# A Classification of Biologic Lipids Based upon Their Interaction in Aqueous Systems

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## Abstract

A classification of lipids is presented, based upon their physical properties in bulk aqueous systems and at the air-water or oil-water interface. This is supported by binary-phase diagrams of the various classes of lipids in water.

The interactions of the lipids of each class with a lipid of another class is illustrated by a series of different ternary-phase diagrams of two lipids in water. The various types of association and the molecular relation of one lipid to another are indicated. The interaction of three classes of lipids with water is illustrated in two examples by quaternary-phase diagrams of the three lipids in water.

As an example of the application of these in-vitro studies, the composition of bile is correlated with a quaternary-phase diagram cholesterol-lecithin-bile salt-water. The correlation shows that human bile behaves as a biologic four-component system the physical state of which is entirely predictable from the quaternary-phase diagram. Although bile is a special case, it is probable that the physical arrangement of the lipids in membranes, cellular organelles, lipoproteins, and adipose tissue can be suggested by studies of the interaction of lipid classes with themselves in water.

## Introduction

THE OLD ADAGE "oil and water don't mix" is true for some lipids but not for others. The extent to which lipids interact with water is crucial to the organization, structure, and function of the living cell. This basic unit of life is really an aqueous system in which are suspended various lipids and complex proteins, the organization and structure of which ultimately dictate its functions. Thus the purpose of this paper is to discuss the interrelation of biologically active lipids with water. Most textbooks of physiological and biological chemistry and books dealing specifically with lipids classify lipids on the basis of their chemical characteristics, their solubility in organic solvents, or their biochemical reactions. However, in view of the fact that all biological systems are basically aqueous, it may be relevant to attempt a classification based on the interaction of lipids with water and to discuss the physical structure of pure lipid species and of mixtures of lipids in water. The physical state and structure of lipids in aqueous systems may help achieve an understanding of the structure of biological membranes, cellular organelles, and lipoproteins. It should also furnish a physical basis for understanding the absorption, transport, storage, and excretion of "insoluble" lipids in more advanced animal species.

A classification of lipids based upon their interaction with water, both in the bulk and at the surface, is proposed. The bulk interactions of the lipid with water will be illustrated by binary lipid-water phase diagrams, and, where it is known, the structure of the lipid in water will be indicated schematically. Next, the interaction of various classes of lipids with one another in aqueous systems will be illustrated with ternary- and quarternary-phase diagrams, and the fine structure of the resulting phases will be schematically illustrated and discussed. Finally, the biological importance of these studies and the relevance of the classification will be illustrated by a particular case, that of the lipids occurring in bile.

The data used in placing certain lipids in the following classification have been derived from both surface studies of lipids (1–25) and bulk studies of lipids (24–51). Most of the lipids were tested for their bulk interaction with water by using the following procedure. A small amount of dry lipid was placed betweeen glass coverslips on a heating stage of a polarizing microscope. The anhydrous phase transformations (crystal, liquid crystal, liquid) were observed as the temperature was increased at a rate of about 1–2C per minute. A second sample was then placed between the slide and coverslip, and changes were observed when a small amount of water was placed under the edge of the coverslip.

These changes indicated the kind of lyotropic mesomorphism (the formation of liquid crystal phases with the addition of water) which takes place (52). If no lyotropic mesomorphism was apparent at room temperature, the sample was heated on the microscope stage in the presence of water up to 100C to test for lyotropic mesomorphism at higher temperatures. Finally, a sample was placed in a vial and equilibrated with water at room temperature or higher temperatures if necessary, and the resulting suspension, emulsion, or solution was examined by direct and polarizing microscope for the presence of crystals, oil droplets, or liquid crystals. Solubility was measured by centrifuging these dilute mixtures and recording the dry weight by microbalance of the supernatant. If fewer than 10 mg of solids per liter supernatant were present, the substance was considered insoluble.

The materials and techniques used in constructing phase diagrams of biologically active lipids have been detailed in several other papers (52–55). Lipids of high purity have been used. Phase diagrams were constructed by observing grossly and by light and polarizing microscope a large series of equilibrated mixtures of the appropriate lipids and water. The fine structure of the individual liquid crystalline phases has been determined and studied by narrow and wide x-ray diffraction (56). The densities of the various lipids in aqueous systems were determined by picnometry. The apparent micellar weights were determined from light scattering and ultracentrifuge determinations (57). The probable structures of the micelles and bile salts and of mixed bile salt-lecithin micelles were deduced from the apparent micellar weight (57), viscometry (56), high resolution NMR (58), surface balance studies (6–11,59), and correlation with Stuart-Breigleb molecular models.

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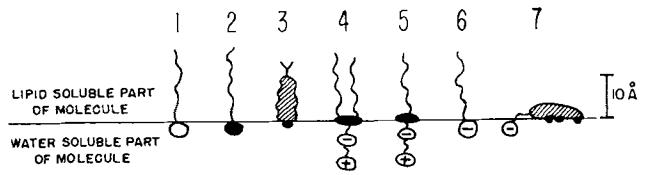


FIG. 1. Schematic representation of lipid molecules mentioned in the text. Molecules are pictured as they would lie at an air-water or oil-water interface. Wavy lines, the lipid-soluble hydrocarbon chain part of the molecule; shaded area, the lipid-soluble cyclic hydrocarbon part of the molecule; open circle, polar head of an amphiphile; closed circle or oval, OH groups or ester groups as the case may be; ⊕ and ⊖, the positively and negatively charged ionic polar groups. 1) the general configuration of aliphatic amphiphilic molecule, 2) long-chained protonated fatty acid, 3) cholesterol, 4) lecithin, 5) lysolecithin, 6) ionized fatty acid, 7) bile salt.

Although lipid is a general word which refers to a heterogeneous group of organic compounds, the present classification is limited to aliphatic compounds with chain lengths 12 carbons or longer and aromatic compounds containing at least three fused rings. Therefore, when the term fatty acid or monoglyceride is used, it refers to long-chain fatty acids with 12 or more carbons in the aliphatic part of the molecule. Simple structural representations of an aliphatic lipid molecule and of the specific lipid molecules discussed at length later are shown in No. 1 of Fig. 1 and No. 2–7 in Fig. 1 respectively.

## Classification by Interaction with Water

Fig. 2 shows the classification of lipids based upon their physical interactions in or on water.

### Nonpolar Lipids

These lipids are primarily made up of long-chain paraffins or unsubstituted aromatic compounds. The following biological compounds fall into this class: octadecane, carotene, squaline, lycopene, gadusene, phytane, pristane, and cholestane. Large aromatic hydrocarbons, some of which are known or potential carcinogens (e.g., benzphenanthracene, benzpyrene), should also be included. This class of compounds is insoluble in the bulk, and in water they form either oil droplets or crystals. When placed on the surface of water, they do not spread to form a monomolecular film but simply appear as an oil drop or crystal resting on the surface of the water. Because of their scarcity in living organisms, these lipids will not be considered further.

### Polar Lipids

*Class I. Insoluble, Nonswelling Amphiphilic Lipids.* These lipids are polar because, even though they contain a long aliphatic chain or a large bulky aromatic structure (such as steroid nucleus), they have at least one hydrophilic group on the molecule. In the bulk they are virtually insoluble and, depending upon the temperature, form either oil or crystals in water. However, on the surface, they can be spread to form stable monomolecular films and therefore have a surface solubility. These lipids are insoluble in water and do not swell in water but possess a surface solubility, as shown by the formation of stable monolayers. Compounds in this class comprise the majority of lipid substances in the higher animals. Examples include the following: di- and triglycerides, long-chain protonated fatty acids, waxes, sterol esters, long-chain alcohols, phytols, retinols, Vitamin A, Vitamin K, Vitamin E, and many sterols such as cholesterol, desmosterol, sitosterol, vitamin D, and a number of hormones.

*Class II. Insoluble, Swelling Amphiphilic Lipids.* Although these substances are virtually insoluble in water, they swell to form certain well-defined liquid crystalline phases. Swelling will only occur at temperatures high enough to render the aliphatic chains partly liquid. This critical temperature depends on the chain length, saturation, branching, and substitution. The result of decreased chain-length, unsaturation, branching, and polar substitution is to decrease this temperature. These molecules also spread to form stable monolayers on the surface of water. Compounds in this class include many of the species of lipid felt to be important in the structure of cell membranes and cellular organelles (lecithins, phosphotidylethanolamines, phosphotidyl inositol, sphingomyelin). The important products of triglyceride hydrolysis in the small intestine, namely, monoglycerides and acid soaps, are also in this class of lipids. Cerebrosides, phosphotidic acid, plasmalogens, phosphotidylserine, cardiolipins, and certain plant sulfolipids are probably also in this class. If these molecules carry a strong net charge (e.g., phosphotidyl serine at pH 7), they may swell to incorporate large-volume fractions of water (45).

