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THE BEHAVIOR OF BIOLOGICAL LIPIDS

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Abstract - Lipids active in biological systems demonstrate a broad range of behavior in water, from hydrocarbons which are insoluble, to molecules such as bile salts that possess potent detergent properties and interact with water rather dynamically. The purpose of this paper is to describe some of the physical properties of lipids with respect to their interaction with aqueous systems, and to classify lipids based on these interactions. The hydrocarbon part of a lipid molecule may be aliphatic or cyclic/aromatic. In predominantly aliphatic lipids, the hydrocarbon part consists of a chain(s), containing eight or more carbons. All such lipids have specific properties related to the hydrocarbon chain packing, while cyclic/aromatic lipids possess unique properties related to the specific hydrocarbon structure. Three basic types of aliphatic chain packing can be found in most predominantly aliphatic chain molecules: 1) a tightly packed aliphatic chain lattice with specific chain-chain interactions and minimum specific volume, 2) an intermediate form of crystalline packing in which specific chain-chain interactions are lost, but the chains are packed in a centered hexagonal lattice, 3) a liquid state in which the $-CH_2-$ groups can move more or less freely. Transitions between these states are characterized by abrupt changes in volume and excess specific heat (enthalpy). The partial specific volume of the $-CH_2-$ group in the aliphatic chain increases from $\sim 23 Å^3/-CH_2-$ in tightly packed chains to $\sim 26 Å^3/-CH_2-$ in hexagonal packing to $\sim 29 Å^3/-CH_2-$ in the liquid state. The enthalpies of the transition are: ~ 1 kcal/$-CH_2-$ from the crystalline lattice (specific chain-chain interaction) to liquid and ~ 0.5 kcal/$-CH_2-$ from the hexagonal chain packing lattice to liquid. Thus, a change in volume of $1 Å^3/-CH_2-$ requires about 0.17 kcal.

The transition from crystalline to liquid chain can occur from either tightly packed or more loosely packed crystalline structures. These transitions are known by a variety of names depending upon the lipid system. For instance, it is called the melting point in alkanes, fatty acids, fatty alcohols, di- and triglycerides, waxes, etc.; the order-disorder transition or gel-liquid crystal transition in soaps, monoglycerides, phospholipids, etc.; the critical micellar temperature or Krafft point in soaps and detergents. This transition temperature is governed by the length of the hydrocarbon chain and the presence of double bonds, cyclic structures, or branches within the chain. The hydrophilic part of the lipid also plays a major part in the chain transition temperatures, as well as in the interaction with aqueous systems. The crystal-liquid chain transition for a given chain length increases in the following order: alkenes < chlorides < alkanes < bromides < aldehydes < alcohols < fatty acids < triglycerides < monoglycerides < Na soaps < phosphatidylcholines < phosphatidylethanolamines < Ca soaps.

Lipids in which the aliphatic chain is in the fluid state, may be classified empirically as nonpolar and polar based on their interaction at the air-water interface and in bulk systems. Nonpolar molecules are insoluble in aqueous systems and do not spread at the air-water interface. Nonpolar molecules include hydrocarbons, waxes, and sterol esters. Polar molecules may be divided into three distinct classes: I. Insoluble Non-swelling Amphiphiles. These molecules spread at the air-water interface to form a stable monolayer but are insoluble in the bulk. This class includes long-chain fatty acids, primary amines, alcohols, cholesterol, di- and triglycerides. II. Insoluble Swelling Amphiphiles. These molecules form stable monolayers, but swell in water to form liquid crystalline

phases. Membrane lipids, such as phospholipids and cerebrosides as well as monoglycerides and acid-soaps, fall in this class. III. Soluble Amphiphilic Molecules. These molecules form unstable monolayers, have some bulk solubility as monomers and form micelles at a critical micellar concentration. Aliphatic molecules of this type swell at low water concentration to form liquid crystals, whereas cyclic/aromatic molecules usually form crystals which dissolve directly to form micelles without first swelling.

