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Lyotropic Mesomorphism in Lipid-Water Systems

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Introduction

When a physical chemist looks at living matter, he sees watery bits and fatty or lipid bits and, in spite of the old saying that oil and water don't mix, there seems to be a lot of mixing and peculiar mixing at that; both lipids in water and water in lipids. Nor is this so strange because the model of lipid phase and water phase lying in contact without any interaction would seem to be a singularly useless one for the complex co-ordinated activities which we call life. Nevertheless the triglycerides are such inert lipids, which is a useful property as depot fats,[1] but one which requires pancreatic lipolysis for intestinal absorption; that is, conversion to lipids which do interact with water and aqueous bile salt solutions.

These lipids contain one or more polar groups which are hydrophilic (water-loving) in a large hydrocarbon molecule which is hydrophobic (water-hating); such molecules are described as *amphiphilic* to describe their dual nature, one of the consequences of which is their ability to form lyotropic mesophases. Such amphiphilic lipids are the major components of the brain, the myelin sheath and they are found in mitochondria, blood corpuscles and in the chloroplasts of plants.

This solubility is not the classical partial miscibility of the text books in cases such as phenol in water and water in phenol; the lipids are insoluble in water and the solubility of water in them is a

localized one around the polar groups. Perhaps one should call it hydration though neither name is entirely satisfactory.

Then we have to accept that in an amphiphilic molecule, the two parts, philic and phobic, may be in different physical states; 2 phases in a single molecule takes some swallowing but I don't think that it would have worried Willard Gibbs because they are not *independently* different. One can think of the prisoner with a large steel ball attached to his leg to stop him running but it makes no difference to his freedom to wave his arms about.

We then see that this potential property of a molecule, which it shows in the presence of water, also shows a characteristic pattern of behavior in the anhydrous substances. We also see that the name amphiphilic originally used to describe the make-up and behavior of the water soluble soaps is now widely used to describe that of water-insoluble amphiphiles particularly in respect of oriented monolayers yet the very important property of the solubility of water in these lipids has been almost entirely ignored.

All these amphiphilic lipids are associated by their polar groups and, because their make up of polar group and fatty chain leads to a layer arrangement, crystallize in bi-molecular layers. Penetration of water therefore forms a sandwich unit of structure.

It is most important to realize that temperature is an important variable in these lyotropic mesoforms; the liquid crystalline phases are limited by the upper temperature at which transition to liquid occurs and the lower one at which the anhydrous lipid crystallizes with ejection of the water.

Now, what are these amphiphilic lipids?

Figure 1 shows their formulae stripped to essential hydrophobic chains and polar groups to show both philic-phobic balance as well as the shape of the molecules which determines their packing in the condensed state—liquid, liquid crystalline or solid. Each type is a family of homologues, the phobic chains ranging from 6 C atoms for unionized and 8 upwards for ionized philic groups to show amphiphilic properties to a marked extent and to make them insoluble in water. All of these substances associate by their polar groups to form bi-molecular layers, the layer structure

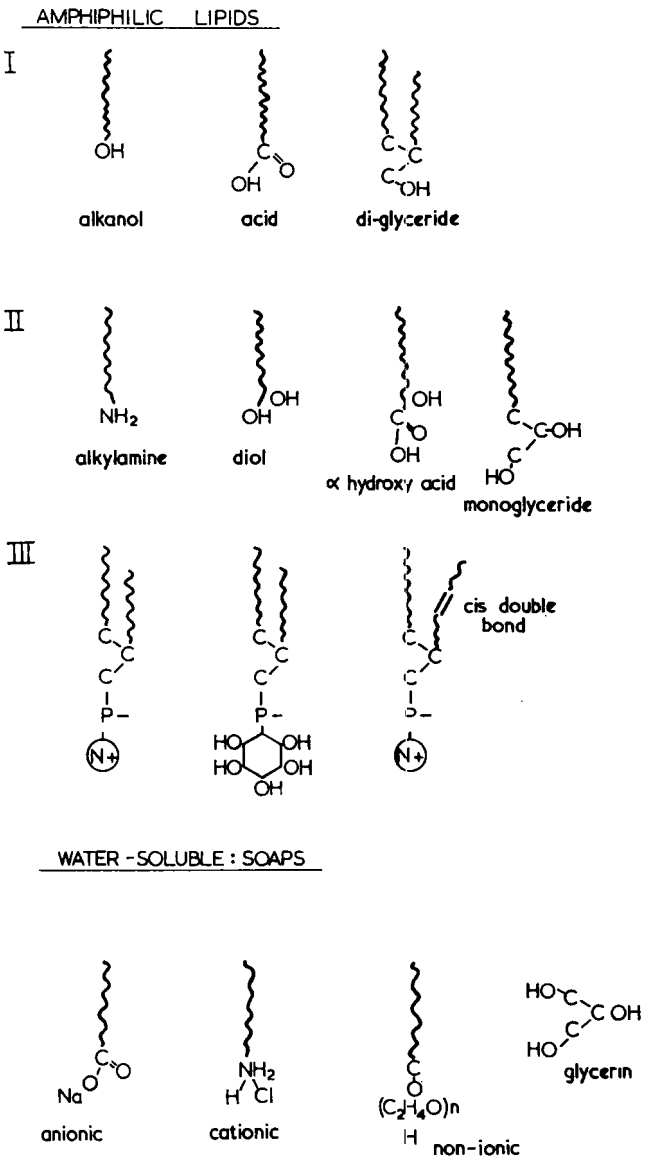


Figure 1. Amphiphilic lipids; skeleton formulae showing shape of molecules and location of hydrophilic groups.

being imposed by the immiscibility of the polar groups with the phobic chains so that the philic groups are planar and this is reinforced by the necessity for the phobic chain also to peak into layer structures.

They fall into three main groups on the basis of their molecular make-up and their interaction with water. Group I consists of the mono-functional unionized fatty acids and alcohols and also includes the important Cholesterol. Group II, also unionized, includes the mono-functional alkylamines, the bifunctional diols such as 1,2 hexadecane diol and all the 1,2 and 1,3 mono-glyceride diols. α hydroxypalmitic acid also belongs here and no doubt its homologues which have not been examined. Group III consists of the ionic lecithins, cerebrosides, sphingomyelins, etc. Figure 1 shows that, if the glycerine molecule is correctly drawn in its zig-zag chain configuration, the geometry of these phosphatides is such that the molecules have the lop-sided tuning fork with a short handle as shown. The two forks are the two fatty acids, not necessarily the same, esterified to the glycerine which is attached to the phosphoric acid with a P^- charge and attached in turn to the " handle " which is always a substance which would, alone, be highly polar and soluble in water. In the lecithins, this is choline giving the lipid its N^+ charge and making it an ampholyte and, alternatively, the choline may be replaced by ethanolamine or by serine, which also has a free COOH group. In the phosphatidyl inositols, the basic group has been replaced by the non-ionic sugar whose hydroxyl groups function as a H bonding powerfully philic moiety.

Group I dissolves up to about 2.5% of water to form either liquid or solid solution of water in lipid; the fatty alcohols reach saturation at the quarter hydrate. Much more water dissolves in the Group II and liquid crystalline phases are formed[2]; with the monoglycerides, up to 50% by volume is dissolved in liquid and *lc* phases but an ionic polar group gives solubility in water and the substances are soaps, not lipids; liquid crystalline phases are however formed by the soaps at higher concentrations in water. Since group III are ionic, two phobic chains are needed

to make the phosphatides insoluble in water as shown by removal of one of them to form water-soluble lysolecithin. Groups I and II have melting points well below 100° but the anhydrous phosphatides have ionic binding and melt around 200° while the soaps are higher still. When, however, water penetrates between the ionic groups the electrostatic binding is, as with classical electrolytes, reduced to a few kT and the thermal behavior now resembles that of the non-ionic types and similar properties are observed below 100 °C. Figure 2 shows the penetration of water diagrammatically; amphiphilic lipids have become almost insoluble in water when the phobic chain has reached a length of 8 carbon atoms; this is the so-called thermodynamic barrier to mixing long hydrocarbon chains with water. No such barrier exists in the case of water in lipid because the water is mixing only with the polar groups in the middle of the bi-molecular layer; penetration of water into this is an intercalation and swelling to form a bi-molecular sandwich with water in the centre. The large faces of flaky aliphatic crystals consist of the terminal methyl groups of the hydrocarbon chains where no penetration occurs; water can enter only around the edges of the thin flakes; this is easily verified by watching the process in the polarizing microscope.[3,4,5]

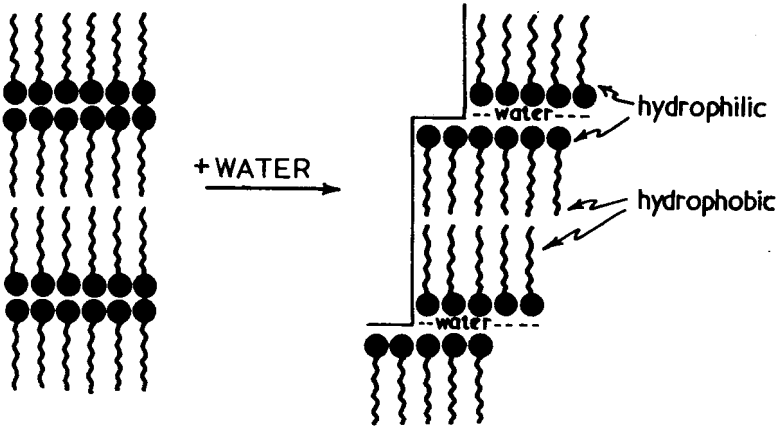


Figure 2. Penetration of water into bi-molecular layer of amphiplilic lipid.

This penetration of water into solid crystals of amphiphilic lipids occurs only at and above a temperature T_{pen} min. characteristic of the substance and it can be observed by heating stage and polarized light microscopy as sharply as a melting point. At first, I visualized the water as loosening-up the lattice but, when it became apparent that the water could also act as a cement, raising melting and transition temperatures, this idea was untenable and it was found that the loosening was thermal.

Thermal Motion and Polymorphism

In 1939 Timmermans drew attention to a large number of molecules, which he described as spheroidal, which had entropies of fusion of less than 5 e.u.[6] These substances are polymorphic so that, on heating, we have:

$$S_1 \rightarrow S_2 \rightarrow L$$

The S_2 thermally disordered form is often named *plastic crystal* because its viscous resistance to flow is considerably less than that of the S_1 low temperature form. Since these molecules approximate to spheres when heated, they pack in the S_2 solid in cubic, usually face-centred, form and at the melting point the chang from thermally disorderly cubic to completely disorderly liquid is unusually small both for ΔH and ΔS. Two special points should be noted; first, that the higher the m.p.t., the more thermal disorder there will be at T_f; e.g. tertiary butanol is a plastic crystalline solid at room temperature up to its m.p.t. of 26 °C; its isomers are not and they melt around − 90 °C. Secondly, that, paradoxically, increase of thermal disorder always leads to higher symmetry of packing in the S_2 form. This exists over a surprisingly large temperature range.

It seems to have escaped attention that the cryoscopic constant K_f of any substance is inversely proportional to ΔH_f so that any substance e.g. camphor or carbon tetrabromide, with a very small ΔH_f will have a very large K_f; a given molar concentration of any miscible impurity will therefore produce unusually large

errors in measurements of transition temperatures, the effect being largest at T_f. In other words, much higher standards of purity are necessary when dealing with polymorphic substances than with those forming one solid only; this applies equally well to thermotropic mesoforms where we have:

$$S_1 \rightarrow lc \rightarrow L$$

In the lyotropic type, water is an exceptionally dangerous impurity because a colligative $-\Delta T$ of a transition which is produced by 18 gm. of water requires from 10 to 15 times as much of any lipid impurity.

When, in the early 1920's, X-ray diffraction was applied to aliphatic substances it was soon shown that the organic chemists' *straight* hydrocarbon chain was in fact zig-zag with the axis straight in the crystalline state. It was not for 15 years or more that the importance of rotation about the C—C bond was established as the origin of aperiodic coiling in polymer molecules and it is only in the last 10 years or so that the existence and importance of thermal motion in classical sized fatty molecules in the solid state was recognized. This had to await not only new theoretical concepts but also new methods by which these modes of thermal motion could be detected and studied. They must be studied because they are the cause of the polymorphism which is so characteristic of lipids, both anhydrous and in the presence of water; it has been known for 50 years or more and innumerable examples have been reported particularly by T. Malkin. The situation is also confused by use of solvents to give one or other form selectively and regardless of temperature.

If we start from the lowest temperature form and apply heat, the various modes of thermal motion increase and alter the shape of the molecule until it no longer fits its original lattice and a phase change occurs to a different packing; thus we may have:

$$S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow L$$

or

$$S_1 \rightarrow S_2 \rightarrow lc_1 \rightarrow lc_2 \rightarrow L \qquad \text{etc.}$$

each transition having its ΔH and ΔS which must be small compared with the simple change:

$$S_1 \rightarrow L$$

One of the most simple and direct methods for detecting such changes is the differential heating and cooling one; Fig. 3 gives an example taken from D. Chapman's valuable book: "The Structure of Lipids".

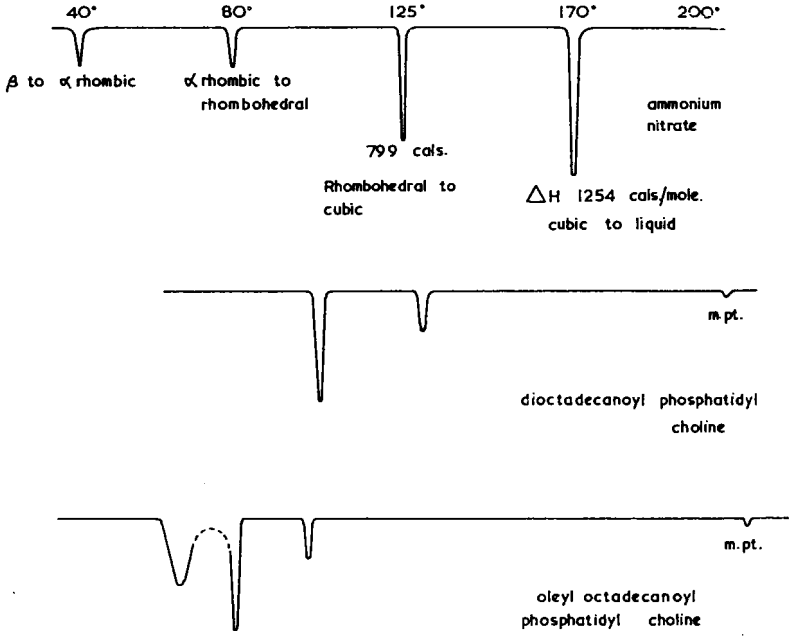


Figure 3. Differential heating curves.

The Alkanols

Some 3 years ago I decided that the alkanols were the simplest type of amphiphilic lipid and that they and their interaction with

water should be examined. I may have been right but simple they certainly are not[7]; they do not form a liquid crystalline phase with water but they connect with those class II and III lipids which do and the physical considerations are similar; they also give valuable information about the effect of mixing upon the temperature range in which any particular phase exists and this is the reason for examining these lipids over temperature ranges far outside those of living matter.

It is not enough to pin-point transition temperatures because we must know which phase exists at any region and it is inadequate and dangerous to go by appearance and use names such as " waxy ", " sub-waxy " and so on. A diagnostic method is needed, preferably one which can scan a temperature range reasonably quickly; X-ray diffraction is very valuable but slow and not suited to metastable phases; infra-red spectroscopy and n.m.r. are both very useful particularly for ease of rapid scanning. It is essential for any worker in this field, perhaps in any physico-chemical field, to realize that these methods give different sorts of information and that problems are not solved by one method alone any more than they are by using all the methods on a single substance.

