lamellar phase, similar to that given by lecithin alone. This phase can hold up to a proportion of one molecule of cholesterol for one molecule of lecithin. On the contrary, the addition to lecithin of sodium cholate, which is a substance soluble in water, produces several paracrystalline phases and an isotropic solution. In addition to a lamellar phase, identical to that given by lecithin alone, can be formed a paracrystalline cubic phase and a paracrystalline phase with a hexagonal structure. These different phases persist on the addition of cholesterol up to a certain limit. In each of the phases the variation of the composition in the different components, and particularly in water, modifies more or less the parameters of the lattice. Hence the long-spacing, the thickness of the water layer, and the thickness of the lipid layer in the lamellar form (neat phase) as well as in the cylindrical form (middle phase) and spherical form (cubic phase) vary depending upon the composition in the different components—bile salt, lecithin, cholesterol and water.

The crystallographic results here presented were obtained in the course of investigations on the problem of the formation of gallstones in relation to the composition of bile. In fact, the main components of bile are bile salts, lecithin and cholesterol, and a large percentage of gallstones are crystalline cholesterol. Some of the results of these investigations and others relevant to the particular pathophysiological problem mentioned above will be published elsewhere.[21]

Acknowledgments

One of us (D. M. Small) wishes to express his sincere gratitude to Professor F. J. Ingelfinger for suggesting and making possible his extended visit to the Institut Pasteur, Paris, France, and also to Dr. Alan Hofmann for his stimulating encouragement.

Both of us wish to express our sincere thanks to Dr. D. G. Dervichian. Without his stimulation, guidance and aid this work could not have been done.

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