INTRODUCTION

Hydrocarbons and bile salts are examples of biological lipids that possess extremely different physical properties. Hydrocarbons are water insoluble, do not spread at air-water interface, and are soluble in organic solvents. Bile salts are water soluble, spread actively at air-water interface, and are insoluble in organic solvents. The purpose of this paper is to describe some of the physical properties of biological lipids interacting with aqueous systems and to classify lipids based on these interactions.

THE PHYSICAL STATES OF LIPIDS

Lipids are medium sized molecules of molecular weight between 150 and 3000 that contain a substantial hydrocarbon moiety. Many lipids also contain a water soluble moiety giving the moleules an amphiphilic character; that is, one part of the molecule is hydrocarbon soluble, the other, water soluble. The hydrocarbon portion of the lipid molecule may be aliphatic or cyclic/aromatic. In predominantly aliphatic lipids the hydrocarbon part usually consists of a single chain. However, two chains are present in some phospholipids and sphingolipids and three chains are present in triacylglycerols. By definition the aliphatic chains should contain at least eight or more carbons. Such lipids have specific properties related to the hydrocarbon chain packing, while cyclic/aromatic lipids derive their unique properties from specific hydrocarbon-hydrocarbon interactions. Aliphatic chain packing assumes three basic configurations: 1) a tightly packed aliphatic chain lattice with specific chain-chain interactions and a minimum specific volume, 2) an intermediate crystalline packing of the hydrocarbon chains in which specific chain-chain interactions are lost, but the chains are packed approximately in a centered hexagonal lattice and, 3) a liquid state in which there is no lattice and the $-CH_2-$ groups move about more or less freely. The characteristics of these configurations are given in Table 1 along with some of their commonly used names.

TABLE 1 Characteristics of Chain Packing

Aliphatic Chain Interaction	Subcell Lattice (a)	Vol/ $-CH_2-$	Surface Area/$-CH_2-$	Motion	Common Names (b)
Specific	Orthorhombic perpendicular Orthorhombic parallel Monoclinic parallel Triclinic parallel Hybrid cells	23-24Å^3	18-19Å^2	Very Restricted	Alkanes = Orthorhombic perpendicular, triclinic, monoclinic, β', γ Acids and Amides = A, B, and C forms Mono, di, and tri-acylglyderols = β and β' forms
Nonspecific	Hexagonal or near hexagonal	~ 26Å^3	~20.5Å^2	Restricted	Alkanes = Rotator phase α Acids, Alcohols, Glycerides = α phase Phospholipids = gel phase, ordered phase, hexagonal phase, L_β (2) Soaps = gel phase

TABLE 1 Characteristics of Chain Packing (Continued)

Liquid	No lattice, but domains of roughly // chains	29 - 30Å^3	23Å^2	Fluid	Alkanes, Acids, Alcohols, di- and triacylglycerols = melt, neat liquid, isotropic liquid phase Monoacylglycerols and Phospholipids = Liquid crystal phase (lamellar or L_α , cubic, hexagonal I and Hexagonal II, etc.) (2) Soap = neat, viscous isotropic, or middle phase; liquid phases Cholesterol esters = liquid crystal, fluid crystal or mesophase (smectic A, nematic, cholesteric), ordered (as opposed to isotropic liquid)

Note a. For a good review of simple and hybrid subcells, see Ref. 1. Hybrid subcells often occur in complex lipids.

Note b. Nomenclature for different states of lipids is complicated and not consistent between lipid classes. This table should be helpful in orientation since many aliphatic molecules undergo transitions from various crystalline states to more liquid-like states. For further discussion of specific states see Refs. 1-4.