The alkanols above C_{10} are insoluble in water and the members from C_{10} to C_{18} have been examined.

These alkanols exist in 3 crystalline forms shown diagrammatically in Fig. 4. The low temperature γ form is monoclinic and X-ray diffraction gives a long spacing, the angle of tilt to the basal plane α, and two short-side spacings d_2 and d_3 and the angle β. On heating this passes to the β orthorhombic form now perpendicular to the basal plane and still giving the d_2 and d_3 side spacings; on further heating the thermal motion is now such that the zig-zag configuration is lost and the chain is now statistically cylindrical and passes to the α hexagonal form with only a single side spacing intermediate between d_2 and d_3.[8] The random thermal motions of the components of the chain result in a tension pulling the two ends together and reducing the long X-ray spacing particularly in the α form. Di-electric measurements

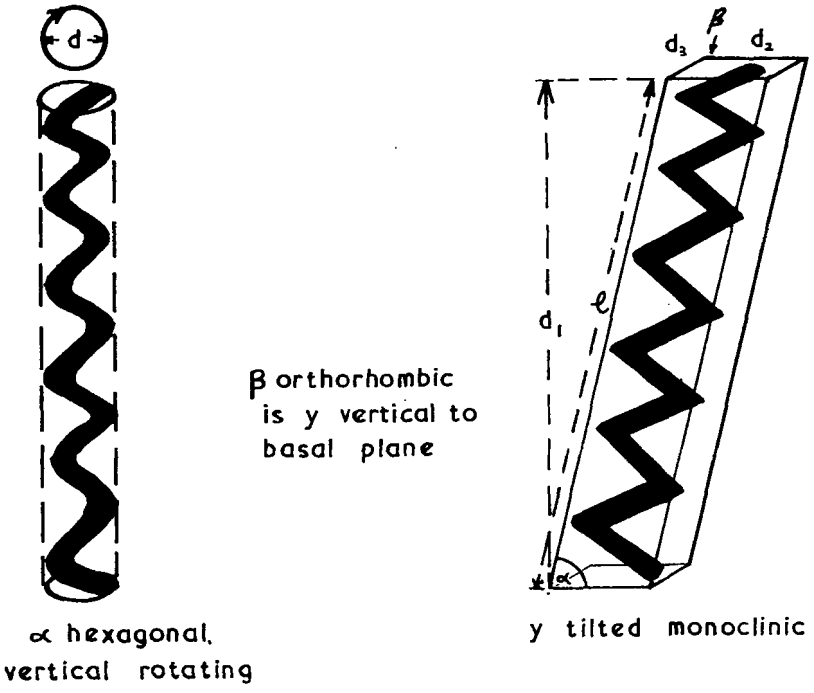


Figure 4. Crystalline forms in fatty alcohols.

indicate rotation of the cylinders. On further heating, melting to liquid occurs. We may note that this involves a greater order to disorder change than that in spheroidal molecules and that ΔH_f and ΔS_f will not be so small especially as T_f is only 58° for the C_{18} alcohol. D. Chapman's results for phosphatides whose T_f is around 200 °C show the much greater disorder in the chains by a very small ΔH_f.

Figure 5 shows the infra-red spectra of liquid, α, β and γ phases of the alcohols in the pure anhydrous state.

Alcohol-water Systems

In 1932 J. D. Bernal showed that dodecanol absorbs water above 16 °C and it crystallizes in hexagonal packing from the melt whereas below this temperature it is monoclinic[10]; in 1958 it was

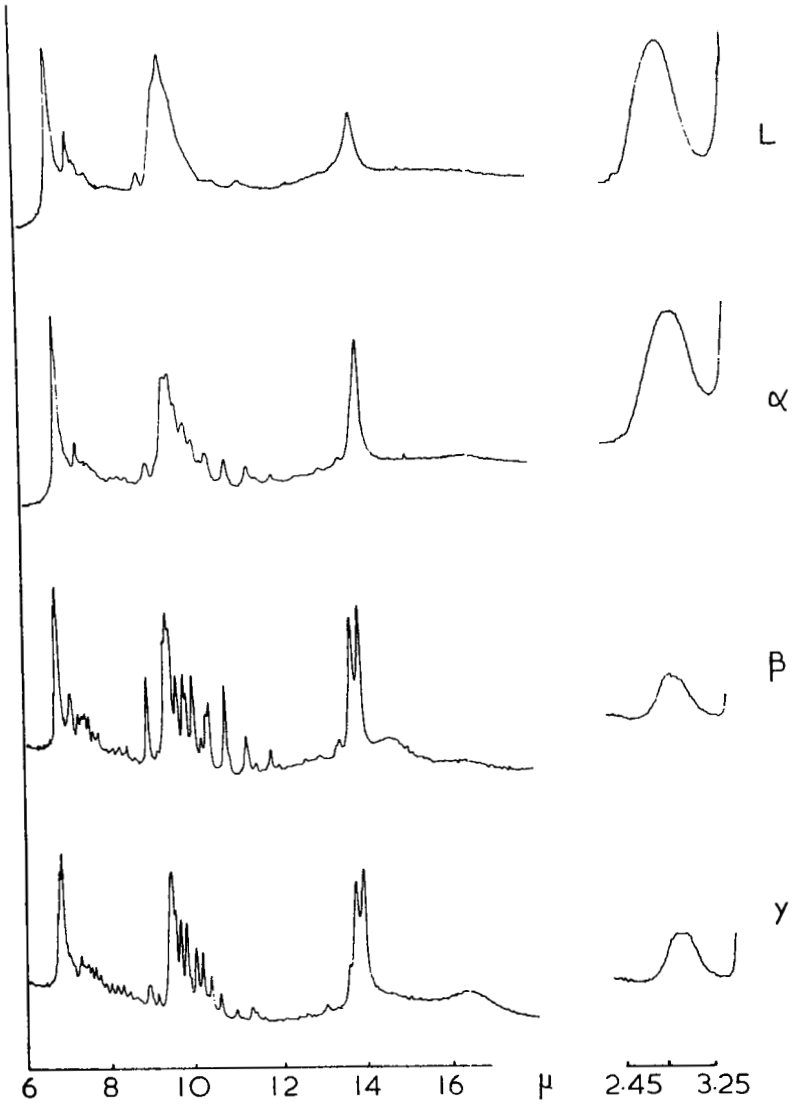


Figure 5. Infra-red spectra of liquid and α, β and γ solid phases of fatty alkanols.

shown by the writer that this behavior is shown by all the fatty alcohols and acids from C_{12} to C_{20}; the minimum temperature at which water penetrates was called T_{pen}[2] and this temperature runs roughly parallel to and below the anhydrous melting points, Fig. 6. Water increases the melting points of the alcohols but not those of the fatty acids. We have found too since that the alcohols are all hygroscopic above their T_{pen} and that the uptake of water is extremely rapid in the vapour phase; normal, iso and sec butanols, stood at room temperature in a dish over water, in a desiccator, took up more than 20% of water linearly over 24 hours; dodecanol at 26° also took up about 80% of its saturation value of 2.45% in the same time; a 20% solution of water in isobutanol placed in a dry desiccator over quick lime lost its water in the same time. Figure 7 shows the vapour pressure of dodecanol-water systems plotted against Temperature; up to T_{pen}, the value is that of water but this is lowered above it; when log vapour pressure is plotted against $1/T$ for water and for the dodecanol hydrate above T_{pen}, two parallel straight lines are obtained; the vapour pressure of the water is reduced but ΔH_{vap} is essentially the same for water from liquid dodecanol hydrate as for water from water. The usual practice of refluxing liquids with a drying agent is to be avoided because heating dissociates the hydrates and drives the water into the vapour phase where it is out of contact with the desiccant. Figure 8 shows the freezing point diagram for the tertiary butanol-water system in which we have complete miscibility and, between the two eutectic minima, the flat topped curve indicative of weak compound formation.

Almost everything about these solubilities is anomalous; the mixing of the alcohols with water at infinite dilution is exothermic, that of the tertiaries up to C_7 being some 3 times that of the primaries with the secondary and iso compounds on a band between them; addition of water to the anhydrous alcohols is small and exothermic for methanol and ethanol and endothermic for the higher ones from propanols to heptanols and the regularity of shape effect is lost.[11] This is not surprising since the barrier to solution in water is that of accommodating the phobic chain in

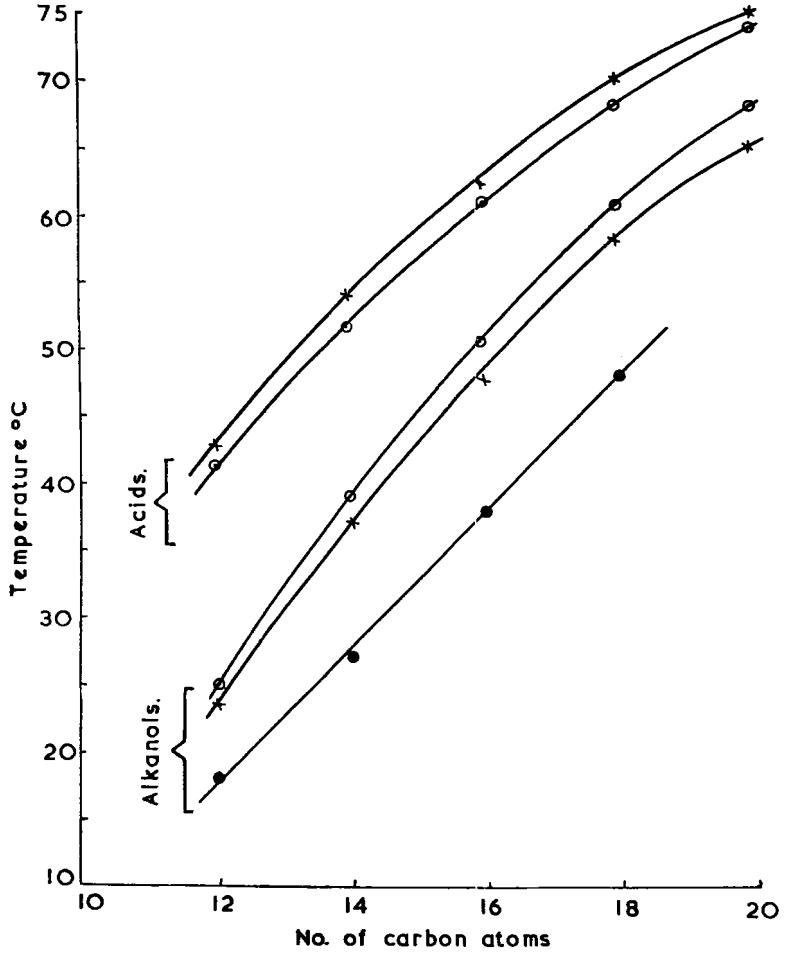


Figure 6. Transitions in fatty acids and alcohols anhydrous x and in presence of water; T_{pen} minimum for alcohols.

—×— Melting point of pure compound
—○— Melting point in water
—●— Loss of birefringence

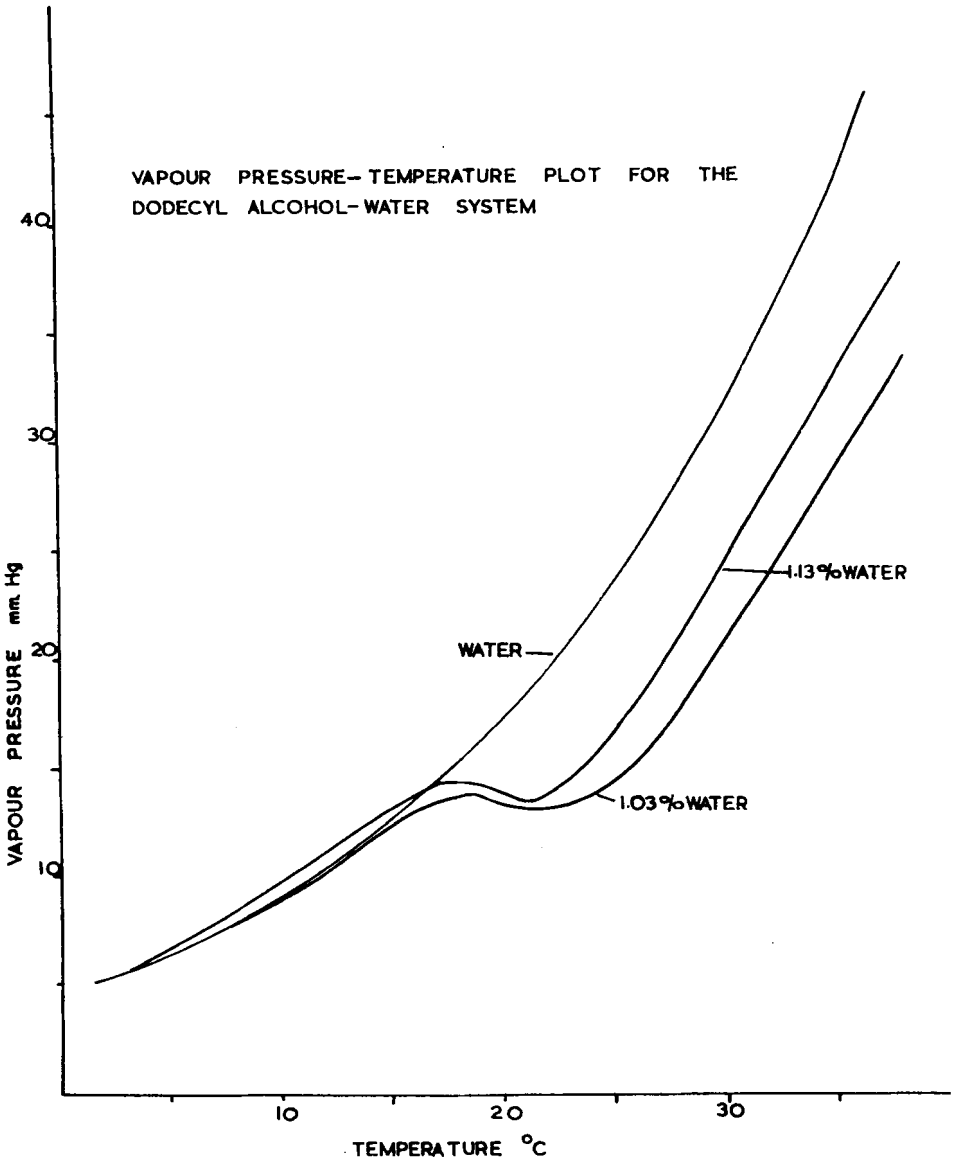


Figure 7. Vapour pressure *vs.* temperature of dodecanol-water mixtures.