*Class IIIA. Soluble Amphiphiles, Type A.* This class of soluble amphiphiles, in general, possesses a clear-cut polarity between the hydrophobic part of the molecule (usually an aliphatic chain which may contain one or two benzene rings) and the hydrophilic part of the molecule. They are distinguished from Class IIIB soluble amphiphiles by the fact that they

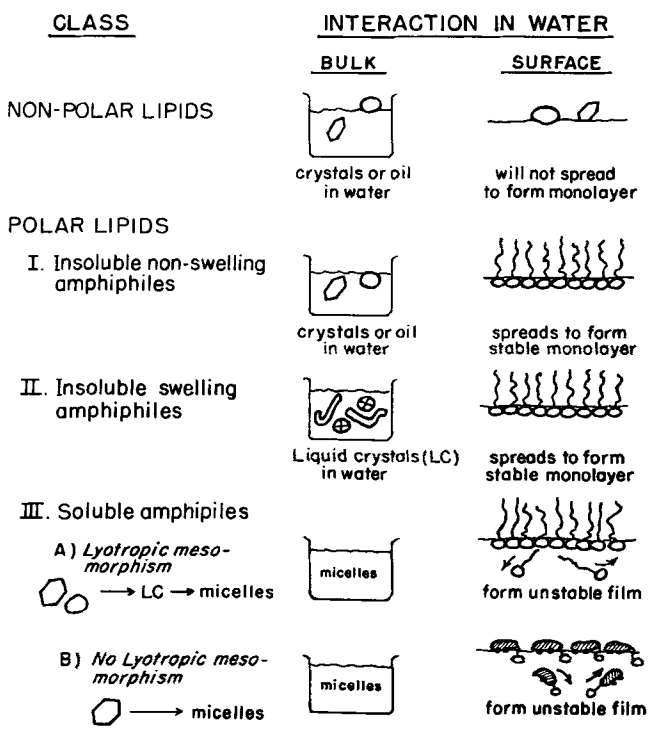


FIG. 2. Classification of biologically active lipids. Column 1 gives the three major lipid classes. Column 2 depicts the physical state of the lipid in bulk aqueous system and on the surface of water. Wavy line represents the aliphatic tails of lipid molecules, and open circles represent polar heads. Cross-hatched area molecules of Type IIIB represent the steroid nucleus of the bile salt.

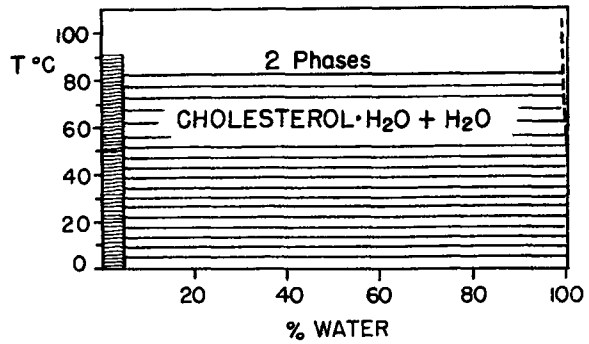


FIG. 3. Cholesterol-water binary-phase diagram (Class I-water). Vertical axis, temperature degrees C; horizontal axis, percentage of water. Line at about 5% water represents cholesterol monohydrate. The dotted line at nearly 100% water is exaggerated and represents slight increase in cholesterol solubility at higher temperatures (e.g., at 85C about 20 mg/L).

form liquid crystalline phases when small quantities of water are added to the compound (lyotropic mesomorphism). This class includes many of the classic anionic, cationic, and nonionic detergents as well as the important biological compound lysolecithin.

*Class IIIB. Soluble Amphiphiles, Type B.* These soluble amphiphiles do not form liquid crystals. In general, they are aromatic compounds with three or more fused rings and although these molecules may have definite hydrophobic and hydrophilic regions, they may possess no clear-cut polarity between the ends of the molecules. The most important biological compounds of this type are the sulfated bile alcohols found in some fish (60) and the bile salts found in higher animals. These compounds are steroids and have a hydrophilic side-chain as well as a nucleus with both hydrophilic and hydrophobic sides (61). Other compounds which appear to belong to this class are the rosin soaps, saponins, and phenanthrene sulfonic acids.

Both Class IIIA and Class IIIB soluble amphiphiles form micelles in relatively dilute solutions, but the micelle structure of the two types is probably different

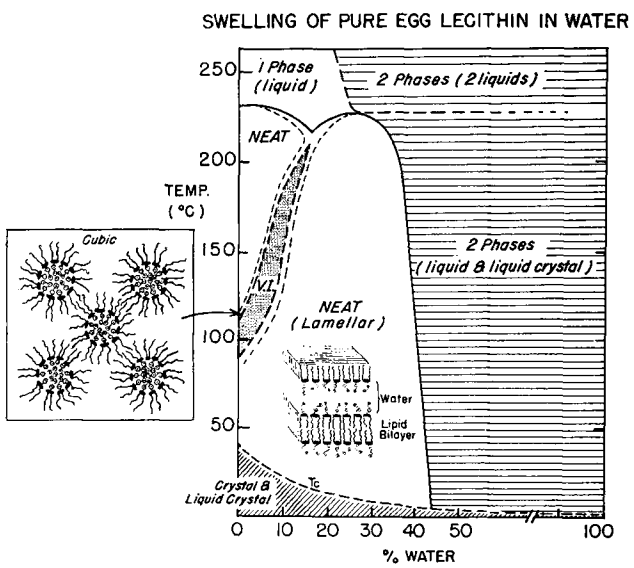


FIG. 4. Egg lecithin-water binary-phase diagram (Class II-water). Vertical axis, temperature degrees C; horizontal axis, percentage of water. VI represents a viscous isotropic (face-centered cubic lattice) phase. The inset on the left represents the author's conception of the arrangement of the molecules in this phase. Tc is an ill-defined boundary of crystal to liquid crystal phase transition. The structure of the lamellar phase is indicated (55).

(57,58). Both types are capable of solubilizing other classes of lipids. However, as will be seen later, there are marked differences between Type A and Type B in the solubilization of the other insoluble classes of lipids. Both types of soluble amphiphiles will form unstable monolayers at the air-aqueous interface and demonstrate an equilibrium between molecules in the bulk phase and those on the surface. Type A-soluble amphiphiles however decrease the surface tension of water much more than Type B-soluble amphiphiles (62,63).

## Interaction with Water (Binary Systems)

McBain (26) showed that the most complete way of illustrating the interaction of various lipids with water is by the construction of a phase diagram. When only one species of lipid and water is involved, a binary (e.g., a two-component) lipid-water phase diagram can be constructed. The construction of the binary phase is relatively simple. Varying mixtures of the two components are allowed to equilibrate at different temperatures. The resulting phases are separated by settling, centrifugation, or filtration and are then analyzed. An example of a simple binary-phase diagram of an insoluble nonswelling amphiphile (Class I) is shown in Fig. 3 (cholesterol-water). Temperature is on the vertical axis and percentage of water on the horizontal axis.

Cholesterol forms a monohydrate with water (64,65), which is represented at the left-hand side of the diagram by a vertical line at about 5% water. This small quantity of bound water can be removed in a dry vacuum to produce an anhydrous crystal. The other side of the diagram demonstrates that the cholesterol is strikingly insoluble in water. It should be noted that the values of cholesterol solubility in water to be found in the older editions of "The Handbook of Chemistry and Physics" (66) are far too high (53,67). They actually refer to the solubility of cholesterol in certain detergent systems. At higher temperatures there is an increase in cholesterol solubility to about 20 mg/L at 80C.

Fig. 4 represents the binary-phase diagram of an insoluble swelling amphiphile (Class II). The diagram shown is that of egg lecithin in water (55). It can be seen that egg lecithin swells in water to take up to about 45% water in a specific liquid crystalline phase, the so-called "lamellar phase." This phase has been called "neat soap" and has a characteristic birefringence by polarizing microscope (30). The structure of this phase as determined by x-ray diffraction is of repeating bimolecular leaflets of lecithin, separated by layers of water.

