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The Control of Lyotropic Liquid-Crystals, Biological and Medical Implications†

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The formation of the different phases of lyotropic liquid crystals is considered as a function of the relative importance of cohesive forces and affinity forces for water. The elementary monolayer being the building stone of these phases, the conditions governing the state of these monolayers, and particularly of mixed monolayers, are analyzed. This shows how the composition of a lipidic mixture determines its physical state in the presence of water: from the solid to the liquid crystalline state, and finally to complete solubilization in water. As biological applications, the case of the bile with formation of gallstones and that of the lipids in the arterial wall with formation of atheroma are given. Finally, the state of the lipids in the model of the cell membrane is considered from a purely physical and crystallographic point of view which fits with the biological concepts.

I INTRODUCTION

Substances of biological intérest, and particularly lipids, can form, in the presence of water, different phases of lyotropic liquid-crystals. The formation of these phases depends on the relative importance of

a) the cohesion forces between the molecules of the substance
b) the affinity of these molecules for water.

We shall examine here the conditions governing the choice of a pure substance, or better of a mixture of substances to get a mesophase of a given type. As a consequence the necessity and the importance of certain mixtures of lipids will appear from the biological and medical point of view.

The most important phase of lyotropic liquid crystals is similar to the well known smectic phase of thermotropic liquid crystals. It is formed by the

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piling up of double layers of long chain polar molecules regularly separated by sheets of water molecules. For this reason it is called the *lamellar* phase. A spectacular evidence of the lamellar structure of phospholipids swollen in water was given by Stoeckenius[1] in 1959 using electron microscopy. The preparation, fixed by osmic acid vapor, imbedded and sliced, showed the regularly superposed layers of lipid molecules.

Using the x-ray diffraction technique, Levine and Wilkins[2] gave in 1971 a precise electron density profile (Fourier synthesis) of egg-yolk lecithin bilayers in a direction perpendicular to the plane of the bilayer. On this profile, the interval corresponding to the middle of the hydrocarbon chains region clearly appears with a low electron density, while peaks of high electron density, situated on the two sides, correspond to the phosphate containing head group region of the molecule.

The pioneer work was accomplished in Germany already in 1939 by Kiessig and Philippoff[3] on the one hand and by Stauff[4] on the other. Not only did they show by x-ray diffraction that the long spacing of the alkaline salts of fatty acids increases progressively with the amount of water added, which led them to propose the lamellar structure of the liquid–crystalline soap phases, but they also discovered that the supplementary addition of a non-polar hydrocarbon such as benzene produced a new increase of the spacing. In this case, a double "sandwich" was produced, sheets of water being located between the polar groups of the oriented soap molecules and sheets of the benzene being situated between the hydrocarbon tails.

Useful informations can still be discovered in the publications of F. O. Schmitt and his colleagues[5–7] which appeared in 1941, describing the first studies on the natural phospholipids. The crude mixtures of lipids extracted from the brain, or fractions of these, were examined in presence of increasing amounts of water. The structure was recognized as lamellar and the authors emphasized that the hydrocarbon region of the double layers of molecules may be considered as essentially liquid. Many authors have since then confirmed and improved our knowledge on the liquid-crystalline structures given by aqueous systems of soaps and phospholipids. They are quoted in general reviews (see G. G. Shipley[8]).

The sonication of the lamellar phase of phospholipids in the presence of an excess of water cleaves or peels off the constituting double layers. In fact the liberated double layers close into bags because the "naked" edges, where the paraffin chains would be in contact with water, are in an unstable state. These bags enclosing water are the so-called "liposomes". Although the size of these liposomes may be as small as 250 A, the curvature of the double layer is relatively small, and the strain imposed to the long chains is compensated by the decrease of free energy resulting from the closing up of the edges.

But to introduce one curvature to form a cylinder, or two curvatures to form a sphere, with a single layer of molecules and without any hollow inside would necessitate extra free energy. Thus, because of the cohesion between the CH_2 groups of the molecular chains, the most stable form is that of the flat lamellar phase. But an enhancement of the interaction of the polar groups of the molecules with water, accompanied or not by a decrease of the cohesion, may furnish the necessary extra free energy for the system to gain one curvature and to lead to the cylinders of the so-called "middle phase", the structure of which was discovered by Luzatti *et al.*[9] With increasing affinity of the molecules for water, relative to the cohesion forces, a second curvature may appear, leading to the well known micelles dispersed in the isotropic solutions of detergent-like substances. Even in the most stable lamellar phase, the affinity for water of the molecules of a substance must be such as to compensate their mutual cohesion. This can be attained by a proper molecular constitution and structure.