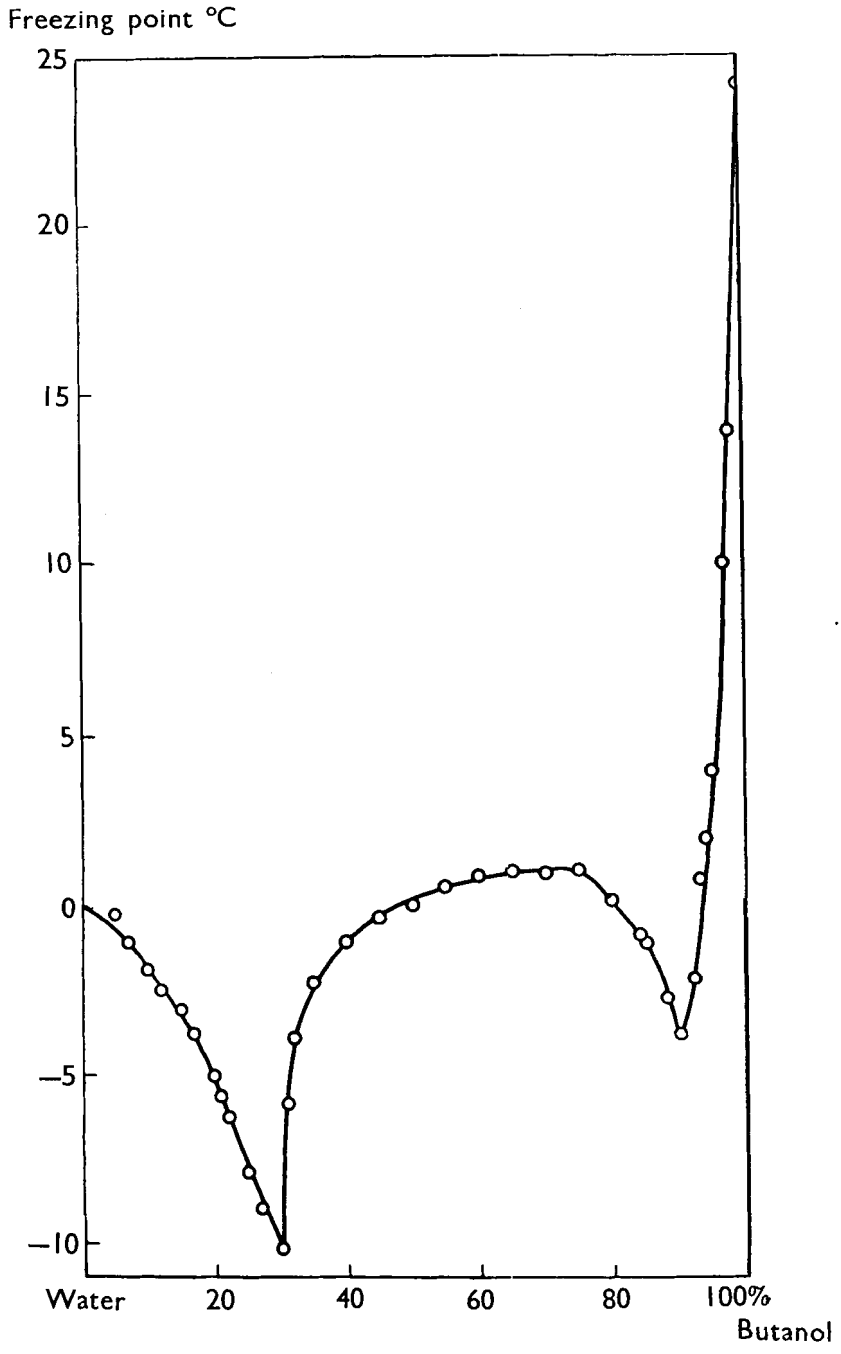


Figure 8. Freezing point diagram for the tert butanol-water system.

it whereas the shape of the molecule affects solution of water in it by the classical steric effect of the large methyl groups around the OH group in the heavily branched alcohols which affects hydrogen bonding of the anhydrous alcohols as well as its bonding to added water. Figure 9 shows the T/C diagram for the partially

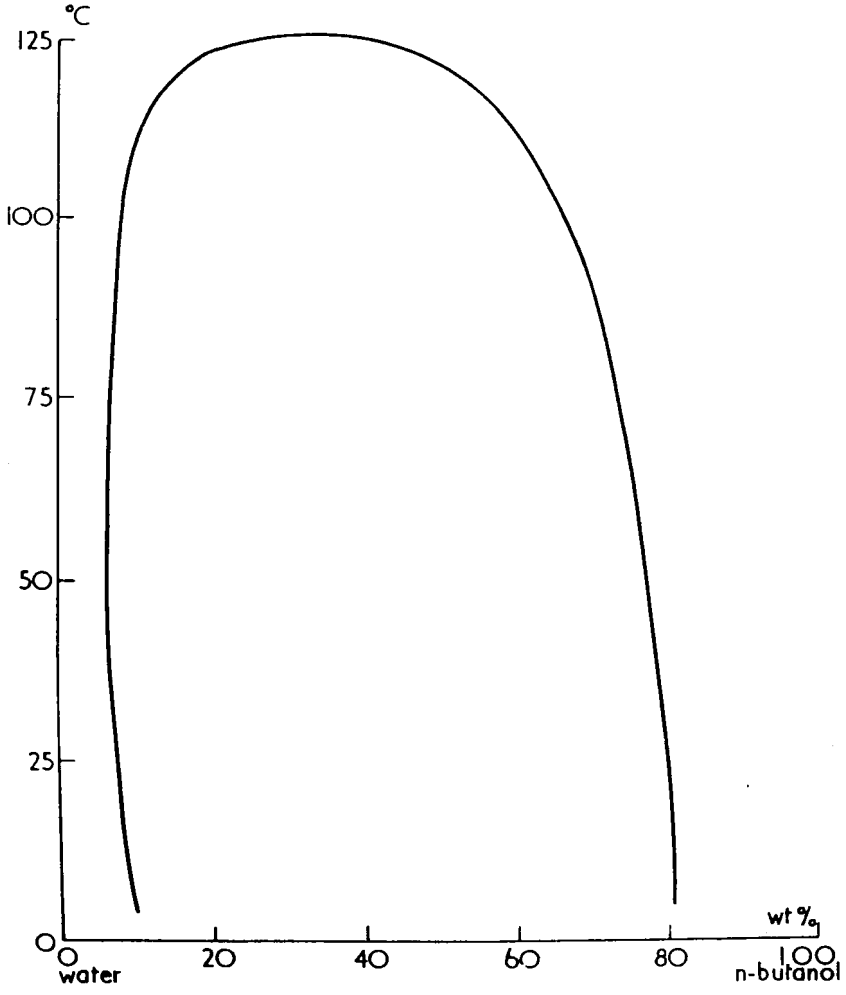


Figure 9. Solubility *vs.* temperature for the *n*-butanol-water system.

miscible *n*-butanol-water system from which two important points emerge; first, that over a wide temperature range, which becomes larger with higher homologues, there is very little change of solubility and, secondly that the solubility of water in butanol is about 3 times as great as that of the alcohol in water—a picture which, with smaller values, is general for the higher homologues. It is also found in the aniline-water system; log of solubility of the *n*-alkanols from butanol to octanol is a linear function of number of carbon atoms in the chain but no such regularity is found for the water in alcohol values.[12] We then measured the solubility

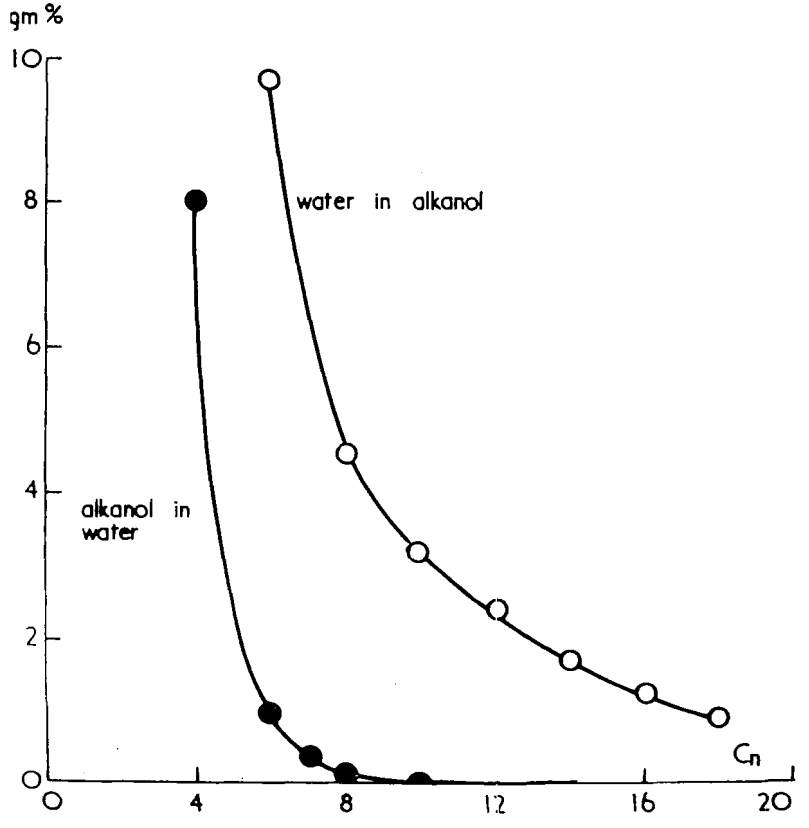


Figure 10. Solubility of *n*-alkanols in water and of water in alkanols as function of number of C atoms in chain.

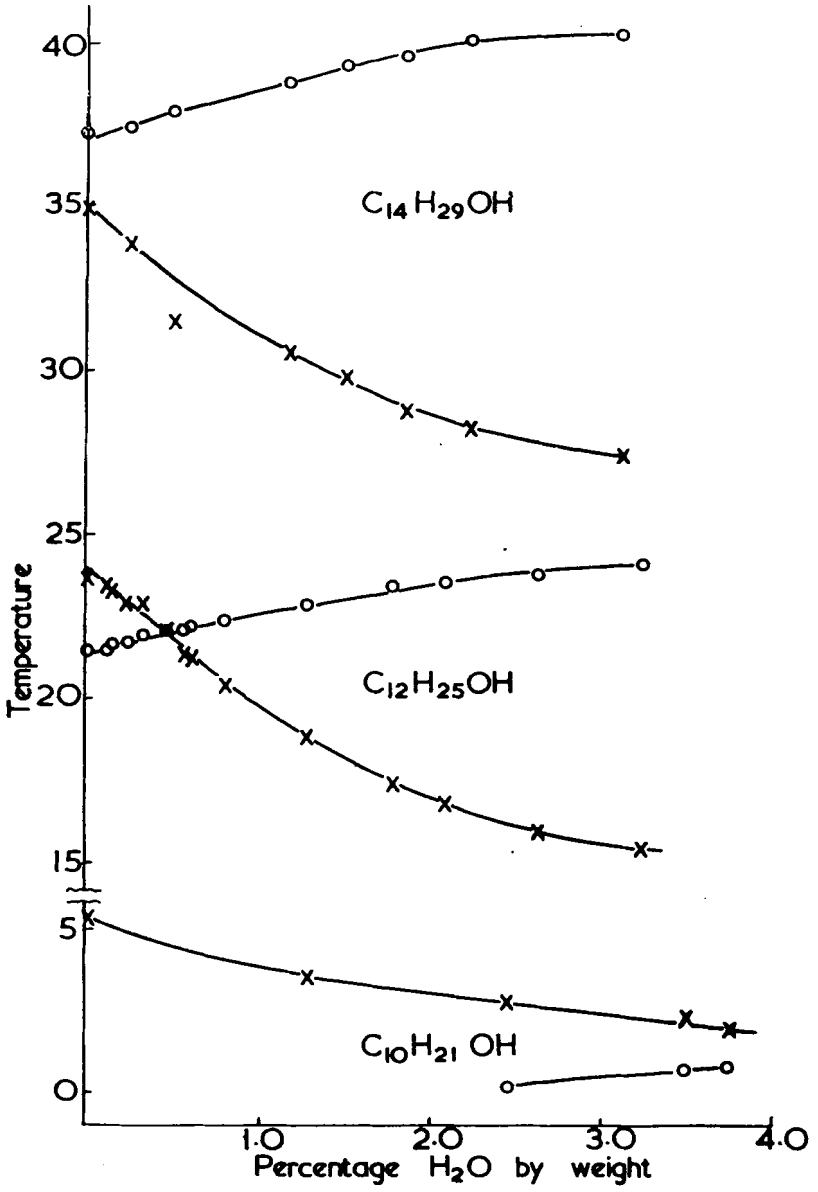


Figure 11. Freezing o and transition points x in tetradecanol, dodecanol and decanol showing transition from enantiotropy to monotropy with reduction of chain length.

of water in the higher members up to C_{13} and obtained the results shown in Fig. 10. If, however, molar quantities are used to express concentrations we find that saturation just above the melting point is reached from C_{12} to C_{16} at a quarter of a mole of water per mole alkanol. This is the same as the solubility in the α phase below T_f.

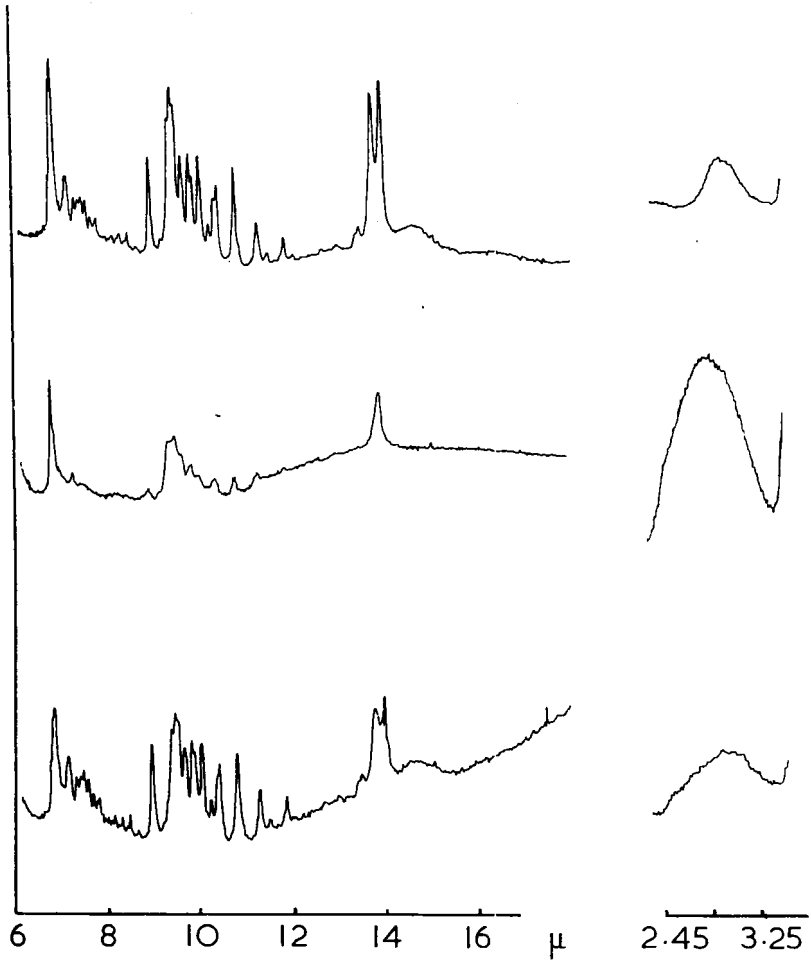


Figure 12. Infra-red spectra of tetradecanol; (a) at 30° anhydrous in β form; (*b*) quarter hydrate in α form at 30° and (c) in β at 25 °C.