As the amount of water incorporated between the bilayer is increased from 12 to 44%, the repeat distance increases from 51.0 Å to 64.1 Å (55,56). The thickness of the lipid bilayer (hydrocarbon parts only) however decreases from 36.3 Å to 30.0 Å (55). This collapse of the lipid layer is probably because of a net repulsion between opposing bilayers of lecithin (68). When the water content of the mixture is increased beyond 45%, two separate phases appear: water and lamellar liquid crystals containing 45% water. At higher temperatures there is a zone of viscous isotropic liquid crystal (VI) which can contain a maximum of about 15% water.

Like the viscous isotropic phases noted for soaps and anionic detergents (42,43), cationic detergents (40,-41,42), and mixtures of bile salt and lecithin (56,59),

the x-ray diffraction data give the spacing of a face-centered cubic lattice. Unlike these examples in which the elements are probably micelles of lipid packed in a face-centered cubic lattice and separated by a thin layer of water (but see Ref. 69), the elements of this lattice are probably spherical groups of the polar heads of lecithin surrounding a small amount of water (Fig. 4). The distance between centers of these groups is about 48.0 Å (55). Phase diagrams of certain monoglycerides (28,29,51) and a mixture of brain phospholipids (42) are similar although they are not identical with the phase diagram of egg lecithin (55).

The phase diagram of a soluble amphiphile (Class IIIA) is shown in Fig. 5 (soap-water). The diagram has been modified from the older works of McBain (26) to show the structure of the important liquid crystalline phases, as described more recently by Luzzati, Mustacchi, Skoulios, and Reiss-Husson (42,-43,70). In the anhydrous state the soap is crystalline, liquid crystalline, or liquid, depending on the temperature. With progressive hydration at a given temperature (e.g., 70C) the soap swells to form a lamellar liquid crystalline phase ("neat soap," Ref. 30), a hexagonal liquid crystalline phase ("middle soap," Ref. 30), a micellar solution, and finally an ideal solution. The lamellar phase contains from 5 to 35% water and the hexagonal phase 40–65% water. At about 65–70% water these long cylinders of hexagonal phase break up into rod-shaped or spherical micelles (71,72).

At a certain given concentration for a specific temperature the micelles fall apart, and the molecules are present in an ideal solution. This last transformation is called the critical micellar concentration (CMC). Usually the CMC of anionic and cationic detergents increases with increasing temperature. Below the line marked "Tc" there exists either a gel or a coagel (a mixture of crystals and water). At Tc the gel or coagel melts sharply to form micelles or a liquid crystalline phase. This line has been called the Krafft Point (73) or, more aptly, the critical micellar temperature (74).

Three conditions are necessary for a soluble amphiphile of Type A to form a micellar solution: it must be above its critical micellar concentration, it must be below the concentration at which it forms a liquid crystalline phase, and it must be above its critical micellar temperature.

Fig. 6 portrays the simple binary-phase diagram of a soluble amphiphile, Type B (Class IIIB) in water. This diagram represents sodium cholate-water systems studied from 0C to 100C. A similar binary-phase diagram for Na deoxycholate was published by R. D. Vold and J. W. McBain more than 25 years ago (75). The striking difference between these diagrams and the one for classical detergents (Class IIIA), shown previously (Fig. 5), is that no liquid crystalline phases are formed (52,59,75) and that the Krafft Point for most of these substances is below 0 (76). The micelles of sodium cholate are not the typical spherical or rod-shaped micelles pictured for soaps in Fig. 5 but are small micelles with an aggregation number of 3 to 8 (depending on counter-ion concentration) (57,76,77,78,79), bonded probably hydrophobically back-to-back as pictured in Fig. 6 (57,58).

These phase diagrams show that polar lipids vary considerably in their bulk relationships with water. Class I nonswelling insoluble amphiphiles have negligible solubility in water, and, in turn, water is nearly insoluble in them. Although by definition Class II insoluble swelling amphiphiles are insoluble in water, they do incorporate water into their liquid crystalline states by swelling. The classical detergents (Class IIIA) swell in water to form several liquid crystalline phases, but in the presence of excess water they form micellar solutions. Finally, soluble amphiphiles of Type B (Class IIIB) form micellar solutions from crystals. They have a high solubility in water and a low Krafft Point but do not form liquid crystals.

### Interactions in Aqueous Systems (Ternary)

In order to express the interaction of two lipids with water in all possible combinations of the three substances, it is necessary to construct ternary-phase diagrams. The method of the construction of such a diagram is simple, in principle. One makes a large series of mixtures containing the three components and allows them to equilibrate. The mixtures are then examined for presence of one, two, or more phases. These phases can be crystalline, liquid crystalline, or liquid. The results of these observations are then

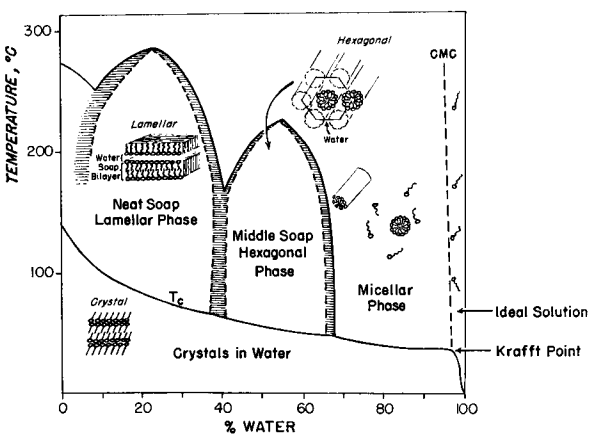


FIG. 5. Soap-water binary-phase diagram (Class IIIA-water), modified from McBain (26) and Luzzati et al. (42,43,70). Vertical axis, temperature degrees C; horizontal axis, percentage of water. This phase diagram is representative of a sodium salt of the long-chain fatty acid in water. The structure of the lamellar phase, the hexagonal phase, and two possible types of micelles (rods and spheres) are shown. CMC, critical micellar concentration. Tc, crystal or gel to liquid crystal or micellar phase transformation.

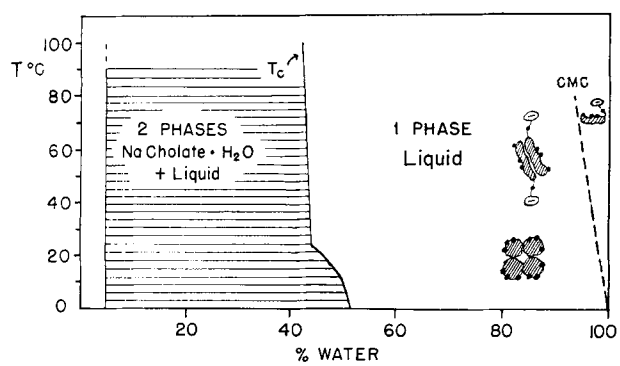


FIG. 6. Sodium cholate-water binary-phase diagram (Class IIIB-water). Vertical axis, temperature degrees C; horizontal axis, percentage of water. The line at about 5% water represents a monohydrate of sodium cholate. This monohydrate is in equilibrium with a liquid phase. The dotted line marked Tc represents the solubility of sodium cholate in water. The solubility increases slightly with increasing temperature. The liquid phase in the dilute region is made up of small micelles (57). The nature of the more concentrated liquid phase is not yet certain, but it appears to be an ordered liquid. CMC, critical micellar concentration.

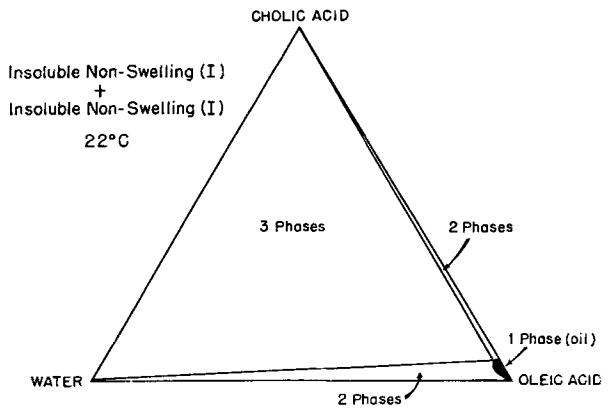


FIG. 7. Cholic acid-oleic acid-water ternary-phase diagram (Class I-Class I-water). All subsequent ternary and quaternary systems are expressed in weight percentage except for Fig. 16 and 17, which are in moles percentage. The only zone of one homogeneous phase is the liquid (oil) at the oleic acid corner. The solubility of cholic acid and water in oleic acid is quite small.

plotted on triangular coordinate paper.[2] The apices of the triangle represent 100% of each of the components, any point along each side of the diagram represents the binary mixture of the components at each end of the line. All points within the triangle represent mixtures of the three components, and the position of the point is determined by the composition of the mixture. When an adequate number of mixtures have been plotted, one can delineate the zones which form one homogeneous phase or zones in which two or three phases are present (52).