It would be a very hard task to give a rigorous theory of the balance between cohesion and affinity forces in the different lyotropic liquid-crystalline phases. But a very good picture can be given by the description of the mechanism of spreading of monolayers at the surface of water. Here rigorous definitions of measurable magnitudes related to cohesion and affinity for water can be given. In fact monolayers are the building stones of the lamellar structure and the spreading of polar molecules on water or the penetration of water between the polar ends of such molecules are two aspects of the same problem.

II THE PHYSICAL BASIS

1 Free energy of cohesion and free energy of adhesion

Consider at the start a flat drop of a liquid b floating on the surface of another liquid a. In order to spread another cm^2, $2\ cm^2$ of surface of liquid b must first be formed: one at the bottom to enter in contact with a, the other on top of the advancing drop. To obtain these $2\ cm^2$, the liquid b can be split along $1\ cm^2$. This requires a work W_b accomplished at constant temperature and pressure against the cohesion forces and produces therefore an increase ΔG_1 of the free energy of the system. The adhesion of the lower $1\ cm^2$ of liquid b to the surface of liquid a will, on the contrary, liberate a work W_{ab} due to the affinity of the two liquids. This will produce therefore a decrease $\Delta G_2 = -W_{ab}$ of the free energy of the system. The process of the spreading therefore occurs only if the total variation of free energy is negative, that is if

$$\Delta G = \Delta G_1 + \Delta G_2 < 0$$

Now, W_b can be measured: it is the work necessary to create 2 cm^2 of free surface of liquid b, that is twice the surface energy of liquid b expressed in ergs/cm^2 (to which corresponds a surface tension expressed in dynes/cm.). On the other hand, the adhesion free energy of liquid b to liquid a, W_{ab}, can be defined as the work necessary to pull apart b from a along 1 cm^2, creating two free surfaces a and b of 1 cm^2 each. This can be easily calculated from the measured surface energies of both liquids and from the measured interfacial energy. In the present process of spreading, instead of being pulled apart, the two liquids enter in contact. The work is therefore negative and equal to $-W_{ab}$.

The total variation of free energy can therefore be written

$$\Delta G = W_b - W_{ab}.$$

Spreading occurs only if ΔG is negative, that is if $W_{ab} > W_b$. *This relation applies rigorously only to isotropic liquids.* Nevertheless, it expresses that spreading occurs when the adhesion forces are superior to the cohesion forces in the spreading liquid. This statement can be maintained in a qualitative way even in the case of states other than the isotropic liquid state.

Interesting properties appear with liquids having elongated molecules. In the 1910's, William Hardy, studying series of long chain substances (paraffins, long chain alcohols, fatty acids, long chain esters), found that, at a given temperature, W_{ab} against water was dependent only on the nature of the end group and that it was practically independent of the chain length (above 6 or 8 carbon atoms). Thus, at 60°, the values for W_{ab} are the following: ca 35 ergs/cm^2 for paraffins, ca 66 for esters, and ca 82 for long chain alcohols and acids. The relative importance of these adhesion free energies W_{ab} corresponds to what one would deduce by comparing the hydrophilic character of the polar groups. Indeed the increase of the solubility of the —COOH acid group by ionization (which is the case with soaps) raises the value of W_{ab} above 100 ergs/cm^2. It was therefore natural to conclude that the affinity for water, measured by W_{ab} was localized on the polar end of the molecules and consequently that the molecules were oriented by the water.

The cohesion work W_b depends both on the nature and the length of the hydrocarbon chain and on the nature of the polar group. It will be shown that the importance of the cohesion, as well as the relative importance of the affinity for water, determine the type of phase of liquid crystals.

2 Physical states of monolayers and of lyotropic liquid crystals

Monolayers can, in certain cases, undergo phase changes by compression at different high surface pressures. But this is not under consideration here.

What will be considered here are phase changes by variation of temperature in the same conditions as ordinary phase transitions, that is when the condensed monolayer phase is in equilibrium with its vapor monolayer (see References 10 and 11). Thus, at each temperature, the molecular area is rigorously defined with conditions of zero variance. In practice, the substance is spread on a surface large enough to allow the two phases (vapor and condensed) to be present. Then, because of the equilibrium, the progressive decrease of the area does not change the very weak surface pressure (constant vapor pressure). Continuing to compress, it is at the point where the whole layer becomes reduced to a single phase and where the surface pressure just starts to increase that the condensed state is defined and its specific molecular area measured.