The effect of water upon the higher alkanols (C_{10} to C_{20} even number members) is shown in Fig. 11; there is the small increase in the melting point mentioned earlier and a much larger reduction of the transition temperatures in the solid which can be described as stabilization of the labile α and β forms to notably lower temperatures than in the anhydrous substances. Figure 12 shows infra-red spectra for tetradecanol; (a) anhydrous at 30° is in the β form whereas its quarter hydrate is still in the α form at this temperature but has changed to the β form at 25°. Figure 13 shows (a) anhydrous and (b) the quarter hydrate of hexadecanol, at 30° where the former is cooled to its γ form while the

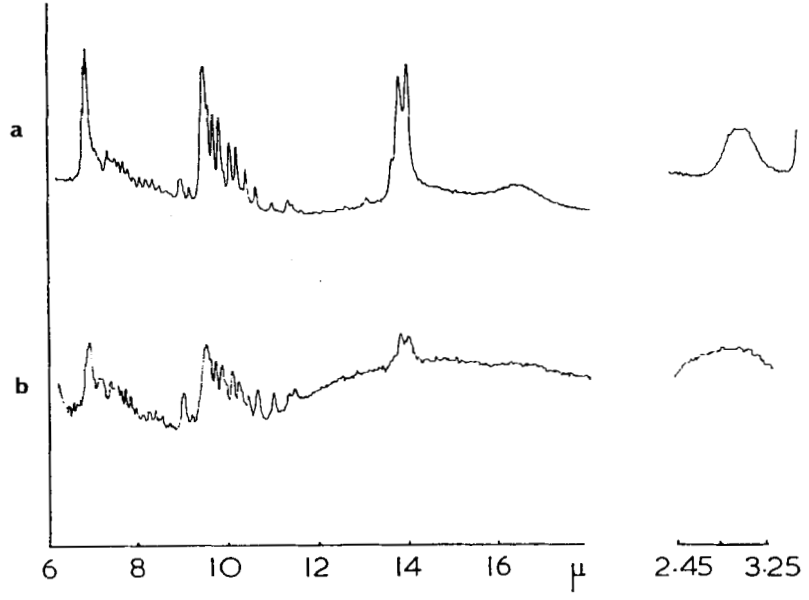


Figure 13. Infra-red spectra of hexadecanol at 30 °C; (a) anhydrous in γ form and (b) quarter hydrate in β.

hydrate is still in its β. It may be noted here that the α hexagonal phase, anhydrous or hydrated, of dodecanol and tetradecanol forms a quite remarkably transparent crystalline mass whereas the higher homologues in this phase are " waxy " looking; one

sees why many workers have spoken of a waxy phase but it is extremely dangerous to rely upon visual appearance.

Figure 14 shows freezing and transition temperatures for the even number anhydrous fatty alcohols and their quarter hydrates; it shows again the extension by water of the α hexagonal phase.

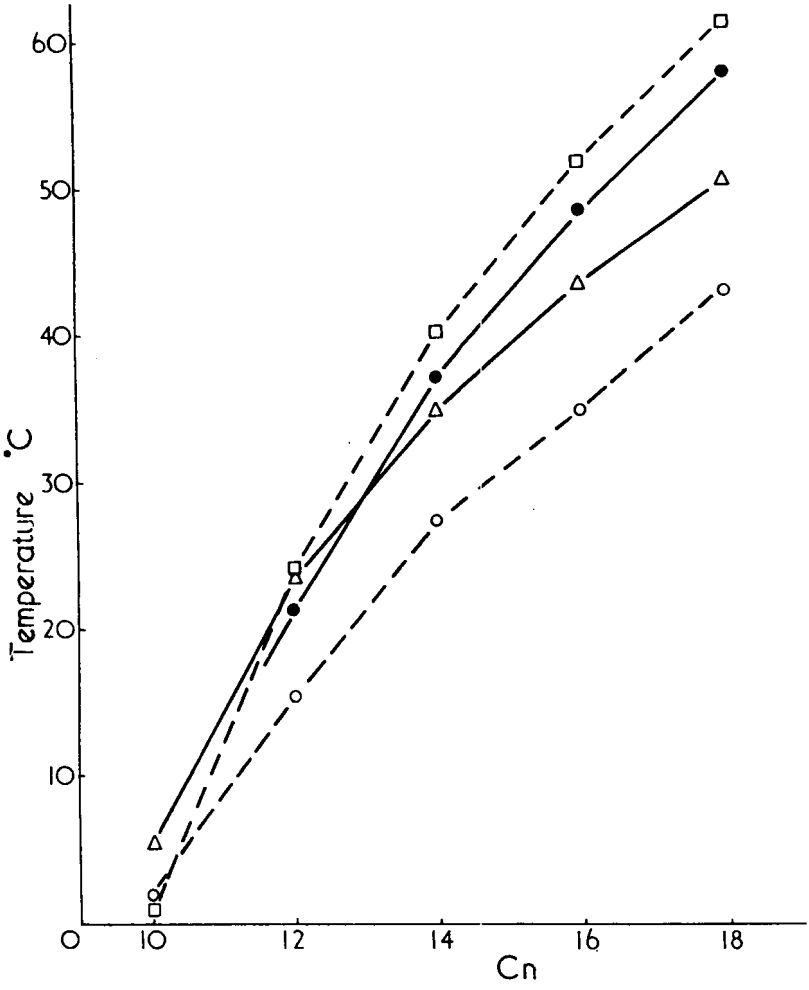


Figure 14. Freezing points ● and transition points △ for anhydrous alcohols; □ and ○ for quarter hydrates.

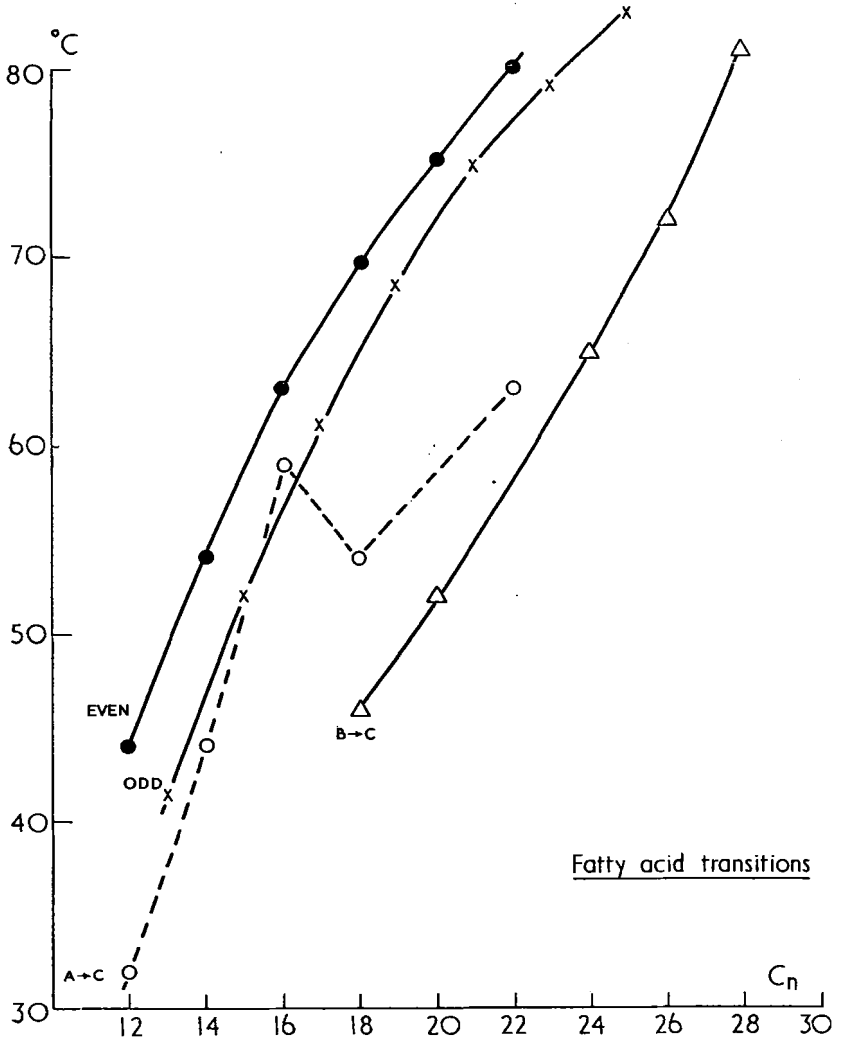


Figure 15. Transitions in fatty acids (von Sydow).

The crossing of the curves between C_{14} and C_{12} shows that the higher members are enantiotropic whereas the C_{12} and also C_{11} and C_{10} are monotropic and form the α phase only when supercooled. Care must be taken to exclude seed crystals of the stable form. When the transition temperature of the quarter hydrates, i.e. at the minimum value at saturation by water, are compared with my old T_{pen} values, they are found to be identical. This is an extremely important point; that there is for each of these amphiphilic lipids a minimum temperature below which there is no mixing with water and no labile solid (or liquid crystalline) hydrated form; the lipid is inert γ monoclinic.‡ Is that T_{pen} above or below blood heat in living matter?

Von Sydow has examined the thermal transitions in the fatty acids with somewhat similar changes, Fig. 15.[3]

The Conditions for the Formation of Liquid Crystalline Phases

Figure 2 shows that a sufficiency of water in a bimolecular layer of amphiphilic lipid provides a centre across which the fluid shear, which is required for the laminar flow of smectic phases, can take place. Throwing a pack of cards across the table is a commonplace example of this sort of flow and if one thinks of the cards being bent round into circles, flow will be telescopic. This latter case, which is important in these liquid crystalline lipids, was first reported by the famous German physiologist Virchow in 1854.[14] In his own words he "soaked a piece of nerve tissue in water for a long time" and then saw this curious "Newgate frill" of tubular looking excrescences under his microscope; although Virchow did not know it, this was the first example of liquid crystals reported and lyotropic ones at that. In 1863 Neubauer observed their formation when ammonium hydroxide solution was brought into contact with oleic acid,[15] again lyotropic; the flow of thermotropic ammonium oleate crystals was reported by Lehmann in 1906,[16] the subject was born and the earlier observation that cholesterol acetate had two melting points, solid to

‡ The odd number alkanols form only α and β solid phases.

milky liquid and to clear liquid at a higher temperature, was explained correctly.

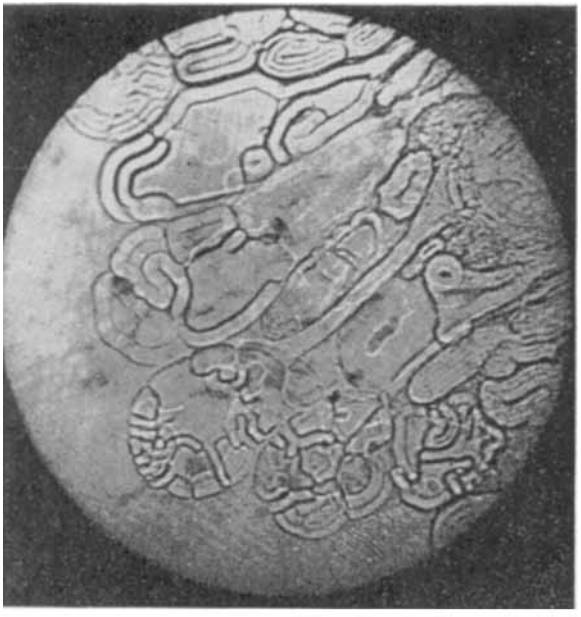
It is tempting to describe myelins as tubular but this is misleading; their cross-section is circular but they are built up of coaxial cylindrical layers each of which is the sandwich of water in a bimolecular layer of amphiphilic lipid. They are therefore birefringent as shown in Fig. 16 where (a) is in white light and (b) polarized light with Nicols crossed. It may be noted that, because the cross-section of the myelin is circular, the width as seen in the microscope can never be greater than the thickness of the specimen; to get large myelins which show birefringence strongly across the lumen, a thick specimen is needed and on no account should the cover glass be pressed down. It took me 7 or 8 years to realize this simple fact and therefore pass the tip on as it is not mentioned anywhere in the literature.

The coaxial layer structure is very beautifully illustrated in the electron microscope pictures obtained by Fernandez-Moran shown in Figs. 17 and 18.

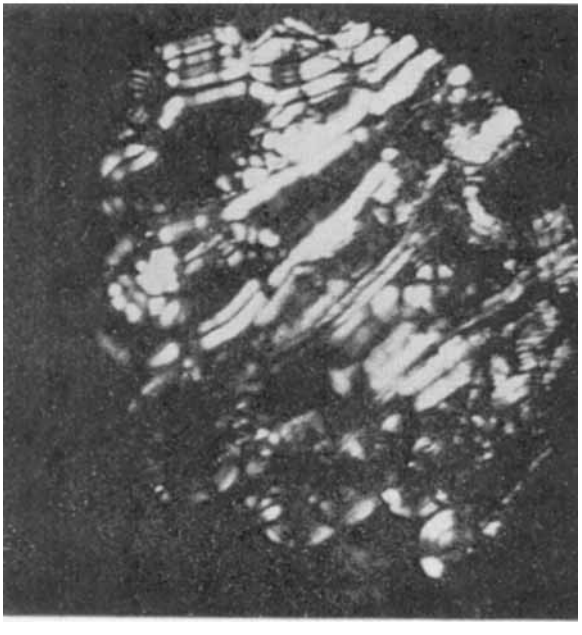
The extrusion of myelins by penetration of water into polar lipid is a process of equilibriation taking place spontaneously at the T_{pen} for the lipid or mixture of lipids; ΔG is therefore negative above T_{pen} and we could define T_{pen} as the temperature at which $\Delta H_m = T\Delta S_m$ where H_m and S_m are the heat and entropy of the mixing process but this includes the ΔH and ΔS of the crystalline transition as well as the terms for the hydration process.

The phenomenon is important because it requires only a minute amount of lipid but also because it is the most simple and direct method for measuring T_{pen}; myelin formation is unrequirocal proof that somewhere in the binary or ternary or indeed poly component system of lipid plus water there exists a bulk liquid crystalline phase. It is always wise to look for T_{pen} because, if addition of water is done too far above this, it may penetrate so rapidly that no myelins are formed, e.g. with mono-olein at 25°.

Figure 16. Penetration of aqueous soap solution into amphiphile with extrusion of myelin forms; (*a*) in white light, (*b*) in polarized light with crossed nicols. × 150.



(*a*)



(*b*)

Figure 17. Electron micrograph of lecithin myelins (micelles),* H. Fernandez-Moran.

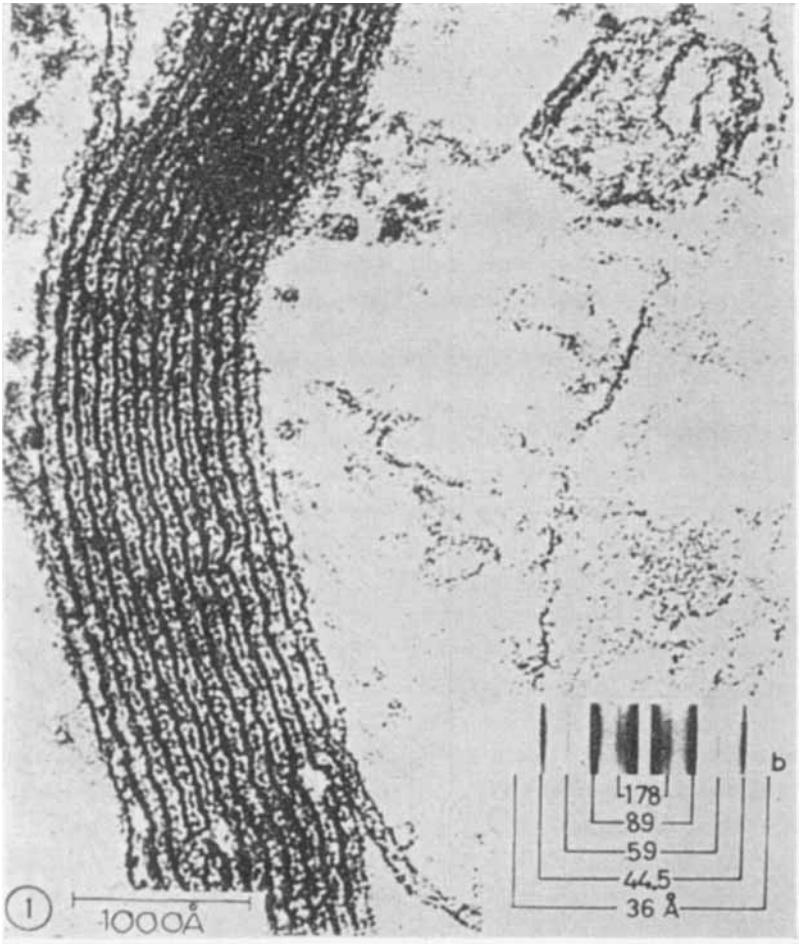


Figure 18. Electron micrograph of myelin sheath (rat sciatic nerve), H. Fernandez-Moran.