Interactions between two types of lipids will be illustrated in the following seven ternary-phase diagrams (Fig. 7–13). These represent most of the combinations of the major classes of polar lipids already discussed (i.e., Classes I, II, IIIA, and IIIB). The following examples of three-component phase diagrams will be discussed in the order of increasing solubility in water: I-I-water (fatty acid-bile acid-water); I-II-water (cholesterol-lecithin-water); I-IIIA-water (fatty acid-soap-water); II-IIIA-water (lecithin-lysolecithin-water); I-IIIB-water (cholesterol-bile salt-water); II-IIIB-water (lecithin-bile salt-water); IIIA-IIIB-water (soap-bile salt-water).

These diagrams are representative of lipid interaction between lipids of different classes. Other ternary-phase diagrams however have been studied and will be mentioned in reference to the above examples.

### I-I-Water

Fig. 7 represents the interactions of two insoluble nonswelling amphiphiles (cholic acid and oleic acid) with water (82). Bile acid, which is crystalline at the temperature of the experiment (22C) dissolves up to about 6% in the fatty acid, which is an oil at this temperature. This oil can dissolve a maximum of 2% water. The other zones are made up of at least two phases. Most of the diagram is made up of mixtures which form three phases: bile acid crystals, an oleic acid-oil phase containing a small amount of bile acid and water, and water containing a trace of cholic acid and oleic acid. Similar types of diagrams would be expected for mixtures of any insoluble nonswelling polar lipids, such as cholesterol and triglyceride, fatty acid and triglyceride, cholesterol ester and triglyceride, etc., although the mutual solubility of the two water-insoluble lipids would vary.

### I-II-Water

The interactions of an insoluble nonswelling amphiphile (cholesterol) and an insoluble swelling amphiphile (lecithin) in water are shown in Fig. 8. Although a number of these systems including lecithin-triglyceride-water (80), phosphotidylethanolamine-cholesterol-water (81), lecithin-bile acid-water (81), lecithin-cholesteryl linoleate-water (81), and lecithin-cholesterol-water (53,56,59) have been studied, the last system is perhaps the most interesting from the point of view of all plasma membranes. As noted before, cholesterol at 22C is virtually insoluble in water. Lecithin swells in water to give a lamellar liquid crystalline phase. The large zone in the lower right-hand part of the diagram (Fig. 8) represents one phase, the lamellar liquid crystalline phase. The maximum amount of cholesterol which can be incorporated into this phase is one molecule per molecule of lecithin; after this, any excess is present as cholesterol crystals. X-ray diffraction measurements (53,56,83) have clearly demonstrated that in the lamellar phase the cholesterol is interdigitated between the lecithin molecules.

These measurements show that, as the cholesterol is added to the lamellar phase containing a given quantity of water, the distance between the layers does not change appreciably, proving that the cholesterol does not lie in between the tails of the lecithin but is interdigitated between the lecithin molecules as suggested in Fig. 8. These leaflets, then, in a sense represent two monolayers back-to-back. The lamellar liquid crystalline phase is an endless stack of bilayers. It is interesting to note that in all the biological membranes (except myelin) thus far studied, the ratio of free cholesterol to phospholipid approaches but does not exceed a molecular ratio of 1:1 (84–86).

The diagrams for lecithin-triglyceride-water, lecithin-bile acid-water (Fig. 15, top right), and phosphotidylethanolamine-cholesterol-water are similar, but the ratio of maximum solubility of the insoluble

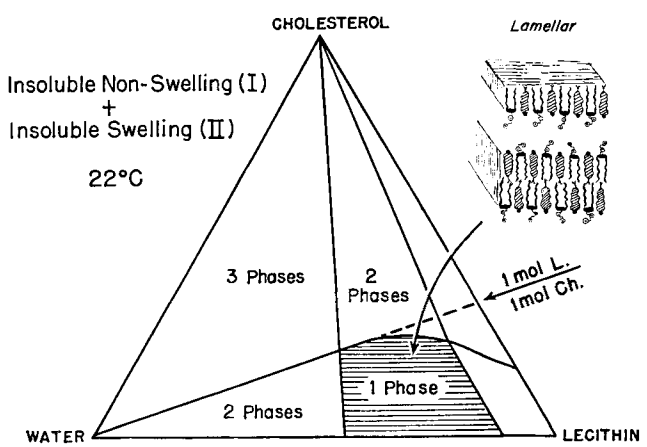


FIG. 8. Cholesterol-lecithin-water ternary-phase diagram (Class I-Class II-water). The inset on right represents the structure of the lipid bilayer composed of lecithin and cholesterol in a 1:1 molecular ratio (maximum saturation of the lamellar liquid crystalline lattice with cholesterol). The cholesterol molecules are interdigitated between the lecithin molecules (53,56,59).

[2] For further explanation of the phase rule see A. N. Campbell and N. O. Smith, Findlay—"The Phase Rule and Its Application," 9th ed., Dover Press, New York, 1951.

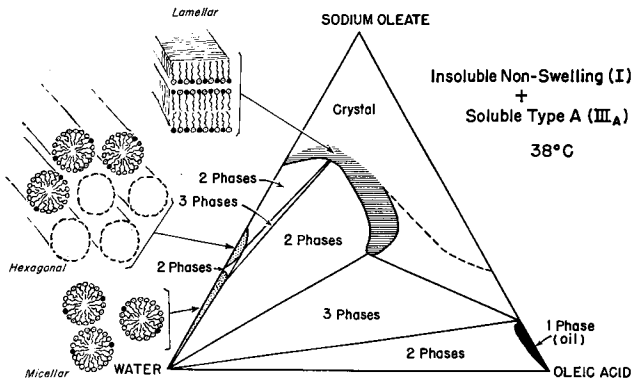


FIG. 9. Oleic acid-sodium oleate-water ternary-phase diagram at 38C (Class I-Class IIIA-water). The representation of the lamellar liquid crystalline phase is that of composition having one molecule of soap for one molecule of fatty acid (acid soap). However much smaller amounts of acid can be incorporated into this phase, provided the quantity of water is small. It will be noted that the zones of hexagonal phase and micellar phase are quite small.

nonswelling lipid (Class I) in the lamellar structure of the insoluble swelling amphiphile (Class II) varies.

### I-IIIA-Water

Fig. 9 represents the interaction of an insoluble nonswelling amphiphile (oleic acid) and a soluble amphiphile of Type A (Na oleate) in water (81). The first studies of the interaction of a soap with its fatty acid in water were reported by McBain and Field in 1933 (87) for potassium laurate and lauric acid. The system sodium oleate-oleic acid-water has recently been examined at a temperature well above the Krafft Point of Na oleate. Although all concentrations of oleic acid are insoluble in water, with increasing concentrations of water sodium oleate forms several different phases (crystalline, lamellar liquid crystalline, hexagonal liquid crystalline, and micellar liquid). Provided the temperature is high enough, mixtures of sodium oleate and oleic acid form a lamellar liquid crystalline phase which can contain up to one molecule of oleic acid per molecule of sodium oleate (i.e., the composition of the acid-soap). Excess oleic acid is present as an oil containing small amounts of sodium oleate and water. However the amount of oleic acid which can be incorporated into the hexagonal phase or into the micellar phase is quite small, representing less than one molecule of acid to 10 of soap.

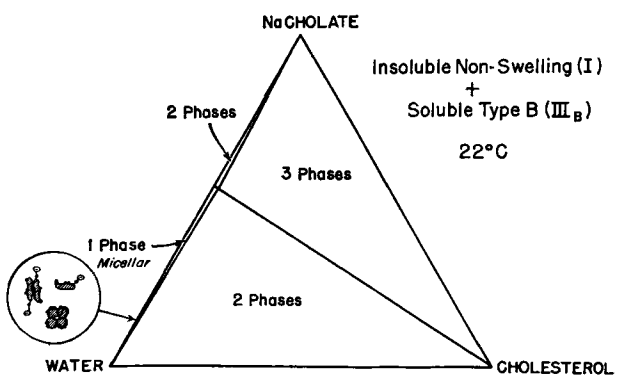


FIG. 10. Cholesterol-sodium cholate-water ternary-phase diagram (Class I-Class IIIB-water). The structure of the bile salt micelles is indicated in the inset. These micelles remain small in the presence of cholesterol. It will be noted that the micellar zone is small and that no liquid crystalline phases are formed in this system (54,59).