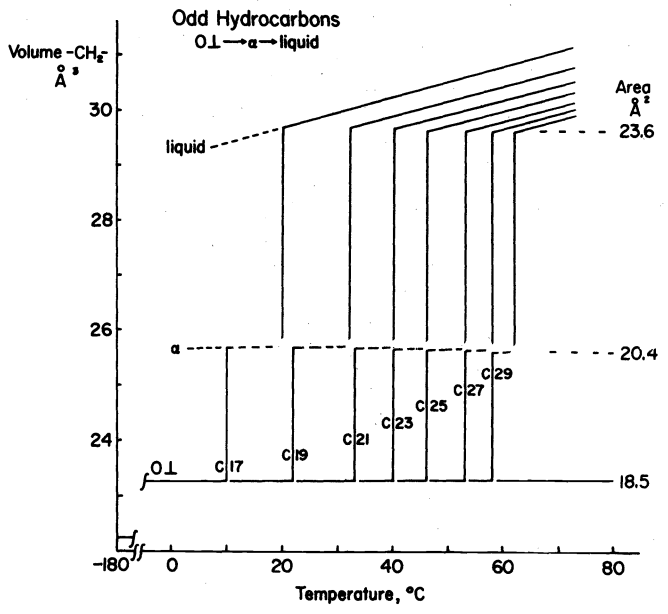


Fig. 1. The volume of the $-CH_2-$ group vs. the temperature for a homologous series of odd carbon numbered hydrocarbons. Orthorhombic perpendicular = O ; alpha = a hexagonal "rotator" phase. Each hydrocarbon undergoes two transitions; one from orthorhombic perpendicular to rotator phase (alpha), and one from alpha to a liquid. The coefficient of expansion of the orthorhombic perpendicular phase is very small, however, that of the alpha phase is appreciable (not shown). The volume per $-CH_2-$ group in the orthorhombic perpendicular phase before the transition to alpha is approximately 23.6Å^3 for each of the hydrocarbons and the minimum area in the alpha phase is approximately 25.8Å^3. In all cases the volume per $-CH_2-$ group in the liquid state at the melting point is approximately 29.4Å^3. Corresponding surface areas per $-CH_2-$ group are given on the right of the diagram.

The simplest molecules to undergo polymorphic transitions between crystals with specific chain-chain interactions and the more loosly packed hexagonal state are normal alkanes as illustrated in Fig. 1 (4).

The odd-numbered hydrocarbons in this homologous series undergo orthorhombic perpendicular to hexagonal transitions and hexagonal to liquid transitions. There are marked and sudden changes in volume for each specific hydrocarbon at a specific transition temperature. Many complex lipids undergo similar transitions and similar abrupt volume changes occur (4). Even hydrated phosphatidyl cholines undergo similar changes at their gel-liquid crystal transition (5).

The changes in excess specific heat (enthalpy) occurring at such transitions are given for a variety of different lipids in Fig. 2. A plot of H versus the number of carbons in the hydrocrbon chain gives a slope which is equivalent to the H/-CH_2- group. The enthalpy of transitions between the three general states of lipid chains outlined in Table 1 are very similar in a wide variety of saturated aliphatic chain lipids (4). When double bonds are present the enthalpy appears to decrease, but the type of chain packing has not been documented in most cases. As a general rule, a change in volume of 1$\AA^3$ per -CH_2- group requires approximately 0.17 kcals.