If the failure of the alkanols to form a liquid crystalline phase is due to insufficiency of water in the bi-molecular sandwich at saturation, then it is necessary to know how this may be increased; we know four methods and can predict a fifth.

I. If, instead of water, concentrated hydrochloric acid is added to dodecanol a liquid crystalline phase is formed with extrusion of myelins; similar behavior is shown by the C_{13} and C_{14} alcohols but not by C_{16} and C_{18} even when the temperature is raised. Dodecanol dissolves 9% by volume of the HCl as compared with 2.45% of water; for tetradecanol the value is reduced to 6%, a trend which would explain the failure of the higher homologues to form any l.c. phase. The liquid crystals of alcohols and water reported by Trapeznikov may be similar.[17]

II. In the monofunctional amphiphiles, the amino group is more strongly hydrophilic than the hydroxyl and carboxyl with the result that the alkylamines from C_6 upwards dissolve considerably more water and form l.c. phases. Ralston reported 2 l.c. phases for the octylamine-water system though one which he describes as " firm semi-solid " may well have been the α hexagonal solid, not then known; he also worked out the phase diagrams for tetradecylamine and octadecylamine and found a number of hydrates.[18] He also remarks upon the looseness of the binding of water to octylamine and, at higher temperatures, a lower consolute temperature change was observed. Figure 19 shows this change and the effect of addition of soap which raises it and, by solubilizing the amine in water, allows the full classical U curve to appear.[19]

III. The philic properties of the monofunctional amphiphiles are greatly increased by the addition of a second polar group; solubility of water in such lipids reaches 40 to 50% and liquid crystalline phases are formed. These are the groups II and III in Fig. 1 which should be sub-divided into non-ionic and ionic types. The non-ionic type melt below 100° and include hexadecane 1,2 diol, α hydroxy-hexadecanoic acid and, no doubt, their homologues down to C_8 and the important class of the monoglycerides, both 1,2 and 1,3 diols.[2] The anhydrous phosphatides

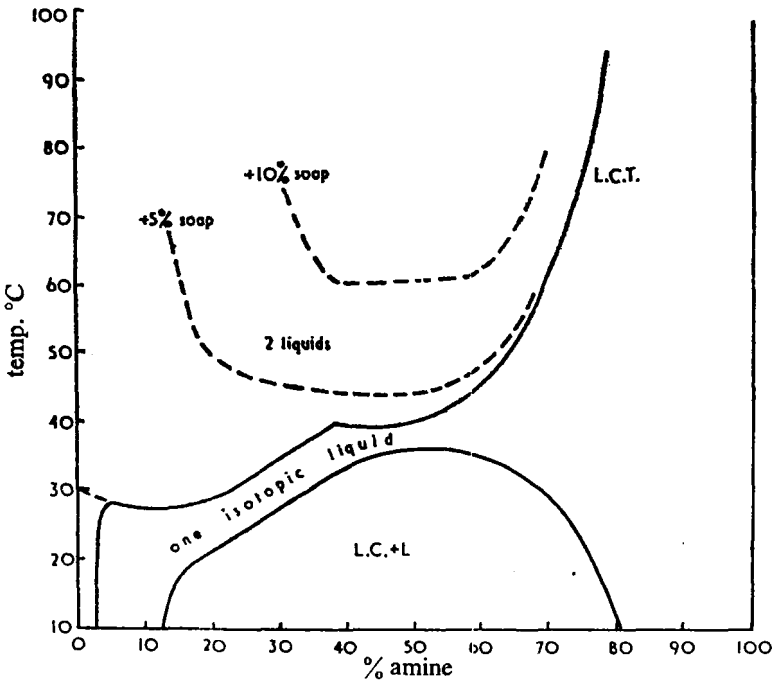


Figure 19. N octylamine-water system; T/C diagram.

have melting points from 190° to 210°[20] due to their ionic binding and the water-soluble soaps have still higher melting points. We may note that the diols are the non-ionic analogues of the lecithins with P^- and N^+ with the phosphatidyl inositols intermediate with one ionic P and the non-ionic H bonding inositol. In both types, penetration by water is accompanied by extrusion of myelins.

Here a question may be asked and needs answering. Do all substances forming lyotropic mesoforms with water also form anhydrous thermotropic ones? The answer is that some do and some don't, the dividing line coming from the point made earlier that the major thermal motion required in the thermotropic mesoform needs a high melting point; otherwise the substance melts normally without passing through a mesophase. Thermotropic mesoforms are therefore found in the high melting ionic

soaps and phosphatides but not in the low melting non-ionic diols and alkylamines. But it must be seen that addition of water breaks the electrostatic binding, as with any ionic salt, and then we see below 100° transitions and behavior similar to those of the non-ionic type.

The Monoglycerides

Figure 20 shows the Temperature-Composition phase diagram for the monolaurin-water system; both 1,2 and 1,3 monoglycerides exist in 2 crystalline forms,[22] the higher melting point one being the stable while the monotropic α form with freezing point from the super cooled melt some 20° lower is seen only when seed crystals of the β form are completely excluded. It will be seen that the transition from l.c. to isotropic liquid for the higher concentrations of water is some 55° above the melting point of the β anhydrous form; the solubility of water in the lipid does not change on crossing this boundary and saturation by water is not determined by the condensed packing in the l.c. phase just as the saturation of the monols by water is the same for liquid and solid α phase. Saturation in the liquid (in sealed tubes) is 47% and, when the temperature is raised to 119°, a lower consolute temperature change to two isotropic liquids occurs, recalling the similar effect in the octylamine-water system where it occurs at much lower temperature. The occurrence of an LCT is usually attributed to breaking of H bonds. Beyond saturation, the system is a suspension of l.c. spherulites in water; this is *not* an emulsion.

Lutton has published phase diagrams for the monoglyceride-water systems including mono-olein[23] and Larsson[24] has reported important X-ray diffraction studies and phase equilibria including the remarkably large area of the cubic phase in the mono-olein-water system. If this phase is mechanically as well as optically isotropic, it cannot be any type of liquid crystal and is, in fact, a soft solid belonging to Timmerman's plastic spheroidal molecular type. It should be noted that no evidence has been adduced to

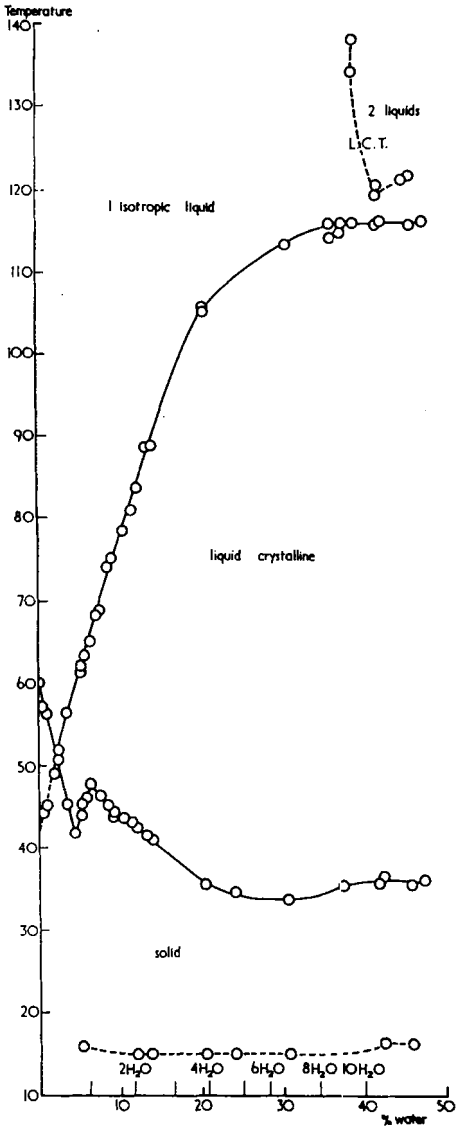


Figure 20. Mono-laurin-water system.

C

show that their flow is plastic; only that the viscosity of the cubic phase is substantially less than that of the lower symmetry form below the transition temperature.

Figure 21(*a*) shows Lutton's phase diagram for the mono-olein-water system and 21(*b*) our results for the increase of vapour pressure with concentration of water at 25°; the vapour pressure of the water in the liquid crystalline bi-molecular layer has reached that of pure water a little above the monohydrate composition, a result which may be compared with the indication of a mono-hydrate in the mono-laurin. Saturation is at about 9 moles of water per mole of mono-olein.

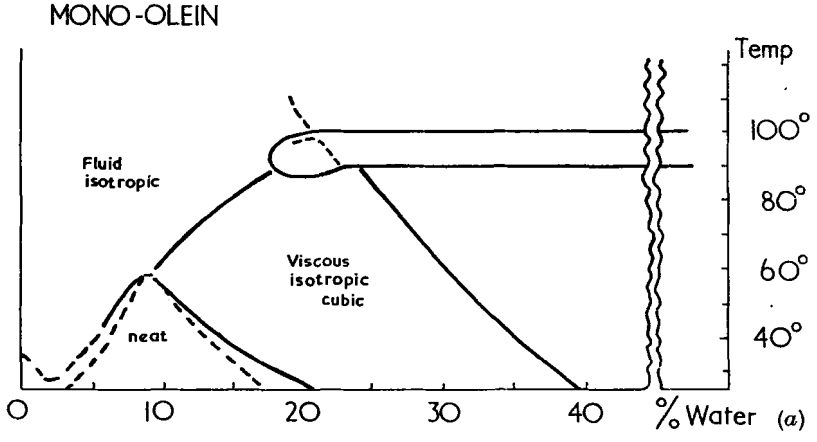


Figure 21. Mono-olein-water system; (*a*) phase equilibria, (*b*) vapour pressure *vs.* water content.

The Phosphatides

These have been separated into the classes with the various end groups such as choline, ethanolamine, serine, inositol and the differently shaped sphingomyelins; each class then consists of a family of substances with permutations of the fatty acids in the di-alkanoyl chain part of the molecule. However, in recent years many single substances have been synthesized and characterized.

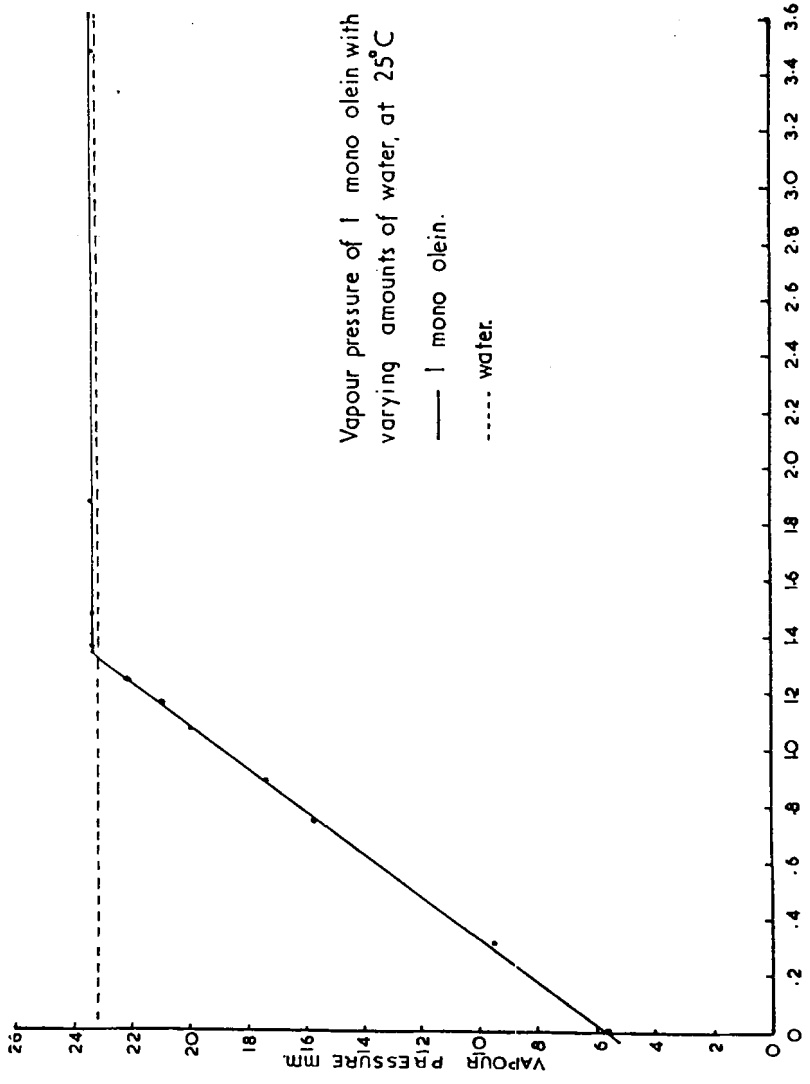


Figure 21(*b*)

Being ionic substances, the anhydrous substances have high melting points around 200 °C; the anhydrous soaps have melting points higher still. It is often suggested that there is much internal compensation of the P^- and N^+ charges in lecithins but this is difficult to reconcile with the solubility of lysolecithin in water which indicates an effective charge.

That water added to lecithin causes myelins to appear and that, with more water, a suspension of liquid crystals is formed has been known since early in this century. However, in recent years it has been claimed that micellar solutions are obtained by supersonic dispersion and that the micellar weight is of the order of 10^6. These are not micelles by the definition of the name by colloid chemists and its seems likely to the writer that they are very small liquid crystalline particles and that the figure of 10^6 merely denotes the limit of subdivision attainable by this mechanical method.

There is too much information about the phosphatides to summarize here and the reader is referred to recent books (see references).

IV. Increase of pressure promotes miscibility in these systems but major effects require several hundred atmospheres, e.g. 800 for complete miscibility of sec. butanol and water.

V. The fifth method upon which there is a large amount of information is the whole of the phenomena, including separation of liquid crystalline phases, included under the name "solubilization"; they are usually discussed as a means of increasing the solubility in water of a water-insoluble substance by addition of a water-soluble amphiphile; i.e. a soap. The penetration of a soap solution into an amphiphile (polar solubilization) is observed conveniently microscopically using a heating or cooling stage; myelin extrusion is observed when the hydrocarbon chain of the amphiphile is 5 normal carbon atoms or more; there is again a T_{pen} which increases roughly parallel with m.p.t. of amphiphile. Figure 22 shows a late stage in the penetration of a stearic acid crystal by soap solution at 50 °C; the characteristic initial form of the myelins is being lost as they reform themselves into rows of l.c. spherulites.[4] The writer has pointed out the great

importance of this liquid crystalline, highly viscous barrier to penetration of soap solution to the dirt, particularly in domestic laundry where the sweat glands have been pouring out a high concentration of fatty acids continuously on to the soiled garments.[25]

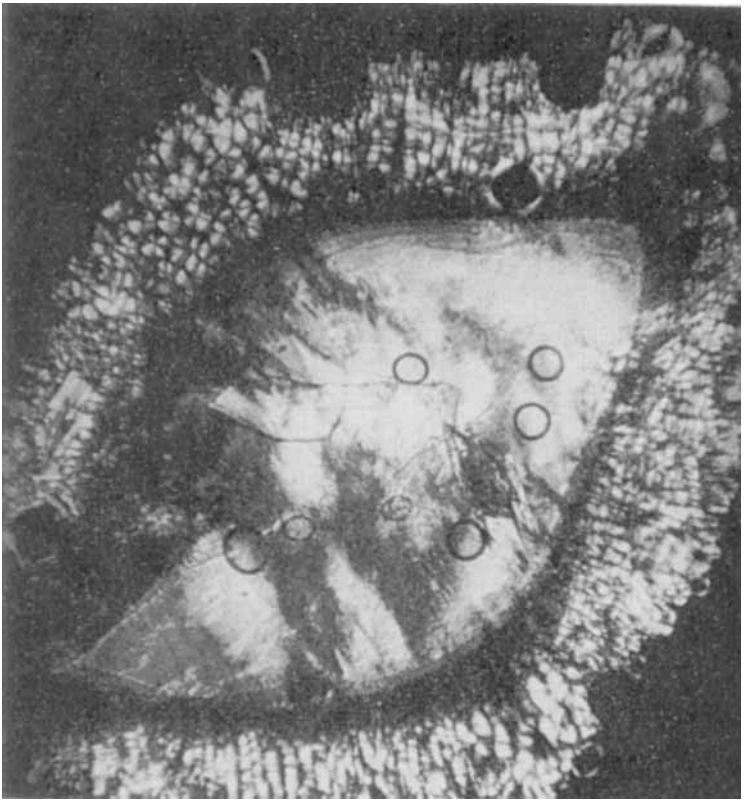


Figure 22. Penetration of soap solution into crystalline stearic acid at 50 °C; Note that the myelin extrusion is around the edges with none on the large face consisting of phobic methyl groups.