### I-IIIB-Water

The interactions of an insoluble nonswelling amphiphile (cholesterol) with a soluble amphiphile of Type B (the bile salt sodium cholate) in water are shown in Fig. 10 (54,59). Both these molecules are steroids, which may account for the fact that no liquid crystalline phases are formed.

The amount of cholesterol solubilized by sodium cholate or by other conjugated bile salts (54,59) in any amount of water is extraordinarily small, less than 2% by weight. In other words, one needs about 35–40 bile salt molecules to solubilize one molecule of cholesterol. Since the sodium cholate micelle in water is small and consists of only two to four molecules (57,76,77,79) and since the addition of cholesterol up to the saturation point (81) does not significantly increase the size of the micelle, where is the cholesterol solubilized? It may be passed rapidly from micelle to micelle like a hot potato.

### II-IIIA-Water

Fig. 11 represents the interactions between an insoluble swelling amphiphile (lecithin) and a soluble amphiphile Type A (lysolecithin) in water (81). This may have some biological relevance as both lecithin and lysolecithin occur in red-cell membranes. This diagram bears some resemblance to the sodium oleate-oleic acid-water diagram (Fig. 9). Lecithin swells in water until at 52C it contains about 40% water. It was necessary to carry out the observations at this temperature because the Krafft Point of this particular lysolecithin was high (36C) and, in order to observe the liquid crystalline phases, the temperature had to be raised well above the Krafft Point. If there are decreasing amounts of lysolecithin in water, there is first a hexagonal liquid crystalline phase between 30 and 60% water, then there is a two-phase zone between 60 and 65% water, and finally at 65% water a micellar phase begins, which extends down to the critical micellar concentration. Both the size of these micelles and the critical micellar concentration have been determined by Robinson and Saunders (88,89).

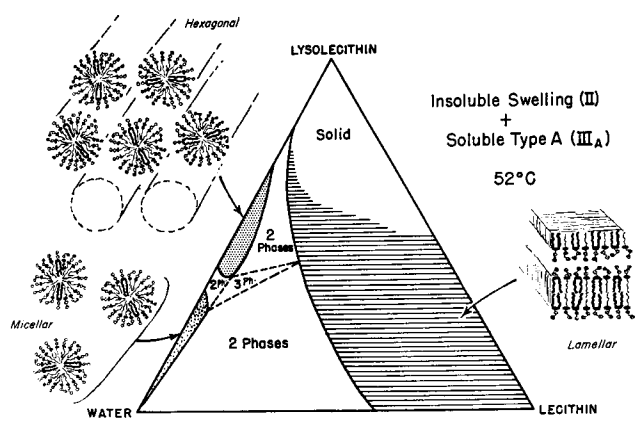


FIG. 11. Lecithin-lysolecithin-water ternary-phase diagram at 52C (Class II-Class IIIA-water). The structures of the lamellar, hexagonal, and micellar phases are indicated by the insets. The lecithin molecules have been drawn in darker characters so that they will stand out. It will be noted that this diagram bears a striking resemblance to the upper one-half of the sodium oleate-oleic acid-water diagram (Fig. 9). Again, the zones of hexagonal phase and micellar phase formed by lysolecithin are small, showing that these phases become saturated with small amounts of lecithin. Lecithin in excess of about one molecule to 10 of lysolecithin separates as a lamellar liquid crystal.

At room temperature they thought that the micelles were spherical and consisted of about 180 monomers. Only a small amount of lecithin can be incorporated into the middle phase or into the micellar phase. Robinson (90) noted that the size of the micelles increases markedly as small amounts of lecithin are added to lysolecithin. When the molar ratio of lecithin to lysolecithin approaches one to 10, the molecular weight of the micelles is more than one million. Although Robinson (90) suggested that the micelles were long and rod-shaped, other asymmetric shapes have not been clearly excluded.

Provided the amount of water is small, lysolecithin can be added to the lamellar liquid crystalline phase in virtually all proportions up to 95% lysolecithin-5% lecithin. The probable structure of the lamellar phase containing one molecule of lysolecithin to one molecule of lecithin is shown in Fig. 11. Most of the rest of the diagram comprises two phases: myelin figures (lamellar aggregates), which contain varying quantities of lysolecithin and lecithin, and a micellar phase, made up of large micelles of lysolecithin which have a small amount of lecithin incorporated into them.

**II-IIIB-Water**

The interaction of the insoluble swelling amphiphile (lecithin) with a soluble amphiphile of the Type B (sodium cholate) with water is shown Fig. 12 (52). This system, lecithin-bile salt-water, is important biologically because the primary constituents, lecithin

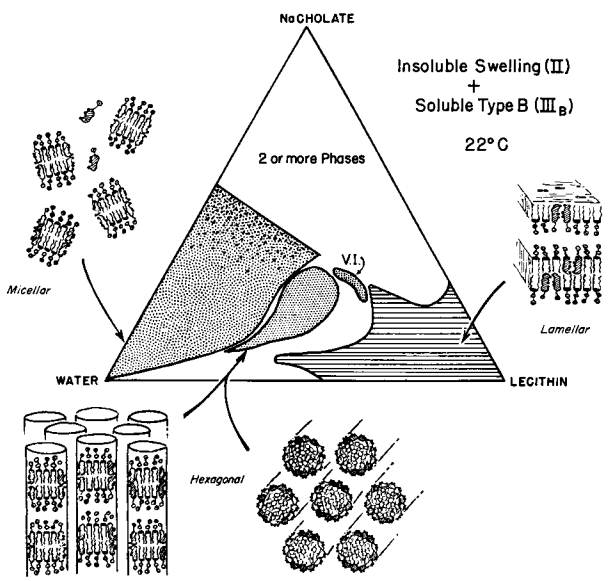


FIG. 12. Lecithin-sodium cholate-water ternary-phase diagram (Class II-Class IIIB-water). The zones of separation of two or more phases have been left out to simplify the diagram. It will be noted that the hexagonal and micellar zones are large. Bile salt (Class IIIB) in contrast to lysolecithin (Class IIIA) is an efficient solubilizer of Class II lipids (lecithin). Only one molecule of bile salt is necessary to solubilize two molecules of lecithin (contrast Figures 9 and 11). This may be because of the arrangement of the molecules in these micelles (inset on left).

The probable arrangement of the molecules in the lamellar phase, the hexagonal phase, and the micellar phase is shown. Both the cross-section of the cylinders and a longitudinal view of the cylinders of the hexagonal phase are represented in the insets at the bottom of the figure. In the lamellar phase the molecules of bile salt form pairs or perhaps tetramers, which are probably hydrogen-bonded through their hydroxyl groups, thus exposing only their hydrophobic backs to the hydrophobic aliphatic chains of the lecithin molecules. The structure of the cubic phase (VI) has not been shown (52,56,58,61).

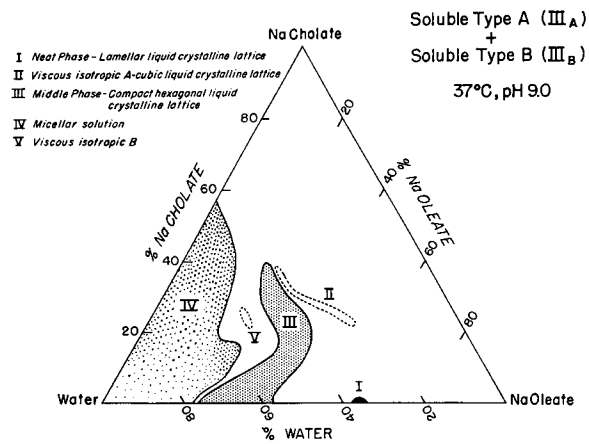


FIG. 13. Sodium oleate-sodium cholate-water ternary-phase diagram (Class IIIA-Class IIIB-water). The various phases have been numbered I-V. The lamellar liquid crystalline phase can incorporate little bile salt into its lattice. The micellar phase is large and includes all possible combinations of sodium oleate and sodium cholate, provided the amount of water is sufficient. The structure of these micelles is not yet known.

and bile salt, are the solubilizing system of bile for insoluble nonswelling amphiphiles, in particular, free cholesterol which occurs in bile in high concentrations. The phases found in this diagram have been studied by a number of techniques including x-ray diffraction (56), light scattering and equilibrium ultracentrifugation (57), nuclear magnetic resonance (58), viscosity (59,81), surface balance (59,81), surface tension (81), and picnometry (81). Sodium cholate forms small micelles in water (Fig. 6) whereas lecithin swells in water to form a lamellar liquid crystalline phase (Fig. 4) (55). Small quantities of bile salt can be incorporated in the lamellar liquid crystalline phase formed by lecithin. The bile salts are probably present as ion pairs, trimers, or tetramers, hiding their hydroxyl groups (by intermolecular hydrogen bonding between the OH groups) from the surrounding lipophilic parts of the lecithin molecules in the manner noted in Fig. 12.