With certain substances, at room temperature, the monolayer is *solid*. This term is used not only because the compressibility is very small, but because the monolayer does not flow and can be broken. Besides, the area per hydrocarbon chain is between 19 and 20 $Å^2$, which is very near to the cross section area per chain in the lattice of the solid crystals of hydrocarbon chain compounds (fatty acids, triglycerides, alcohols, etc.). The monolayer may also be fluid, quite compressible and show areas of the order of 45 to 60 $Å^2$ per chain. With areas of this order, the mean intermolecular distances are comparable to the mean distances between molecules in the ordinary liquid state of the same substances. The qualification of *liquid* applied to this state in monolayers is justified by the existence of an intermediate phase, also fluid but more condensed, with an area per chain which expands with increasing temperature from 24 $Å^2$ only to not more than 32 $Å^2$. To call this phase *mesomorphous* seems so much more justified that the behavior of the monolayers, when the temperature is varied, resembles very strikingly that of substances giving crystal liquids. Indeed, with a solid monolayer, when the temperature is increased, a first definite point is observed at a temperature T_1 at which the monolayer becomes fluid but shows an area per chain of ca 24 $Å^2$. From there, the area gradually expands, with increasing temperature, reaching ca 32 $Å^2$ per chain at a second definite point T_2 at which a *sudden* expansion is observed to ca 45 $Å^2$ per chain. This is the real melting point from the mesomorphous state to the liquid, while the first point T_1 corresponds to the transition from the solid to the intermediate phase.

It is obvious that the temperatures T_1 and T_2 vary with the nature of the substances as is notoriously the case also in ordinary phase transitions. Thus, these temperatures rise with the length of the hydrocarbon chain. With unsaturated chains, they are lower than with saturated chains.

Another point should be strongly stressed. The range of transition temperatures in monolayers is well below that of the melting points in the bulk for the same substances. Thus T_2 for a monolayer of palimitic acid is 30 , while

the ordinary melting point of this acid is 64°. With dipalmitoyl phosphatidylcholine (C_{16} lecithin), T_2 in the monolayer is ca 12°, while the ordinary melting point is ca 235°. It is easy to understand that in the fatty acid crystal the cohesion is strengthened by two hydrogen bonds between pairs of the polar groups, while when spread on water these bonds are already broken. With the lecithin, very strong ionic bonds between the polar groups exist in the solid crystal, so that the melting is comparable to that of a salt. While, when spread as a monolayer, the ionic bonds are dissociated. In both cases the cohesion in the monolayer is practically reduced to the Van der Waals forces between the hydrogen chains. This is also the case in the lyotropic liquid crystals: e.g. Pottassium Palmitate in the presence of water forms lyotropic liquid crystals already at 40°, when the melting point of this soap, when anyhydrous, is 380°.

The fact that the transition temperatures depend on the nature of the substances, implies that, at a given temperature—say at 37°—different substances are in different states, either in monolayers or in bulk aqueous systems. The state can therefore be controlled by the choice of a substance or of a mixture of substances. Here again the study of monolayers can help to understand the problem of lyotropic liquid crystals. From what was said above, we conclude that the choice of the chain length and of its degree of saturation (absence or presence of double bonds) determines the state of the system. In fact, it determines W_b. The choice of the polar group influences not only W_{ab} but also W_b and necessarily the spreading energy $W_{ab} - W_b$. To play on the importance of the spreading energy, a choice is possible on the relative proportions of chains and polar groups in the same molecule. Thus, triolein, with three hydrocarbon chains and without hydrophilic groups, is completely insoluble in water and does not even swell. Lysolecithin, on the contrary, disperses in water because the molecule has only one hydrocarbon chain and contains a strongly hydrophilic phosphorylated amino-alcohol group in addition to the free OH group of the glycerol. The balance is definitely in favor of W_{ab}. Lecithin stands between these two extreme cases, with its two hydrocarbon chains counterbalancing the hydrophilic character of the phosphorylated choline group. Therefore, lecithin swells to give a liquid crystal although it does not dissolve readily in water.