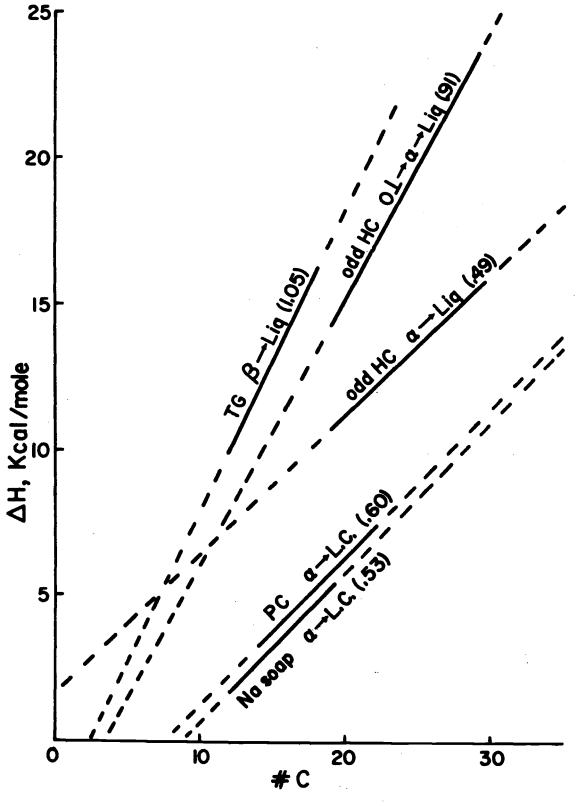


Fig. 2. The enthalpy (ΔH) of chain melting transitions of a variety of lipids plotted against the number of carbons in the aliphatic chain. Triglyceride = triglyceride β to liquid; odd hydrocarbons = orthorhombic perpendicular to liquid; odd hydrocarbons α (rotator phase) to liquid; PC = hydrated phosphatidylcholines, α (L_β) to liquid crystal; and Na soap = sodium soaps, hexagonal to liquid crystal. The enthalpies of the transitions from the tightly packed crystalline forms (β, orthorhombic perpendicular) - liquid are about 1 kcal/-CH_2-, whereas the transitions from the more loosely packed hexagonal (alpha phase) to the liquid are roughly 0.5 Kcal/-CH_2-. The numbers given in parentheses are the enthalpies per -CH_2- group (4).

The hydrophilic portion of the molecule also plays a major part in chain transition temperatures. The transition temperatures of a homologous series of several different aliphatic chain molecules are given in Fig. 3. Those with the strongest polar lattices appear to have the highest melting transitions. Thus, both the polar and hydrocarbon parts of the molecules affect the transition temperatures.

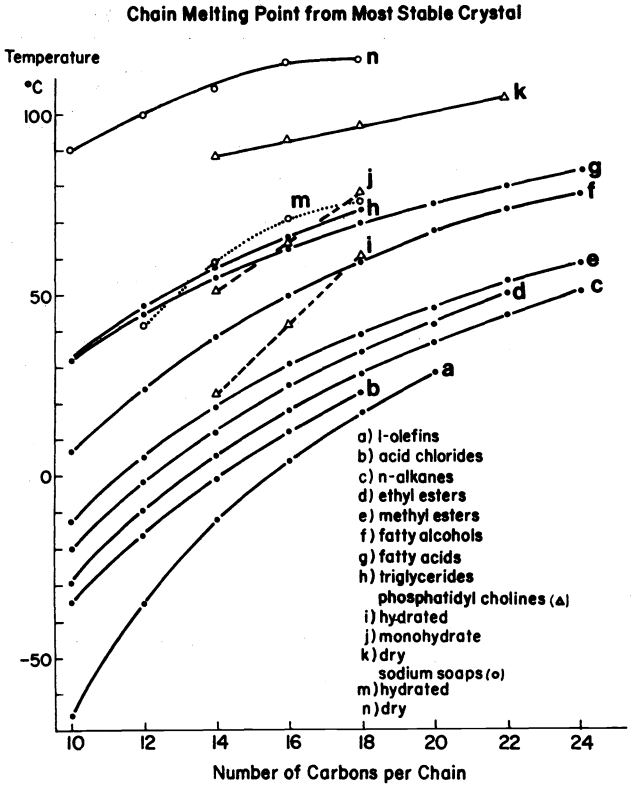


Fig. 3. The major chain melting transition temperatures of a variety of lipids are plotted against the number of carbons in the aliphatic chain. The stronger the Tm interaction of the polar group the higher the melting point. Note that the melting transitions increase with the increasing hydrocarbon chain length, and even in water (see soaps and phospholipids) the transition temperature increases with chain length. However, the presence of water in general lowers the chain transition (4).

A CLASSIFICATION OF LIPIDS BASED ON INTERACTION WITH WATER

The interactions of lipids in aqueous systems can be discussed with respect to their bulk properties (behavior in water) and surface properties (behavior at the air-water interface). In water, some lipids are completely insoluble, while others swell, undergo lyotropic mesomorphism,(that is, form liquid crystals) or dissolve to form micellar solutions. At the interface, lipids may not spread, may spread to form stable or unstable monolayers, or may dissolve. The surface and bulk properties of a given lipid depend upon the relative strength of the hydrophilic and the lipophilic or hydrocarbon portions of the molecule; that is, the hydrophilic-lipophilic balance (HLB). If the hydrophilic part is stronger than the hydrocarbon part, then the molecule tends to be water-soluble. Strong hydrophilic groups include ionized carboxyls (H_2COO^-), sulfates, phosphates, quaternary amines, sugars, etc. Conversely, if the hydrocarbon part is stronger than the hydrophilic part, the HLB is tipped towards the lipophilic side and the molecule tends to be water-insoluble. Small (6,7) has proposed a simple classification which groups molecules according to their behavior at an air-water interface and in bulk aqueous systems (Table 2).