This is strongly suggested by the fact that the x-ray diffraction analysis of lecithin-bile salt mixtures at 25% water shows a decrease in the thickness of the bilayer as bile salt is added, suggesting that the bile salt is, in fact, collapsing the lecithin bilayer slightly (56). That hydrogen bonding can occur between molecules of this type in organic solvents has been recently shown by Bennet, Eglinton, and Kovac (91). In the area of the diagram containing fairly large amounts of bile salt but not much water, large amounts of bile salt can be incorporated into the lamellar liquid crystalline phase without becoming a separate phase. This is probably owing to the fact that there is not enough water present to penetrate between the OH-bonded bile salts.

Two other interesting liquid crystalline phases are formed by these systems. The cubic phase, the structure of which is not known, is probably made up of mixed micelles packed in a face-centered cubic lattice (56). The hexagonal phase, which covers a wide range of compositions, gives the typical "middle soap" (30) anisotropy by polarizing microscope (52) and the spacings of a two-dimensional compact hexagonal lattice. Exhaustive x-ray data on more than 80 mixtures in this phase have been carried out (56), and these data, coupled with studies with Stuart-Breigleb molecular models, observations of mixed monolayers

of lecithin and bile acid (81), and nuclear magnetic resonance studies (58) suggest that the structure of this middle phase differs from that of soaps and other detergents (Fig. 5). The probable structure is that of cylinders of lecithin, coated with bile salt molecules which are separated by water and packed in a compact hexagonal array (Fig. 12 for both longitudinal and cross-sectional views).

This type of cylinder differs in structure from that of soaps in that the cylinders are chopped up into short discs and contain some water within the cylinder itself. As one adds more water to these mixtures, the cylinders break off into discs which are surrounded by bile salt molecules to form peculiar mixed micelles. The light-scattering and ultracentrifugation studies show that the micellar size decreases as the amount of bile salt increases (59,78,81). Further, x-ray analysis shows that the diameter of the cylinders in the hexagonal phase decreases in a like fashion (56). With the addition of more bile salt, it is possible that two species of micelles are present: one of pure bile salt and the other of lecithin and bile salt with a fixed ratio of roughly 50% bile salt and 50% lecithin by weight.

Bile salts are commonly used to solubilize membranes and to clarify phospholipid solutions. The mechanism whereby bile salts solubilize membranes is possibly by simple penetration and formation of mixed micelles with the phospholipids of the membrane. In the mixed micelle, the bimolecular leaflet structure of the phospholipid has not really been altered by the bile salt, it has simply been chopped up into small discs. If one dialyzes a solution of these mixed micelles against a buffer, one finds that the bile salt dialyzes out and, in time, the phospholipids reaggregate to form larger lamellar structures (81).

#### IIIA-IIIB-Water

Fig. 13 is the final in the series of triangular diagrams and represents the last possible combination, that of the two soluble amphiphiles, Type A and Type B. The example given is the ternary-system, sodium cholate-sodium oleate-water (82). Sodium oleate, with increasing hydration, forms a small lamellar liquid crystalline phase (Fig. 13, Zone I), then a hexagonal phase (Zone III), and finally a micellar phase (Zone IV) whereas sodium cholate simply forms a micellar phase. As sodium cholate is added to sodium oleate, the lamellar phase (Zone I) disappears abruptly, showing that little bile salt can be incorporated into the lamellar liquid crystalline phase formed by sodium oleate.

At low concentrations of water, a viscous isotropic phase which has a face-centered cubic lattice by x-ray is present (Zone II). There is a large zone of hexagonal liquid crystalline phase (Zone III), which can contain up to 65% sodium cholate by weight. Although this phase has been studied by x-ray diffraction, the detailed molecular arrangement in the hexagonal lattice has not yet been established. Another small zone of viscous isotropic liquid crystal (Zone V) is present between the middle and micellar zones. Its liquid crystalline lattice has yet to be determined. When large proportions of water are present, a micellar phase (Zone IV) is formed, which is probably made up of mixed micelles of sodium cholate and sodium oleate. The characteristics of these micelles are presently under study. All proportions of sodium cholate and sodium oleate form a micellar solution when an excess of water is present.

These examples of the interaction of lipids of two classes with water, represented as triangular-phase diagrams, have expressed most of the possible combinations of major classes of lipids: insoluble nonswelling (I) with insoluble nonswelling (I), insoluble nonswelling (I) with insoluble swelling (II), insoluble nonswelling (I) with soluble (IIIA or IIIB), insoluble swelling (II) with soluble (IIIA or IIIB) and soluble (IIIA) with soluble (IIIB) amphiphiles. It is important to stress that insoluble nonswelling amphiphiles can be of several types, either a ring structure such as cholesterol, a long-chain structure such as alcohol or protonated fatty acid, a combination of aromatic and aliphatic structures (e.g., sterol esters of long-chain fatty acids), or a multichain structure such as triglyceride. There are some differences in the three-component phase diagrams obtained with each of these types of nonswelling insoluble amphiphiles.

### Interactions Between Classes (Quaternary)

To study the interactions of three lipids in an aqueous system it is necessary to construct a regular tetrahedron. Each corner represents 100% of a given substance. Each edge represents a binary mixture of the compounds at the ends of each edge. The four faces of the tetrahedron (all equilateral triangles) represent three-component phase diagrams of the components at each corner. The mixture of the three components in water (i.e., four-component systems) all fall within the interior of the tetrahedron.

Fig. 14 represents the quaternary systems cholesterol-lecithin-sodium cholate-water (I-II-IIIB-water) (54,56,59). The ternary-phase diagrams (cholesterol-lecithin-water, cholesterol-sodium cholate-water, and lecithin-sodium cholate-water), described earlier (Fig. 8,10,12), represent three of the faces of the tetrahedron. The fourth face, cholesterol-lecithin-sodium cholate, consists of only solids and has not been studied completely. Because it is difficult to represent a three-dimensional figure in two dimensions,

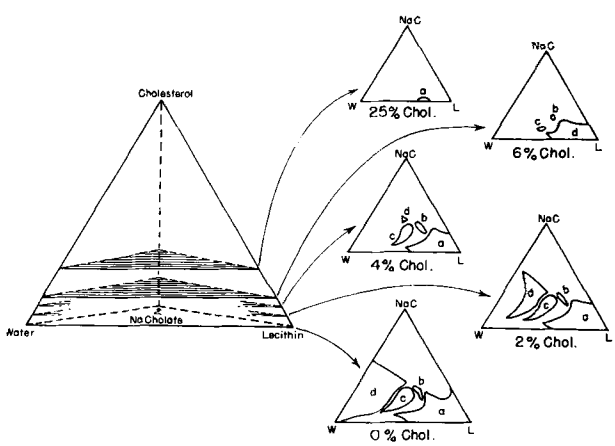


FIG. 14. The quaternary system cholesterol-lecithin-sodium cholate-water (Class I-Class II-Class IIIB-water). The tetrahedron on the left is a representation of this four-component system. The five triangles of decreasing size at the right of the diagram schematically represent cuts taken parallel to the base of the tetrahedron (lecithin-sodium cholate-water), to which a given amount of cholesterol at different over-all cholesterol concentrations (2%, 4%, 6%, 25%) has been added. Chol, cholesterol; L, lecithin; NaC, sodium cholate; W, water. Also a, lamellar liquid crystalline phase; b, cubic liquid crystalline phase; c, hexagonal liquid crystalline phase; d, micellar phase (54, 59).