The same sort of balance can be obtained by spreading as a monolayer a mixture of two substances with different chains and different polar groups.[12] A two-dimensional array of molecules with different polar groups is formed, so that the hydrophilic character of the whole is more or less the weighted average of the individual affinities. Thus, W_{ab} can be varied at will with varying proportions of the components. But, the mixture of the hydrocarbon chains behaves like an alloy, showing properties which are not the weighted

average of the cohesive properties of the different sort of chains. It is well known that, in mixtures of fatty substances, the melting point, for certain proportions, is below the melting points of both components. There are therefore very unexpected actions on W_b when substances are mixed. Particular proportions exist where discontinuities appear in the properties, pointing sometimes to an arrangement in a hexagonal array: 1 molecule of X surrounded by $6Y$ and vice-versa 1 molecule of Y surrounded by 6 of X and corresponding to the proportions of 1 to 2 or 2 to 1.

3 Penetration of monolayers

So far the two substances forming the mixed monolayer were both insoluble in water. We are here interested with the case where one of the components is readily soluble. Suppose a monolayer of cholesterol is maintained under constant surface pressure and thus covers a defined and measured area. Suppose now that a very small amount of a long chain detergent (i.e. sodium cetyl sulphate or sodium oleate) is injected in the underlying water. An important increase of the area is observed in the course of time, reaching a definite value. This is similar to the change of volume which occurs when gases react under constant pressure. Here, the increase of the area corresponds to the adsorption of the dissolved molecules of the detergent which penetrate between the molecules of cholesterol, forming a mixed monolayer. It should be stressed that, in the absence of the monolayer of cholesterol, very little of the detergent, at the concentration used, would adsorb at the surface of the water. It is primarily a phenomenon of the same nature as partition between two solvents: once adsorbed, the detergent molecules are maintained on the surface by the cohesion forces between their chains and the hydrocarbon part of the cholesterol molecules. The resulting mixed monolayer is a very stable one, with a W_{ab} much more important than that of pure cholesterol, but weaker than that of the detergent. The study of this monolayer penetration was generalized by J. H. Schulman *et al.*[13,14]

4 Lyotropic phases given by mixtures

Let us now turn to the mesomorphic bulk phases obtained with mixtures. As early as 1908, C. P. White had observed, by microscopical examination, that myelin figures (a form taken by lamellar lyotropic liquid crystals) were formed at the surface of cholesterol crystals added to a solution of soap. After a time, all the sholesterol crystal would disappear as a result. From what was said above on the penetration of monolayers, it is easy to deduce that the resulting lyotropic phase is simply formed by the piling up of mixed double layers of cholesterol and soap molecules. Cholesterol alone is insoluble in

water, soap is completely soluble, but the mixture of the two has the right balance between cohesion and affinity for water to swell without dissolving and to give a lamellar liquid-crystalline phase.

This was generalized and mixtures of more than 50 pairs of substances (one insoluble and the other soluble) were examined in the presence of water by Dervichian, Joly and Magnant.[15–17] According to the relative importance of cohesion and affinity for water, the mixtures gave lyotropic liquid-crystalline phases, or an isotropic rather concentrated phase in equilibrium with the excess of water (coacervation) or produced complete solubilization. More recently quantitative studies were undertaken by different authors, establishing complete phase diagrams. Just one example will be given with some details as it will serve to illustrate a biological and medical application.

Lecithin by itself, in the presence of water, swells to give the lamellar lyotropic phase. This swelling is limited since, because of the presence of the two hydrocarbon chains in the molecule and despite the action of the very hydrophilic phosphorylated choline ionic group, no more hydration is possible. But, nevertheless, the affinity for water is strong enough to afford the addition of cholesterol molecules between the lecithin molecules and still obtain the lyotropic lamellar liquid crystal. Indeed the polar OH group of the cholesterol is far from being as much hydrophilic as is the polar group of the lecithin and, with the mixture of the two, the overall value of what is symbolically represented by W_{ab} is evidently reduced. But, at the same time, the perturbation introduced by the penetration of the steroid hydrocarbon skeleton of cholesterol between the hydrocarbon chains of lecithin must probably reduce the cohesion term W_b. As a consequence, up to one molecule of cholesterol per molecule of lecithin (i.e. one steroid hydrocarbon skeleton for two aliphatic chains) can be added in the lyotropic lamellar phase. Any additional cholesterol remains out of the phase.[18,19]

What happens if, on the contrary, a molecule much more soluble than lecithin itself is added. This is the case with sodium cholate, a bile salt, which has an ionized carboxylic group at the end of the molecule and three OH groups located on the steroid skeleton. At room temperature, the solubility of sodium cholate in water is 55 per cent. Here again a certain quantity of this substance can be accommodated in the lamellar phase with the lecithin molecules. But with more than one cholate for two lecithins and if enough water is added, the structure turns to that of the cylindrical (or hexagonal) liquid crystalline phase. If, continuing to increase the proportion, more than 5 molecules of cholate for 3 of lecithin are added, an isotropic solution is obtained where lecithin and bile salt are dispersed under the form of mixed micelles. The complete ternary phase diagram was established for this system[20] by varying the proportions of each of the components (lecithin, bile salt and water) from 0 to 100 per cent and determining the nature of

the different phases (or mixture of phases) both by x-ray diffraction and by optical observation with the polarizing microscope.