Table 2

Classification of Biologically Active Lipids (note c)

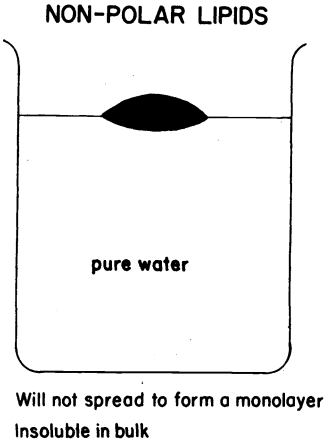
Class	Surface properties at air-water interface	Bulk properties in water	Examples
Nonpolar	Will not spread to form monolayer	Insoluble	Long-chain, saturated or unsaturated, branched or unbranched, aliphatic hydrocarbons with or without aromatic groups, e.g., dodecane, octadecane, hexadecane, paraffin oil, phytane, pristane, carotene, lycopene, gadusene, squalene. Large aromatic hydrocarbons, e.g., cholestane, benzpyrenes, coprostane, benzphenantrocenes. Esters and ethers in which both components are large hydrophobic lipids, e.g., sterol esters of long-chain fatty acids, waxes of long-chain fatty acids and long chained normal monoalcohols, ethers of long chained alcohols, sterol ethers, long chained triethers of glycerol.
Polar Class I insoluble nonswelling amphiphilic lipids	Spread to form stable monolayer	Insoluble or solubility very low	Triglycerides, diglycerides, long chained protonat fatty acids, long chained normal alcohols, long chained normal amines, long chained aldehydes, phytols, retinols, vitamin A, vitamin K, vitamin cholesterol, desmosterol, sitosterol, vitamin D, un-ionized phosphatidic acid, sterol esters of very short chain acids, waxes in which either aci or alcohol moiety is less than 4 carbon atoms lon
Polar Class II insoluble swelling amphiphilic lipids	Spread to form stable monolayer	Insoluble but swells in water to form lyotropic liquid crystals such as lamellar cubic, hexagonal	Phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, sphingomyelin, cardiolipin, plasmalogens, ionized phosphatidic acid, cerebrosides, phosphatidyl serine, monoglycerides, "acid-soaps", alpha hydroxy fatty acids, monoethers of glycerol, mixtures of phospholipids and glycolipids extracted from cell membranes or cellular organelles (glycolipids and plant sulfolipids).
Class IIIA soluble amphiphiles with lyotropic mesomorphism	Spreads but forms unstable monolayer due to solubility in aqueous substrate	Soluble; form micelles above a CMC. At low water concentrations forms liquid crystals.	Sodium and potassium salts of long chained fatty acids, many of the ordinary anionic, cationic, and nonionic detergents, lysolecithin, palmotyl and oleyl coenzyme A and other long chained thioesters of coenzyme A, gangliosides, sulfo cerebrosides.
Class IIIB soluble amphiphiles, no lyotropic mesomorphism	Spreads but forms unstable monolayer due to solubility in aqueous substrate	Forms micelles but not liquid crystals	Conjugated and free bile salts, sulfated bile alcohols, sodium salt of fusidic acid, rosin soaps, saponins, sodium salt of phenanthrene sulfonic acid, penicillins, phenothiazines

Note c. Taken in part from ref. 7

In applying this classification to any specific lipid, one must consider the water concentration and the temperature. Certain molecules may be quite insoluble at low temperature, but on passing to a higher temperature undergo chain melting which allows water to penetrate to the hydrophilic region, thus causing the formation of liquid crystals or micelles. The transition temperature from the tightly packed crystal or even the relatively loosely packed hexagonal chain to the more liquid state determines the physical characteristics of the lipid. Although little data are available, it is possible that acyl chain lipids packed in a crystal with specific chain-chain packing can hydrate appreciably without undergoing chain melting to a less tightly packed lattice. Certainly, lipids with predominantly hexagonal chain packing can hydrate appreciably without undergoing chain melting. A change in tilt or perturbations in the lattice that cause rippling of the basic crystalline bilayer may accompany hydration (2,8,9). The following classification applies only to lipids with fluid chains.