One may equally well observe the penetration of water into an intimate mixture of anhydrous soap and amphiphile; water is always essential to the mixing of the non-ionic polar group of the amphiphile with the ionic one of the soap.

Dealing as we are with a three component mixture, equilibria must be shown by triangular diagrams, a typical example of which is shown in Fig. 23.[26] The ternary mixtures are L_1 which is amphiphile in (micellar) soap in water or, more precisely, amphiphile co-micellized in soap in water; this involves the two mixing processes, soap polar group plus amphiphile polar group and water on the outside of the micelle with the two hydrocarbon chains mixed in the interior.

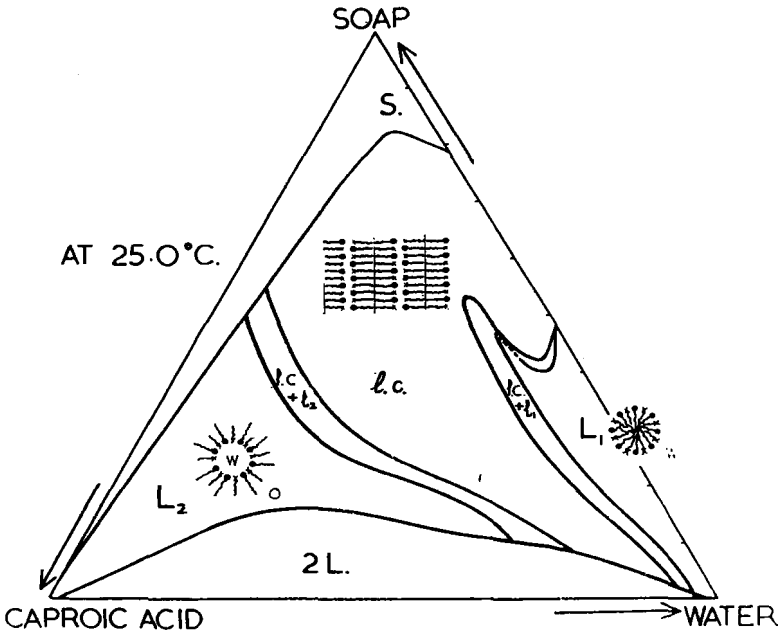


Figure 23. Phase diagram for soap-water-amphiphile system.

As concentration of insoluble amphiphile increases, the ternary liquid crystalline phases separate out in the area marked l.c. and the simple layer l.c. structure is shown. In the left side of the diagram, the amphiphile-rich L_2 is water in soap in amphiphile, best described by Schulman's name " micro-emulsion ". It will be seen that this extends upwards from the solubility of water in the amphiphile while the L_1 area is likewise based upon the

solubility of the amphiphile in water; the ternary l.c. phase area has the binary soap-water l.c. phase as its base on the right hand side of the triangle; in it are found middle, neat and cubic phases.[27]

The transition from molecular dispersion to micellar is one to a condensed state and the electrical state of an ionogenic group in the surface of the micelle is now conditioned by its distance from its neighbours and not by the much greater distance between the micelles; this surface concentration corresponds to about 5 Molar; there is heavy binding of counter-ions and consequently ionization is only about 20% in a micellar soap solution. When non-ionized amphiphile is co-micellized, there is a dilution in the surface of a kind which is peculiar to amphiphiles; if half the soap molecules in a micelle are replaced by an alcohol the surface concentration of ionic groups will be halved, bound counter-ions are unbound, Fig. 24, and conductivity rises. Figure 25(*a*) shows what happened when we did this experiment using a 20% solution of sodium dodecyl sulphate; the very large increase of equivalent conductance as *n*-hexanol is co-micellized is paralleled by the increase of sodium ion concentration measured by sodium sensitive glass electrode.[28]

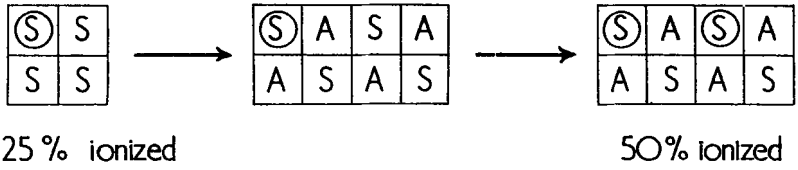
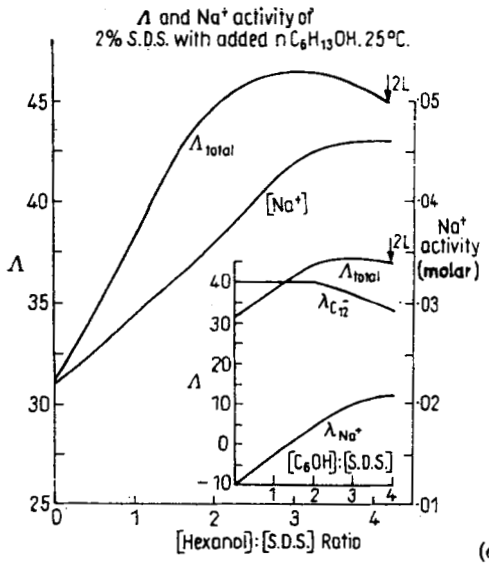


Figure 24. Increase of ionization following surface dilution of soap by non-ionic amphiphile.

Figure 25(*b*) shows the effect of varying the chain length of the alcohol added and illustrates again the rapid change of distribution between water and lipid (in this case micellar) with variation of hydrocarbon chain length particularly at the lower end of the homologous series; the vertical arrows show the saturation concentrations. Figure 26 shows formally the small swelling of monols compared with the large swelling to l.c. of diols and of monol plus soap.



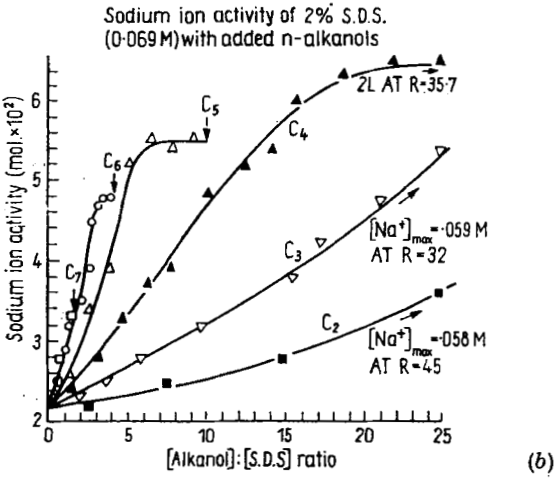


Figure 25(*a*). Effect of addition of amphiphile (*n*-hexanol) to 2% solution of soap (sodium dodecyl sulphate) upon conductivity and sodium ion activity; insert shows fraction of current carried by anion and cation; (*b*) Effect of chain length of alkanols upon sodium ion activity; the increase of p_{Na} is an indication of the distribution of alcohols between micelles and water.

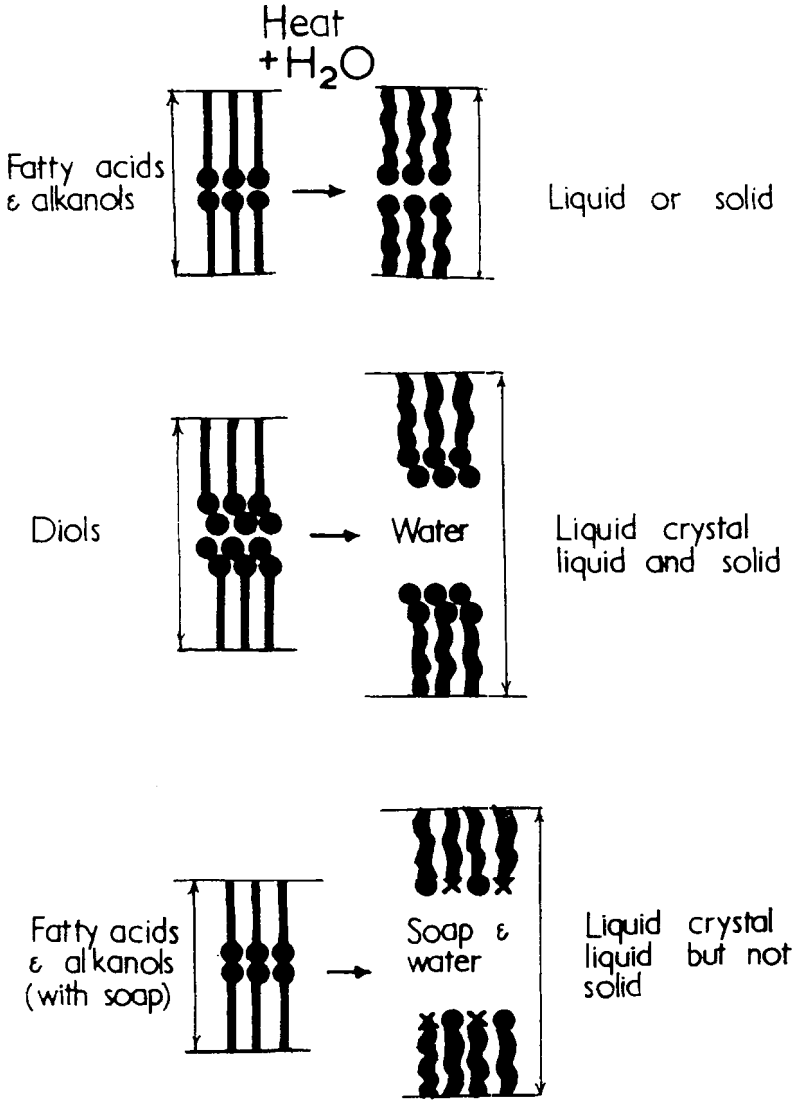


Figure 26. Diagrammatic summary of penetration of water into monols, diols and monols plus soap. Note that below T_{pen} the chain is drawn as a rod and above it the thermal motion is denoted by the wiggly chain.

Hydrotropy

The name " hydrotropy " was first used in 1916 although the phenomenon had been studied by Engler and Dieckhoff in their early paper of 1892[29]; however, they and some later workers did not distinguish between hydrotropy and solubilization. Freundlich and later Durand defined the relative increase of solubility of a solute R as R_1C^n where C is the concentration of hydrotrope and R_1 and n are constants for each system. The early work of Setschenov[30] is almost unknown and so is the later important paper by Long and McDevitt who discuss salting in and salting out.[31] Lumb has presented hydrotropy results as triangular equilibrium diagrams for these 3 component systems.[32]

Hydrotropes, almost always salts of organic acids or bases, at very large concentrations in water dissolve both non-polar and amphiphilic substances insoluble or poorly soluble in water; the minimum concentration required may be 20, 40 or 60% whereas solubilization by soaps starts at the critical micelle concentration which is usually less than 1%. In my laboratory we have examined the ternary systems: hexanol-water-alkyl trimethyl-ammonium bromides with 18, 16, 14, 12, 10, 8, 7, 6, 5 and 4 carbon atoms in the chain; these are soaps down to C_8 below which properties change abruptly and the lower members are hydrotropes.[33,34] Figure 27 shows the results for the 4, 5, 6, 7 and 8 members with n-hexanol and water at 25°. The base shows the small solubility of hexanol in water and the larger one of water in hexanol; the 2 liquid area boundary must start and end at these two points, so long as addition of hydrotrope has no effect upon the solubility of hexanol in water, a line parallel to the r.h.s. of the triangle gives the saturation solubility and the phase boundary between 1 and 2 liquid areas. For the C_4 compound solubilization clearly does not start until a concentration of 56% is reached; for C_5 it is down to 32.5%, for C_6 to 14% and to 6% for the C_7 hydrotrope; in C_8 TAB, solubilization starts at the cmc. Tie lines are drawn through the analyses of 3 conjugate solutions in each 2 liquid area; these are of the highest importance because

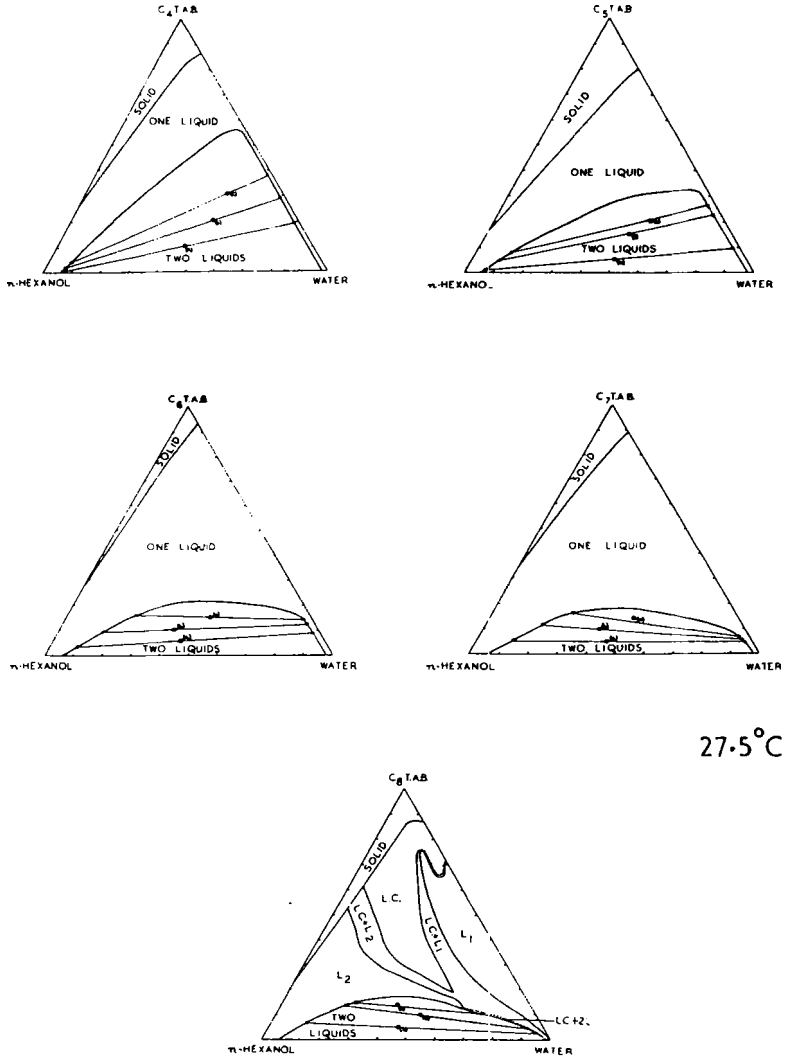


Figure 27. $C_{4,5,6,7}$ alkyltrimethylammonium bromide (hydrotropes)-water-hexanol phase diagrams, with C_8 soap system for comparison.

they indicate the distribution of the hydrotrope (or soap) between the 2 liquids; the C_4 lines slope upwards towards the water side showing the expected distribution of this short chain salt much in favour of the water; the slope is smaller for C_5 and approaching horizontal in the C_6 compound; that is phobic and philic tendency equal (for hexanol and water). The slope is reversed in C_7 and, for the higher homologues, the distribution moves increasingly in favour of the amphiphile (it would not do so in a non-amphiphilic lipid such as olive oil or even hexane).