Even at its saturation concentration (55 per cent) sodium cholate enables only 1 per cent cholesterol to dissolve in water. But, in the presence of lecithin and bile salt, much larger quantities of cholesterol can be solubilized in water. In fact, cholesterol can be incorporated into each of the various phases of the ternary system water—bile salt—lecithin without changing their general structure. It simply modifies the frontier of their corresponding areas on the diagram. Thus, according to the proportions of the other components, up to 25 per cent cholesterol can be incorporated in the lamellar phase, 6 per cent in the cylindrical (hexagonal) phase, and 4 per cent in the isotropic solution. The phase diagram of this quaternary system[21] is normally represented by a tetrahedron (i.e. a pyramid with four triangular faces), the four apexes correspond respectively to 100 per cent of each of the four components: water, bile salt, lecithin and cholesterol. These are the four main components of bile in the gallbladder and here we begin to approach some biological and medical applications.

III BIOLOGICAL AND MEDICAL APPLICATIONS

1 Bile and gallstones

The presence of both lecithin and bile salt explains therefore why, although containing 0.5 per cent cholesterol which is practically insoluble in water, normal bile is a clear solution. In fact, there is about 92 per cent water in bile and in order to study the effect of the variation in proportions of the other components, it is necessary to examine the section of the phase diagram tetrahedron by the plane corresponding to the constant proportion of 92 per cent water. This is an equilateral triangle with the three apexes corresponding respectively to bile salt, lecithin and cholesterol. On this triangle appear the outline of the region where the system is an isotropic solution. With any composition outside the border line of this region, other phases appear which are either the solid cholesterol crystal or one of the liquid-crystalline mixtures.

How does this compare with medical facts? The answer is unambiguous. Analysing the composition of the bile of a large number of normal and diseased subjects, Redinger and Small[22] found that the points representing the composition for all normal gallbladders fell inside the isotropic solution region on the triangular diagram and for some on the border line itself. The points for all bladders containing gallstones fell definitely outside. An important remark is that the formation of gallstone does not necessarily

correspond to an excess of cholesterol, but sometimes to a relative decrease of the lecithin. As the physical-chemical diagram shows, it is a matter of relative composition.

2 Atherosclerosis

Atherosclerosis lesions are characterized by the accumulation of lipids, specifically cholesterol and its esters in the *intima* (i.e. inner layers) of large arteries. Phospholipids are naturally also present. It appears from the data of E. B. Smith and her colleagues[23–25] that the lipid composition of the intima is a function of age. The cholesterol esters (linoleate or linolenate) at first absent below the age of 15, increase in quantity with age. The histological aspect of the inner layer of the arteries also changes with age: fat droplets and crystalline deposits appear which become very important in the atherosclerosis lesions.

Small and Shipley[26] have drawn the attention on the physical state of the lipid deposit (solid, liquid-crystal, liquid) as a function of the composition. First of all, they have examined artificial mixtures of the natural lipidic components of the intima. Varying the proportions of cholesteryl esters, cholesterol, lecithin and water, they have established a quaternary phase diagram in the form of a tetrahedron. Except for the lamellar phase rich in lecithin which can contain up to one molecule of cholesterol for one of lecithin (i.e. 33 per cent), the other regions with a single phase are rather small. While cholesterol esters, by themselves in the anhydrous state, give well known thermotropic liquid crystals, they cannot be readily incorporated in the structure of the lamellar lecithin + water phase because of the absence of a hydrophilic group in their molecules. Only 2 to 3 per cent are incorporated in the presence of water. When more than 2 to 3 per cent cholesterol ester is present, the excess ester separates as an oily birefringent phase. This oily phase can dissolve up to about 8 per cent cholesterol.