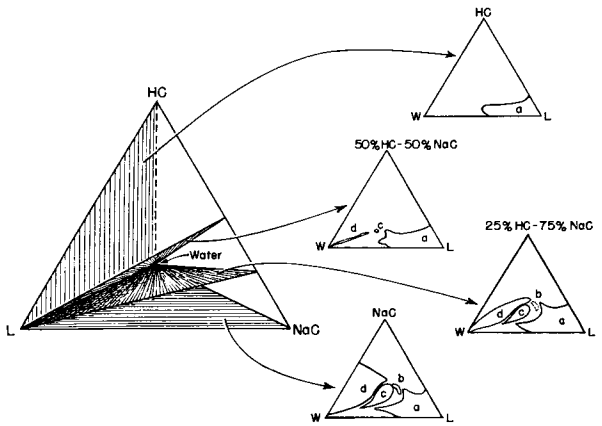


FIG. 15. The quaternary system cholic acid-lecithin-sodium cholate-water (Class I-Class II-Class IIIB-water). The tetrahedron at the left represents this quaternary system. HC, cholic acid; L, lecithin; NaC, sodium cholate; W, water. Also a, lamellar liquid crystalline phase; b, cubic liquid crystalline phase; c, hexagonal liquid crystalline phase; d, micellar phase. The shaded areas represent four sections of the quaternary system, taken at different proportions of cholic acid and sodium cholate. These sections are, from top to bottom: cholic acid-lecithin-water; 50% cholic acid; 50% sodium cholate-lecithin-water; 25% cholic acid, 75% sodium cholate-lecithin-water; and sodium cholate-lecithin-water.

mixtures containing all four components have been represented as sections or cuts through the tetrahedron, parallel to the base (lecithin-sodium cholate-water), to which a fixed amount of cholesterol has been added.

The resultant triangular sections are illustrated by representative cuts on the right side of Fig. 14. Obviously, as the amount of cholesterol is increased, each phase becomes progressively saturated with cholesterol. Thus the micellar phase becomes saturated at 4% cholesterol, and the hexagonal and cubic phases at 6% and 7% cholesterol respectively. The lamellar phase however can contain up to and beyond 25% cholesterol. It would appear that the cholesterol and the bile salt compete for places in the lamellar liquid crystalline lattice.

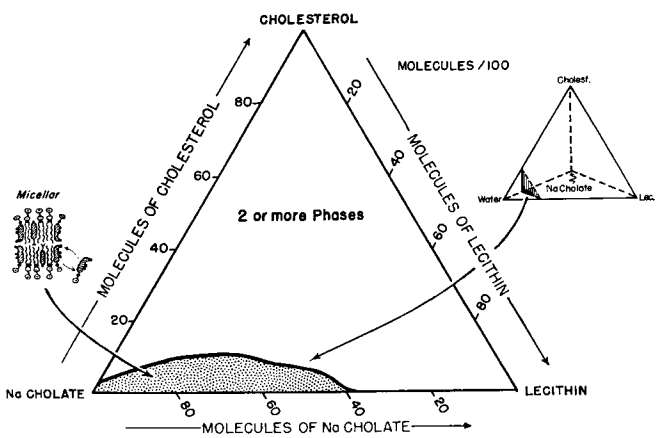


FIG. 16. The quaternary system cholesterol-lecithin-sodium cholate-water (Class I-Class II-Class IIIB-water). The small tetrahedron at right is the same as that shown in Figure 14 except that a cut taken at 90% water, parallel to the side cholesterol-lecithin-sodium cholate, is shown (expressed in moles%). The large triangle at the left is an expanded picture of that cut. Two zones are apparent, a micellar zone at the bottom left-hand part of the diagram made up of mixed micelles of lecithin, cholesterol, and sodium cholate; and the rest of the triangle, which is made up of mixtures that separate into two or more different phases (61,76,94).

Fig. 15 (left side) is a regular tetrahedron that represents the cholic acid-lecithin-sodium cholate-water systems (I-II-IIIB-water). Representative cuts have been taken through the tetrahedron and are illustrated by the sections in Fig. 15. The only difference in each of the cuts is the proportion of free cholic acid to sodium cholate. Thus these cuts are, from bottom to top: the base diagram lecithin-sodium cholate-water (Fig. 12), the diagram taken at a cut with 25% cholic and 75% sodium cholate, the diagram taken at a cut with 50% cholic acid and 50% sodium cholate, and the ternary-phase diagram cholic acid-lecithin-water. These diagrams demonstrate that, as cholic acid replaces sodium cholate, the micellar phase d, the hexagonal phase c, and the cubic phase b all decrease and disappear at about 50% sodium cholate, 50% cholic acid. However the lamellar liquid crystalline phase a can incorporate up to about 15% cholic acid by weight in its lattice. The structure of this lamellar liquid crystalline phase, as suggested by x-ray diffraction, is similar to that shown for the lamellar liquid crystalline phase formed by lecithin and bile salt (Fig. 12). However in the present case, dimers, trimers, and tetramers of cholic acid are interdigitated between the lecithin molecules.

## Pathophysiological Correlation

The major organic components of bile are conjugated bile salt, the phospholipid lecithin, and free cholesterol (92,93). These components make up about 85–90% of the dry weight of bile. They are representative of Lipid Classes I, II, and IIIB. Bile from normal human beings or animals is a clear one-phase micellar solution whereas in people with cholesterol gallstones the bile is obviously a two-phase system, the liquid bile and the cholesterol stones. By assuming that the physical state of bile could be predicted by the quaternary-phase diagram cholesterol-lecithin-sodium cholate-water (Fig. 14), a section at 90% water (the average water content of human gallbladder bile) was made in the tetrahedron representing this quaternary system (Fig. 16). On examination of this section, two distinct zones were encountered, a small one-phase micellar zone at the lower left-hand part of the diagram and the rest of the diagram, which consists of a micellar solution in equilibrium with other phases (e.g., cholesterol crystals or liquid crystals of varying composition). To correlate this in-vitro four-component system with the physical state and composition of human bile, gallbladder bile from 66 patients with cholesterol gallstones and from 25 normal subjects obtained at operation was assayed for total phospholipid, total bile salt and total cholesterol; the results were expressed as percentage of the total of these three components (94). These data were plotted on triangular coordinate similar to that shown in Fig. 16.

Fig. 17 shows the results of these studies. The composition of bile samples in all of the normal subjects fell in the micellar zone. In other words, their biles are less than saturated with cholesterol. In contrast, the composition of the biles from the patients with cholesterol gallstones fell either close to the line separating the two zones or clearly in the zone where cholesterol crystals would be found in equilibrium with the micellar solution. This fact proves that the physical state of bile is indeed predicted from the quaternary-phase diagram cholesterol-lecithin-bile salt-

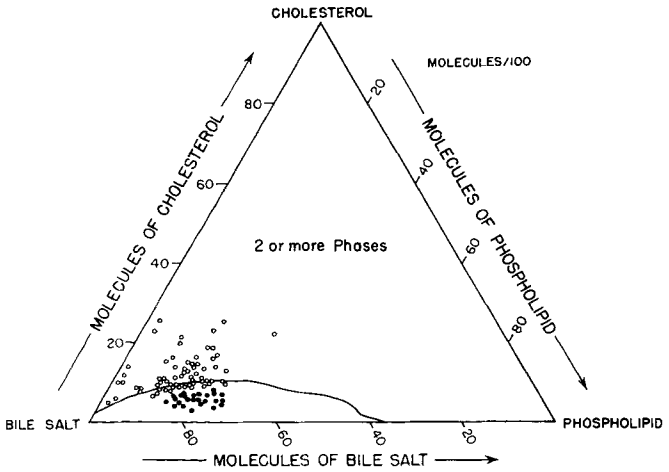


FIG. 17. Pathophysiological correlation of bile with the four-component system cholesterol-phospholipid (lecithin)-bile salt-water. Closed circles represent the composition of biles (moles %) taken from normal subjects, and open circles represent the composition of biles (moles %) taken from patients with cholesterol gallstones. A clear separation of these two populations of biles is evident (94).

water and that other components (inorganic salts, bile pigments, proteins, etc.) do not influence the solubility characteristics of cholesterol in bile.

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## Discussion

DR. SHIMON GATT (Hebrew University, Jerusalem): Would you like to comment on the system in which cholic acid or sodium cholate would be used on an intact membrane? We found that several enzymes from brain which we have been studying could not be extracted with aqueous media. We isolated the intact membrane which had the protein on it, and when we treated it with sodium cholate we extracted and obtained what we thought was a soluble enzyme. What kind of system is this?