Figure 28 shows the effect of varying the chain length of the amphiphilic solute while keeping the chain length in the hydrotrope constant. This very large increase of what we may call "the critical hydrotrope concentration" is not found in solubilization by soaps.

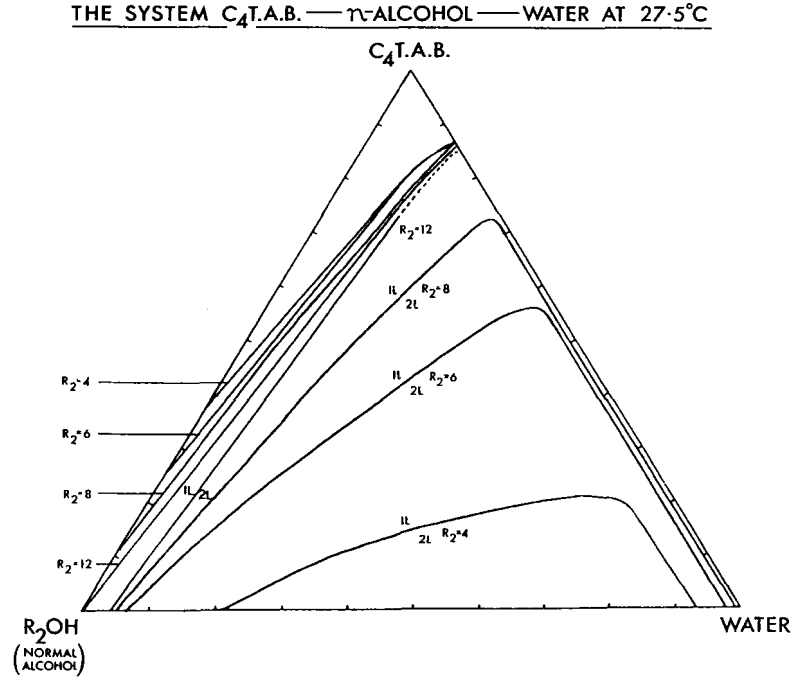


Figure 28. Effect of chains length of alkanols in aqueous hydrotropic system.

Figure 29 shows our Perspex 4 component model for hexadecyltrimethylammonium bromide-water-*n*-hexanol-cyclohexylamine hydrobromide at 27.5°. 100% hexanol is at the apex of the triangles furthest from the camera and somewhat obscured by specular reflections from the Perspex; the inverted U curve on

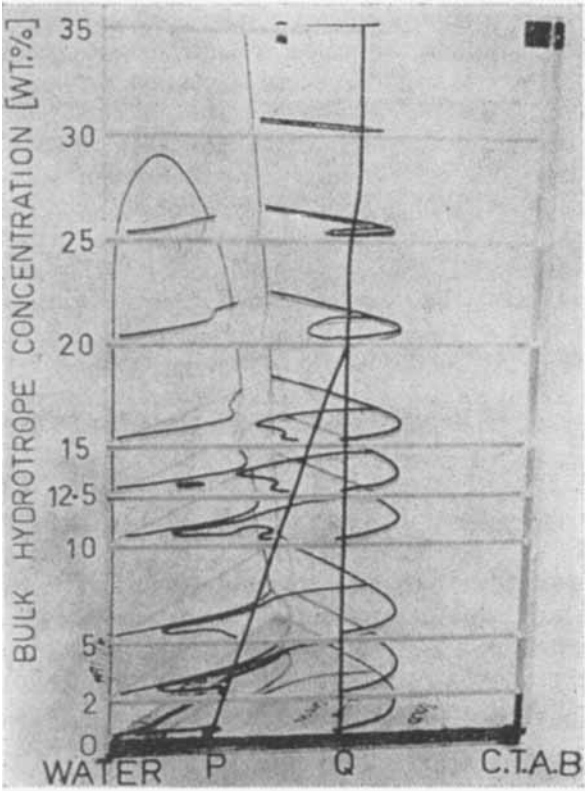


Figure 29. 4 component phase model showing hydrotropic solubilization of hexanol and peptization of binary soap-water liquid crystalline phase, PQ.

the left, the water-hexanol face, shows clearly the increase of miscibility of hexanol and water caused by the hydrotrope and complete miscibility with 28%.

It is clear that hydrotropes are molecules containing a hydrophilic polar group and a hydrophobic part not large enough to

make it a soap; this need not be a normal paraffin; it can be branched, cycloparaffin, aromatic ring, indeed any hydrophobic group. It is to be noted that these organic ions, which are active structure breakers, are, in their unionized forms of acids and bases, structure builders as amphiphiles when added to soap solutions. The peptization of the binary liquid crystalline soap-water phase by the hydrotrope ion is also seen in Fig. 29.

Figure 30 shows the process of structure breaking; as usual we must have the dual mixing processes, polar groups with polar

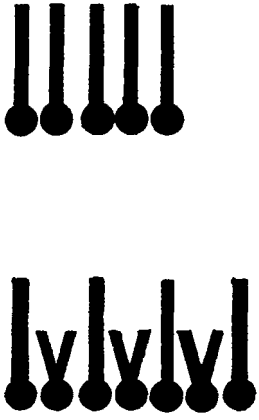


Figure 30. Long-chain structure breaking by hydrotropes.

groups and water plus the two phobic parts mixing; the hydrotrope acts as a wedge separating the long chains and so changing the l.c. layer structure to liquid. Interpolation of shorter chains is a process of making holes in the longer chain lattice; if these are too large to be filled by the random thermal motion of the chains, unmixing will occur; e.g. as a ternary l.c. phase going to 2 liquids when hydrotrope is added or, as with 2 lipids with common polar group, by passing from solid solution when the " hole " is one or two CH_2 groups to an eutectic when it is larger. Hydrotropes are fairly small molecules; the acetyl choline ion is one. The best penetrant through a heterogeneous lipid-water system would be the hydrotrope which had a 50 : 50 distribution ratio between

water and *that particular* lipid. It is not suggested that solubilization in water by hydrotropes at these very large concentrations is important biologically but it is suggested that it is the amphiphilic lipid-solubility of hydrated hydrotrope that is in cases of penetration including drug action and the transport of particles through heterogeneous lipid-water material. It should also be noted that there is no reason for restricting the name to salts since non-ionic hydrotropes such as *n*-butanol, tert pentanol are obvious ones and there may well be many other cases where philic properties come from a sugar moiety of a larger amphiphilic molecule; the most clear outsider now coming up to the front in this field is the glyco-lipids.

Conclusions

Biologists may find so much talk about transitions and structures at temperatures far above those of living matter pointless, but it is necessary first to make the chemical analytical step, to take the problem to pieces and examine the properties of each piece; until this is done we cannot know what are the mixing effects and rules. Insofar as the physical states of these lipids are concerned, most of the mixtures show eutectoid behavior although occasionally solid solutions are found; this eutectoid effect is not just the simple case of lowering of freezing points but lowering of transition temperatures in the solid state also; that is a systematic lowering of the temperature range in which the various physical states and structures exist. In the high melting point phosphatides, the electrostatic binding is broken by addition of water, the various eutectoid lowerings due to the different fatty acids in each family plus similar effects due to mixing the polar groups, choline with inositol and sphingomyelins etc. will bring the key property of T_{pen} down to below 100° and then Old Mother Nature's ace of trumps; the *cis* configuration of an unsaturated fatty acid usually on the β carbon atom of the glycerin drops it by another 50° or so and brings the liquid crystalline phase temperature down to cover blood heat. This is shown diagrammatically in Fig. 31,

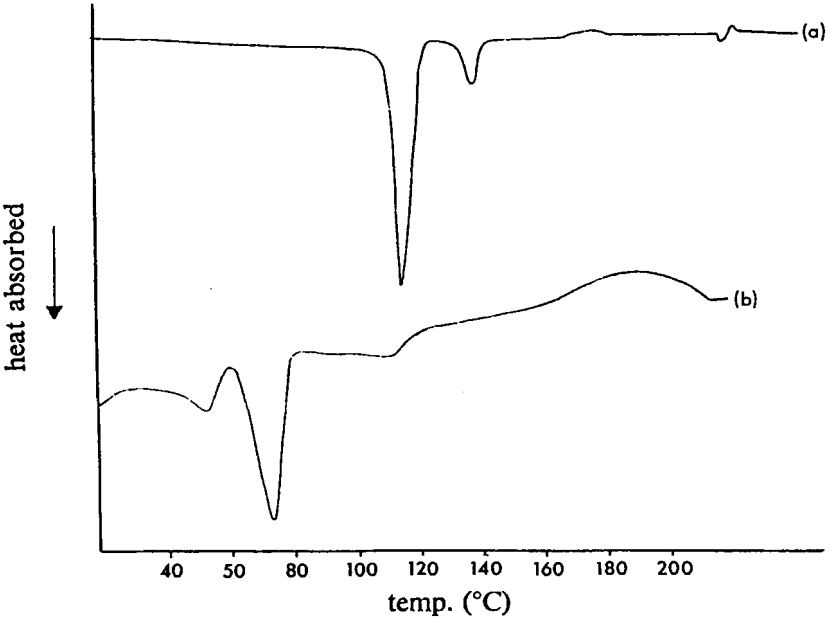


Figure 31. Differential thermal analysis curves showing lowering of transition temperature; (*a*) dimyristoyl and (*b*) 2 oleyl 3 stearoyl—D L phosphatidylethanolamine.

in which we see the lowering of the large peak by some 60 °C.[35] Figure 32 shows the structural features of the formation of the lyotropic liquid crystalline phase. It seems unlikely that it would matter physically whether the best *cis* chain is on the α or the β carbon; the *cis* configuration is a tailor-made disorder, equivalent, so far as crystalline packing is concerned, to heating a normal hydrocarbon chain of the same length by some 50° as is well illustrated by the melting points;

(*trans*) stearic acid	69.6°	octadecanol	58°	mono-stearin	81°
cis = oleic acid	16°	oleyl alcohol	2°	mono-olein	35°
trans = elaidic acid	43.7°	elaidyl alcohol	36.5°	mono-elaidin	58.5°

We must however be wary of regarding these effects as a simple move of all the transitions and each of the phases between them down the temperature scale; an actual lowering of 60° is accompanied by an equivalent reduction of the thermal motions such

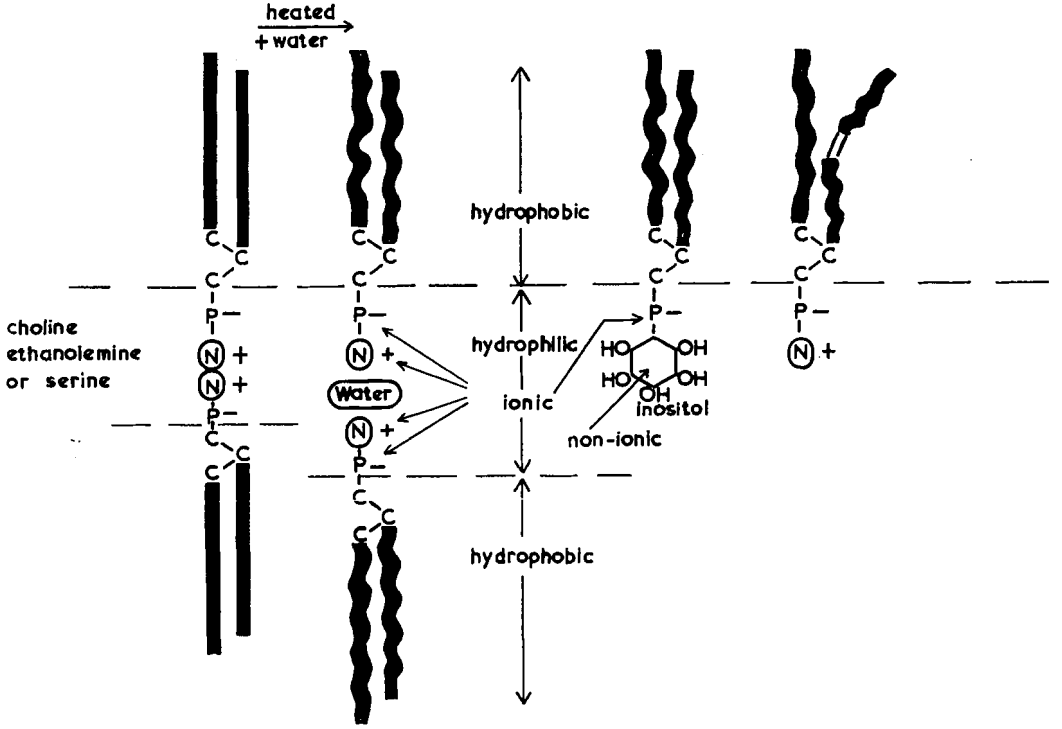


Figure 32. Swelling of phosphatides to form liquid crystals above T_{pen}; thermal disorder is shown by wiggly chains and the large built-in disorder due to the *cis* configuration is indicated.

that phases may be lost and the phase sequence changed. In general, with the sort of mixtures which have been discussed here, it is easy to achieve large lowerings of the higher temperature transitions but not of that to the lowest temperature form—call it T_{pen} or Krafft Point—which is the final stage of un-mixing of the Lipid with anything and cannot therefore be lowered.

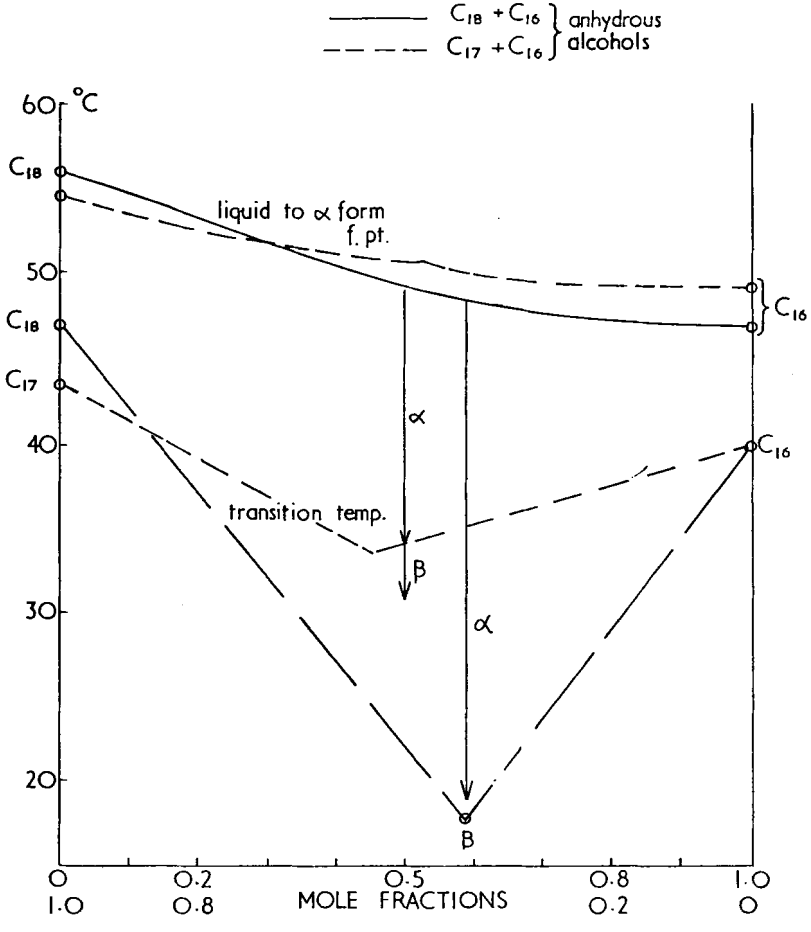


Figure 33. Freezing and transition temperatures in binary fatty alcohol mixtures.