Nonpolar lipids (Fig. 4)
Lipids belonging to this class are insoluble in the bulk and do not spread to form a monolayer on the surface; that is, they have a negative spreading pressure. These molecules either have no polar constituents (pure hydrocarbons) or possess a hydrophilic part so small or so buried in the center of the molecule that it cannot interact with water, thereby preventing the molecule from spreading. This is the case with esters of long chain fatty acids and bulky monohydroxyalcohols (waxes) and sterol esters.

Fig. 4

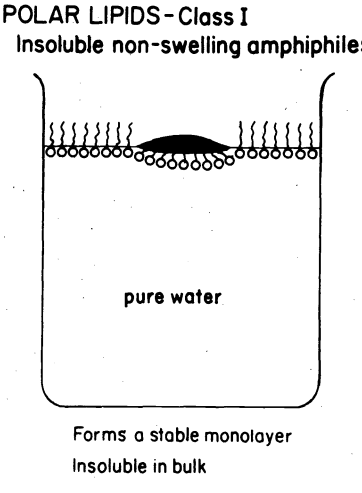


Polar lipids

These lipids are surface soluble and form stable or unstable monolayers.

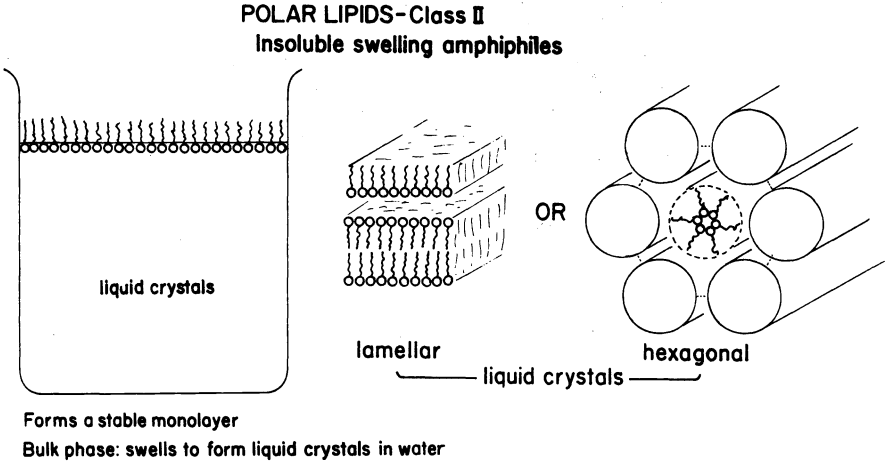
Class I: Insoluble, nonswelling amphiphiles (Fig. 5): Lipids of this class are virtually insoluble in the bulk but will spread at the interface to form a stable monolayer. Thus, they have a positive spreading pressure. Triglycerides, long-chained un-ionized fatty acids, cholesterol, and many fat soluble vitamins (vitamins A, D, E, and K) are members of this class.

Fig. 5



Class II: Insoluble, swelling amphiphiles (Fig. 6): These lipids are virtually insoluble in water. However, they undergo lyotropic mesomorphism to form liquid crystalline phases. In other words, while the lipid is not soluble in water, water is soluble in the hydrophilic part of the lipid, and therefore it swells. Some lipids have a finite capacity to swell, particularly those with smaller polar groups which form an inverted hexagonal phase (e.g. phosphatidylethanolamines). Phosphatidylcholines (8-10), sphingomyelin (11,12), and cerebrosides (13) which form a lamellar phase incorporate up to 45% water by weight into the polar region of their liquid crystal structures. Other lipids, however, appear to swell to a much greater extent. Ionized phosphatidylserine swells to form lamellar liquid crystals which are at least 90% water by weight (14). These lipids form stable, insoluble monolayers and have a positive spreading pressure. The principal lipids in this class are the lipids that constitute biological membranes, such as phospholipids and cerebrosides.