DR. SMALL: This morning I have been talking about lipid-lipid and lipid-water interactions and have not discussed lipid-protein interactions. Proteins, and often enzymes, are an integral part of many biological membranes. If I may speculate, concerning your question, bile salts probably penetrate the biological membrane and form micelles with the lipids of the membrane. These micelles would be similar to those discussed in my paper for the lecithin-bile salt-water or the cholesterol-lecithin-bile salt-water systems (refer to Fig. 12 and 14). I cannot say whether the protein, under these conditions, remains attached to the micelles, is solubilized itself by the bile salts or is itself soluble. Nuclear magnetic resonance studies or erythrocyte membranes [D. Chapman, V. Kamat, J. deGier and S. Penkett, Nature *213,* 74 (1967)] suggest that the hydrocarbon chain parts of the phopholipids are normally buried in the membrane, but become free when the membrane is treated with the bile salt sodium deoxycholate. These studies, however, do not tell us what happens to the membrane protein in the presence of bile salt. A definitive answer to your question must await further studies on lipid-protein interactions and the effect of bile salts on these interactions.

DR. BALWANT S. AHLUWALIA (Worcester Foundation, Shrewsbury, Mass.): Using isolated liver mitochondria, it appears that calcium stimulates their rate of swelling. I would like to ask for your comments regarding this effect on the swelling mechanism. Another question, mitochondrial swelling is observed in essential fatty acid deficiency, I wonder if calcium and essential fatty acid deficiency bring about the swelling in the same manner?

DR. SMALL: Concerning the action of calcium, Leathes (23) in 1925 reported that the growth of myelin figures by crude egg lecithin in water was inhibited by calcium. More recently, Shah and Schulman (21) have demonstrated in monolayers of lecithins that when the fatty acid chains of the lecithins are saturated or partly unsaturated, calcium binds to the phosphate groups, probably giving a net positive charge to the monolayer. Calcium not only binds to cardiolipin films but also contracts the films. A net negative charge on artificial membranes (suspensions of lamellar liquid crystals) have been shown by Bangham (45) to increase the swelling in aqueous systems. Although calcium had little effect on the swelling of phosphatidyl choline liquid crystal spherules, it has been shown to cause considerable swelling in liquid crystal spherules formed by phosphatidyl serine or mixture of phosphatidyl serine and phosphatidyl choline [D. Papahadjopoulos and A. Bangham, Biophys. Acta *126,* 185 (1966)]. Calcium apparently increases the permeability of these liquid crystals to both cations and water. Whether the increased swelling with calcium of these liquid crystals is related to the swelling of mitochondria is not known. Beef heart mitochondria contain considerable phosphatidyl ethanolamine as well as phosphatidyl choline, cardiolipin and phosphatidyl inositol [S. Fleisher, H. Klouwen and G. Brierly, J. Biol. Chem. *236,* 29–36 (1961)]. Phosphatidyl serine is not present in large quantities in beef heart mitochondria, therefore, this mechanism may not pertain.

DR. H. E. CARTER (University of Illinois, Chemistry Department, Urbana, Ill.): In connection with this comment I was interested that you classified phosphatidyl inositol in Group II as an insoluble, nonswelling lipid. I would guess that the calcium salt of phosphatidyl inositol falls in this category but I doubt very much that either the sodium or the potassium salt would. More attention needs to be given to the effects of the counter ion on the solubility distribution coefficients and chromatographic behavior of acidic phospholipids.

DR. HERBERT L. DAVIS (University of Nebraska, College of Medicine, Omaha, Nebrsaka): I should like to ask two questions. Is it sufficient merely to say "lecithin?" Would it not make considerable difference if there are one or two double bonds in the fatty acids involved? In the first question I had in mind that double bonds seem to have high affinity for water—sodium oleate is much more dispersible than sodium stearate. One would then expect the HLB values to be shifted toward hydrophilic by double bonds. My other question refers to the term amphipath. We are essentially dealing with surfactant molecules, containing a hydrophobic R group and a hydrophilic group such as hydroxyl, carboxyl, phosphate. My question is, have we any measure of the hydrophilic character of the polar groups? In other words, how big a balloon (the non-polar R group) does it take to pull the hydroxyl ion clear out of the water—to make it insoluble? Perhaps the second question could be answered by considering the solubility of the homologous series of alcohols—by octyl alcohol ($C_8H_{15}OH$) solubility in water has dropped to very low value.

DR. SMALL: To answer the first question, unsaturation does make a difference. It does not greatly alter the basic phase diagram (Fig. 4) for lecithin, but does lower or raise the transformation from crystalline to liquid crystalline. For instance, in water distearyl lecithin will not form a liquid crystalline phase at room temperature. However, at 70C the hydrocarbon parts of the molecules melt and the crystalline phase transformation takes place. On the other hand, dilinoleyl lecithin will form liquid crystals at 0C in water. The temperature of the crystalline-liquid crystalline transformation of lecithins having less double bonds than dilinoleyl lecithin are intermediate between these two examples.

Regarding the second part of the question, I have used the term aphiphile simply to suggest that one end or one part of the molecule is hydrophilic and the other part of the molecule is hydrophobic. Concerning the relative strength of the hydrophilic groups, there have been attempts to define this balance, particularly in the field of emulsions by the use of hydrophile lipophile balance (HLB) numbers [Griffin, W., J. Soc. Cosmetic Chem. *1,* 311 (1949) and Davies, J. T., Proceedings of the International Congress on Surface Activity, 2nd, London, *1,* 426 (1957)]. Al-

though some equations have been suggested to calculate HLB numbers from the chemical formula of the compound, these have not been altogether satisfactory and the experimental methods for determining HLB numbers are laborious. This has not been attempted in the present paper.

DR. DENIS A. HAYDON (University of Cambridge, Department of Colloid Science, Cambridge, England): I have a question arising from what Dr. Small has just said and from some work that was recently presented by Dr. Parsegian. This concerns the addition of water to egg yolk lecithin, and the examination by X-ray diffraction techniques of the swollen liquid crystals. If the hydrocarbon density is assumed constant, then the thickness of the hydrocarbon region of the lecithin lamellas seems to decrease as the water content increases. The first part of my question is, how reasonable is it to assume the hydrocarbon density to be constant? The second part of the question is to ask whether there is any comparable analysis of data for the addition of water to lecithin and cholesterol.

DR. SMALL: We have calculated the density of myelin figures of egg yolk lecithin at 25C. The value found was 1.013 g/cc. This corresponds well with the figure 1.015–1.016 given by Elworthy (J. Chem. Soc. 1951, 1959) for ultrasonically irradiated aqueous preparations of egg lecithin. The density of dry egg lecithin is about 1.04 g/cc. However, at 25C dry egg lecithin is partly crystalline and remains so until about 12% water (55). Since the major change in density of hydrocarbons occurs with melting, it would seem reasonable to assume that the higher density of the dry lecithin was due to its partly crystalline state. Although we have not specifically measured the density of the lecithin in different states of hydration, I doubt that it would vary significantly in the liquid crystalline state.

In reference to the density of the lecithin-cholesterol systems, the density of lecithin and cholesterol myelin figures increase from 1.013 g/cc for pure lecithin to 1.056 for myelin figures containing 30% cholesterol and 70% lecithin. Mixtures having compositions between pure lecithin and the 30–70 cholesterol-lecithin mixture have densities in between these two values.

DR. GATT: Have you tried replacing the sodium cholate with a cationic detergent, such as Cetavlon: would it make any difference? The reason I'm asking this, is that in the enzymatic studies which we conducted, we found that one type of detergent (i.e. cholate) will activate while some other type (i.e. Cetavlon) will completely inhibit. The question is whether this is due to different dispersion of the substrate, or to the charge imparted by the detergent.

DR. SMALL: We haven't tried that. I don't know whether I can answer the question until I have tried it.

DR. H. T. TIEN (Michigan State University, East Lansing, Michigan): Do you believe that the phosphatidyl choline groups are sticking straight out into the water, or do you think that they are lying flat parallel to the bilayers?

DR. SMALL: According to Parsegian (68) the phosphate group and the quaternary amine act somewhat like the carboxyl and cation of a soap. That is, the positively charged quaternary amine behaves almost as a diffusable ion, limited only by the 5 Å distance between the phosphate and the center of the quaternary amine. Therefore, in the liquid crystal state the quaternary amine group may sometimes be parallel, sometimes be perpendicular and sometimes be someplace in between.