The mixing effects which Mother Nature uses (like any other applied scientist) may be classified into two groups; phobic chain mixing and philic polar group mixing in the presence of water. In the first group, there is the mixing of the various chain lengths in a homologous series, e.g. the fatty acids which give eutectoid mixing and the alkanols which, surprisingly form solid solutions of α phase at the freezing point but eutectoid behavior for the transition from α to β. Figure 33 shows our preliminary results for the mixed C_{18} and C_{16} fatty alcohols and J. C. Smith's for $C_{15} + C_{16}$.[35] A simplification arises when we find that, on a molar concentration basis, the quarter hydrate, which is of course 0.2 mole fraction, gives the same $-\Delta T_{trs}$ as octadecanol does; i.e. we are dealing with simple colligative lowering of T_{trs} equal to the cryoscopic transition constant of the solvent multiplied by the mole fraction of the second component whatever it is. Figure 34 shows the Infra Red Spectra all at 30° where

(*a*) pure C_{16} alcohol is the γ,

(*b*) + 5% of $C_{18}OH$ is in β and

(*c*) $C_{16}OH$ + 30% of $C_{18}OH$ is in the α form.

Similar mixing of *cis* and *trans* isomers both as single substances and as different acids in the di-alkanoyl phosphatides may be expected. Nothing is yet known of the further lowering of T_{trs} which water may produce except my old observation that T_{pen} for aqueous soap solution into mixtures of stearic and palmitic acids gives a eutectoid graph below that for the anhydrous materials but with minima at the same concentration.[5]

It should be noted that these fatty alcohols, anhydrous or hydrated, have a ΔH_{fusion} considerably larger than $\Delta H_{transition}$ in contrast to the phosphatides (Fig. 36) at much higher temperature; Fig. 35 shows a typical differential cooling curve for hexadecanol. The cryoscopic constant K_f (for liquid to α solid) is therefore smaller than the K_{trans} which means that a given amount of miscible second substance must always give a larger $-\Delta T_{trans}$ than the $-\Delta T_f$; i.e. that the α form will always be stabilized in the sense of existing over a wider temperature range than in the pure substance.

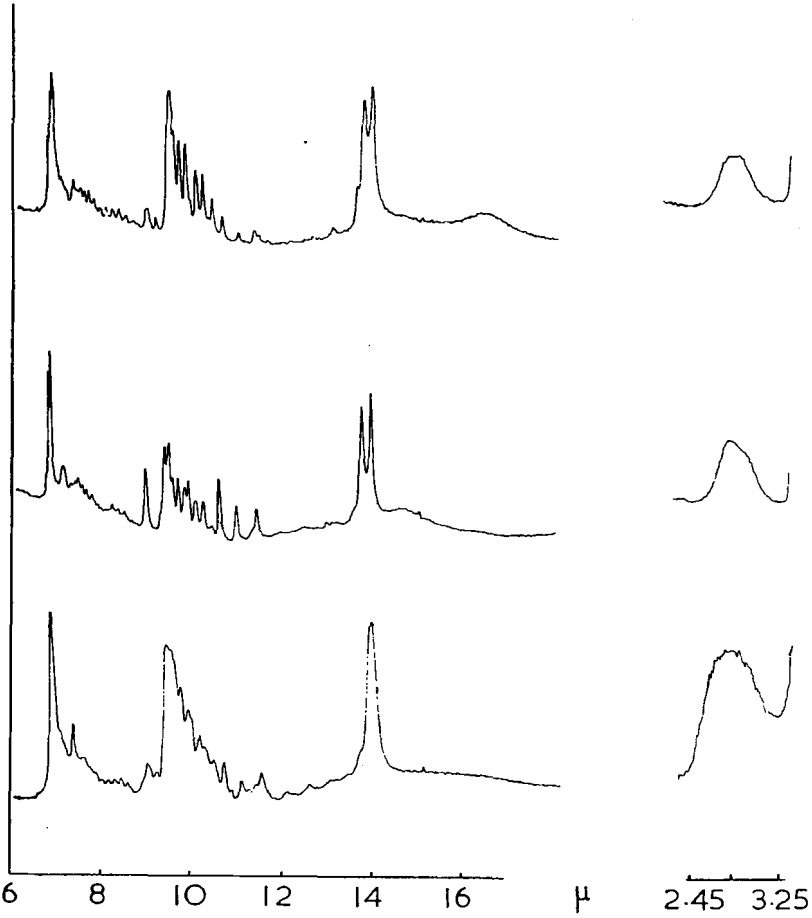


Figure 34. Infra-red spectra of (*a*) hexadecanol; (*b*) 5% and (*c*) 30% of octadecanol, att at 30 °C.

Timmermans has given a value of 8.9 kcals for ΔH liquid to α form from cryoscopic results; subtracting this from M. Davies[36] calculated value for ΔH liquid to γ form gives 5.6 kcal for the transition α to γ, a figure in good agreement with our Fig. 36.

A mixture case where the hydrocarbon chain lengths are the same but the two non-ionic polar groups different is the monolaurin-lauric acid system; Fig. 36 shows that the freezing point

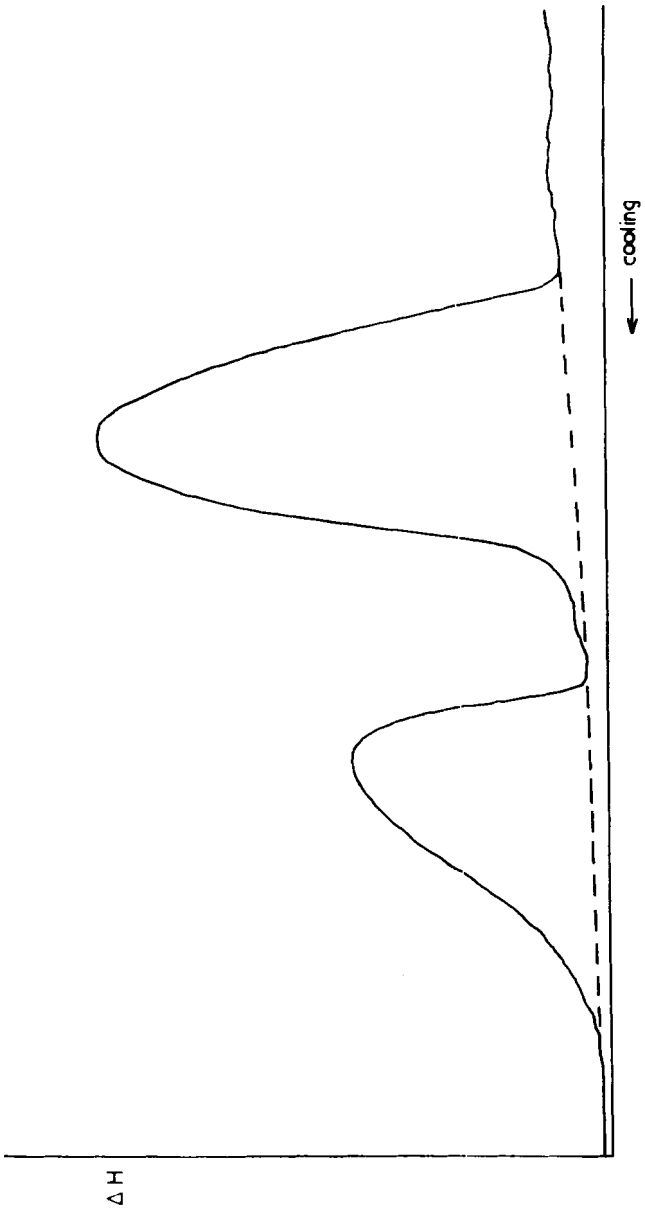


Figure 35. Differential cooling curve for fatty alcohol.

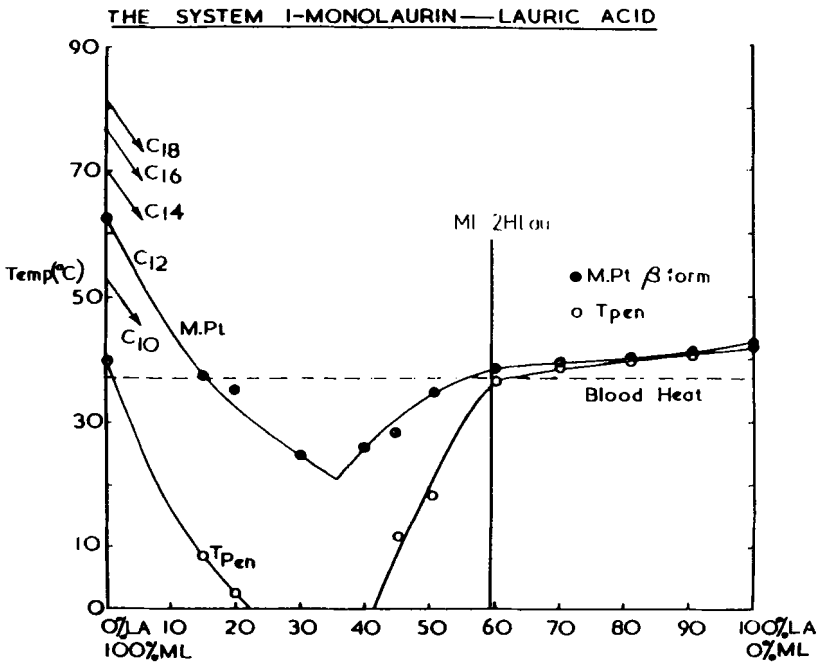


Figure 36. Mono-laurin-lauric acid system.

curve for the anhydrous materials is a rather flat eutectic while the T_{pen} one is so steeply falling that it goes below 0 °C.[2] Values for T_{pen} for the higher homologous monoglycerides are also indicated and it is clear that, for the saturated fatty acids T_{pen} is above blood heat. In the presence of fatty acid, however, it is below and spontaneous entry of water will form a liquid crystalline phase. Pancreatic lipolysis converts the triglycerides, inert to water, into the amphiphilic monoglyceride and two molecules of fatty acid; water penetrates with transient formation of l.c. which is then co-micellized by the Bile salts to form a solubilized lipid sol.[37] These Bile salts contain 2 or 3 OH groups on one side of the hydrophobic steroid ring which gives them extra philic properties, rather than soaps. The net result of all these rapid interactions is to form a liquid of low viscosity in which the lipid is very finely dispersed. It will be seen that the effect of the fatty

acid extends only to 2 molecules per molecule of monoglyceride. In practice, of course, the chain lengths are not all the same which will cause some smaller further eutectoid lowering of transition temperatures.

More complex systems in which both phobic and philic parts of the two components are different; Fig. 37(*a*) shows our results for the mono-laurin-water-sodium deoxytaurocholate system at 45° in which the binary l.c. phase is non-ionic and the Bile salt ionic; (*b*) shows D. Small's lecithin-water-cholesterol system where the binary l.c. phase is the ionic component plus water.[33] The broken tie line indicates formation of a 1 : 1 complex and, if a similar line is drawn tangentially to the top of the l.c. phase in (*a*), 1 : 1 complexing is also indicated. D. Chapman has recently shown that, in the presence of water, lecithin takes up cholesterol as a l.c. phase up to a maximum of 1 mole per mole, an observation of the highest interest in view of the large amount of cholesterol in the brain.[39] Does the mixing of the cholesterol OH group with water and the ionogenic polar group of the lecithin produce electrical effects similar to those recorded above when the alkanol OH mixes with the ionic soap group? Does the inositol (or other sugar groups) mixing with ionic phosphatidyl choline also produce such changes? Bangham's work on the effect of alkanols upon ionic permeability of liquid crystalline lecithin and water is highly relevant[40]; it also illustrates the distribution for the homologous alcohols between water and lipid.

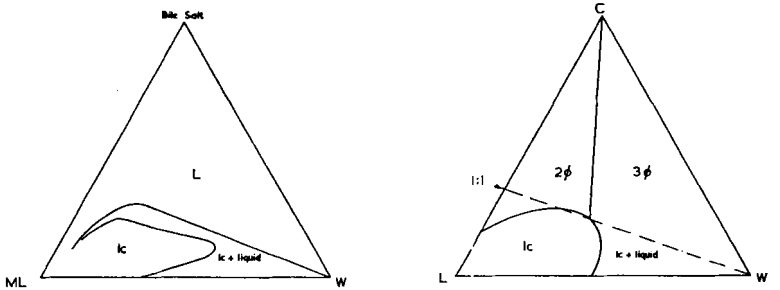


Figure 37(*a*). Mono-laurin-water-sodium deoxytaurocholate system at 45 °C; (*b*) Lecithin-water-cholesterol system.

Four component systems have been worked out for the cases; soap-water-amphiphile-hydrotrope[26] and for lecithin-water-cholesterol-Bile salt.[41]

In a famous paper presented to the Paris Academy of Sciences in 1838, Dr. F. M. Ascherson wrote[42]: " · · · our general physiological knowledge concerning fat has progressed but little, . . . a body which in the living organism is usually the faithful companion of inactivity ". Though the inactivity referred to is of the body and not of the fats themselves, we can see to-day that the problem is not physiological but the physical chemistry of amphiphilic lipids (Thudicum's classical work on the phosphatides of the brain did not appear until 1881) and of the conditions of constitution, composition and temperature under which they are in a labile physical state at body temperature; of their activity in mixing locally with water and forming layer structures in which the molecules are arranged with the regularity of thermally disordered solids or liquid crystals. A melting point to liquid is the upper temperature limit to their existence and T_{pen} the lower one below which unmixing with water occurs, and the lipid passes to its inert low temperature crystalline form. This structural and disorderly model of the interior of a membrane is much more attractive to the physical chemist considering permeability than the biologist's favourite little drain pipes.

It has often been said that the complexity of living matter is so extreme that there is no hope of interpreting it in terms of its molecular components and by the methods and rules of the exact sciences. So far as the lipids are concerned, this is nonsense; all of the lecithins in an organelle can be taken as one component; the same for the phosphatidyl ethanolamines, inositols and the sphingomyelins. We do not even want to know their mean thermal charges over a wide range of temperature: only what physical state the mixed homologues forming each family are at blood heat. We do then urgently need to know the mixture rule of each pair of terminal groups choline, etc. keeping the fatty acid chain length the same. The mixtures are, of course, in the presence of water.

The cases described in this paper are water—in lipid and they

therefore have a hydrophobic exterior but the simple rule that, of two immiscible liquids, the one in excess will always be the continuous phase does not apply to these amphiphilic lipids where there is the limited local miscibility with water. Water in excess of this must be outside and the exterior of the lipid hydrophilic to satisfy surface energy requirements but it is far from clear what determines this saturation or indeed what is the meaning of a saturation in this context.

Is then the liquid crystalline state biologically useful? Perhaps it is only that its combination of lability and lateral cohesion that is important; a single bi-molecular membrane or layer cannot be or not be liquid crystalline; this requires 2 or more piled on top of each other and, if one bi-molecular layer can be a membrane, why not a unimolecular one consisting of a very long chain say 30 methylene groups with $\alpha\omega$ substitution by two polar groups, e.g. OH or COOH? It would not work at 40 °C but it might at 60 °C or above.

The original work described here forms part of the joint research being carried out by the writer and by Dr. M. P. McDonald in the University and in the Sheffield College of Technology. Valuable assistance has been given throughout by Mrs. J. Stevenson, by Dr. A. Bingham in the use of infra-red spectroscopy and by A. al Mamun who has carried out the work on the fatty alcohols and alcohol-water systems. The vapor pressure measurements on the mono-olein-water system were made by S. Jenkinson at Chesterfield College of Technology.[43] Financial assistance from Messrs. Unilever, the Royal Society and the Science Research Council, is acknowledged gratefully.

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