Fig. 6



Class III: Soluble amphiphiles: Because of their bulk solubility, soluble amphiphiles form unstable monolayers at the interface and micelles in aqueous solutions. There are two general types of these compounds. Type IIIA lipids (Fig. 7) exhibit lyotropic mesomorhpism at low water concentration, and form liquid crystals, as do Class II lipids. However, at higher water concentrations, these liquid crystals dissolve to form rod shaped or spherical micelles. Aliphatic molecules, such as Na^+ or K^+ soaps, lysolecithin, and many detergents, are representative of Type IIIA lipids. In Type IIIB lipids (Fig. 8), bulky aromatic ring systems often comprise the hydrophobic component of the molecule. These compounds form micelles, but as a general rule do not form liquid crystals. Molecules typical of this system are bile salts, rosin soaps, and a variety of pharmacological agents.

Fig. 7

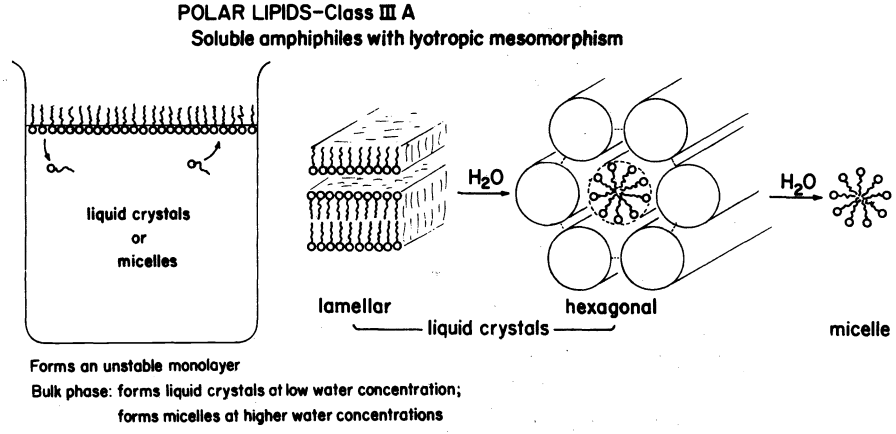
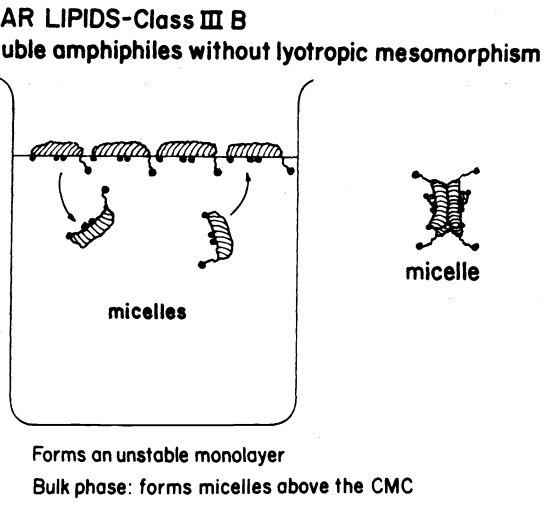


Fig. 8



Interactions between different classes of lipids in aqueous systems may in part be predicted from the classification presented above. Phase equilibrium studies of interactions between different classes have been presented (6). For instance, a Class I lipid such as cholesterol may be solubilized appreciably in a lamellar liquid crystalline phase by phospholipids such as lecithin and sphingomyelin. Cholesterol and Class IIIA lipids combine in roughly equal molar ratios to form a lamellar liquid crystalline phase and the two behave together as a Class II-like lipid. Only when large amounts of Class IIIA detergents are added is cholesterol dissolved into micelles. The usefulness in biology of such phase equilibrium systems has been pointed out (6,7) and presumably processes such as membrane budding and fusion may ultimately be explained by the composition and states of lipids taking part in these processes.

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