Here again, since the arterial intima is basically an aqueous system containing about 60 to 70 per cent water by weight, the section of the tetrahedron diagram corresponding to a constant 70 per cent water composition must be examined for comparison. This triangular section with respectively cholesterol ester, lecithin (or some other phospholipid) and cholesterol at its apexes shows on each side two very narrow zones, one corresponding to a lamellar liquid-crystalline phase containing mainly phospholipid and varying amounts of cholesterol ester and cholesterol, the other corresponding to the oily liquid phase of cholesterol ester containing up to 8 per cent cholesterol. Between them is a wide region where these two phases exist in equilibrium. All the remaining surface of the triangle represents a region of three phases: the lamellar phase saturated with cholesterol and cholesterol ester, the oily

cholesterol ester phase saturated with cholesterol and thirdly cholesterol crystals.

If now normal and pathological dissected sections of arteries are examined[23–27] and a parallel study of the composition is made,[27] it clearly appears that the different aspects taken by the intima at different ages, with or without lesions, are mainly related to the states of the lipidic components of the tissue and that these states correspond to those predicted thanks to the phase diagram, when the composition is known.

3 The cell membrane

The most speculative application will now be considered. For many years, biologists have been led to the idea of a membrane surrounding the living cell, constituting a "wall" separating the living organism from the environment and working as a sort of "filter" discriminating between substances, allowing some to enter other to go out. The adoption of this idea has been encouraged by the electron microscope pictures of cells showing a thin region on the border of their section, having a thickness of the same order as a double layer of lipid molecules.

The first naive model of a membrane with pores of different diameters working like a sieve was later replaced by a more complex one with lipidic regions and protein regions to allow the passage of both lipid soluble and water soluble substances. Nowadays, the phase changes in lipids with the composition having been more commonly known, biologists and biophysicists are considering membranes with regions corresponding to different phases. The problem of phase *separation* (to be distinguished from phase *transition* as underlined by McConnell) is examined particularly by the group of McConnell,[28] studying the lateral diffusion of molecules in artificial or natural lipid layers by different methods and especially by paramagnetic resonance spectroscopy with a spin label molecule.

IV CONCLUSION

Two important points have to be stressed to understand the surface properties of living cells. What we call the "membrane" must be plastic or fluid: both in the proper and in the figurative sense of the term.

1) First in the proper sense. The surface of the living cell undergoes uninterrupted movements, ameboid movements, pinocytosis, which prove its fluidity or plasticity. We saw how, as soon as a solid phase appears, it is rejected by the living organism: the insoluble solid cholesterol is rejected as gallstones in the bladder and as plaque formation in atheroma. On the

other hand, the exchange of the membrane components would be considerably slowed down if they became solid: it is well known that lipolytic enzymes do not act on solid lipids.

2) Secondly, the membrane must be fluid in the figurative sense. Here the formation of mixed monolayers by penetration and association helps to understand. There is not a once and for all established membrane; lipids are continually renewed at a very rapid rate. Lipolytic enzymes are on the spot hydrolyzing glycerides and phospholipids and liberating fatty acids. Other enzymes are present which transfer fatty acids from lipids to cholesterol to make cholesterol esters or resynthesize fats and phospholipids.

Besides, by the mechanism of monolayer penetration, a continuous exchange occurs between the lipidic bilayer and the lipids circulating in the fluids which bathe the cells: lipids which are solubilized either under the form of lipoproteins or under the form of micelles. Exchange occurs also with the lipids synthesized or stored inside the cell.

In fact, as far as the lipids are concerned, their permeation into the cell or out of the cell is bound to this continual renewal of the lipid membrane itself, the equilibrium being continuously broken by the association in the bilayer with new coming lipid molecules, while others leave the bilayer as a consequence of the hydrolysis or synthesis under the action of enzymes.

This sort of mechanism has recently been proposed by Scow[29] for the uptake of lipids by the fat tissues of the body from the blood stream. It seems that there is a continuous lipid membrane going from the surface of the chylomicrons to the surface of the fat cells, working like a travelling band, carrying the fatty components which have first been hydrolyzed and which eventually, when reaching the tissue cells, are resynthesized into esters to be stored.

The study of the penetration of monolayers by molecules coming from the underlying solution shows that the composition of the resulting mixed monolayer cannot be indeterminate: the addition of new coming molecules can occur only in certain proportions. This is with binary mixtures and it is probable that, with a larger number of components, the composition of the monolayer may be more versatile. Nevertheless, from a purely physical-chemical and crystallographic point of view, here again the components cannot be in any proportion: any excess is rejected, any want is provided. As far as the cell membrane is a lipidic double layer, its composition must be submitted to these conditions which sound very like the regulation familiar to biologists.

Thus, purely physical-chemical and crystallographic considerations lead to the conclusion that what we call the structure and the composition of the membrane is simply a description of a steady state equilibrium. This is

indeed one of the basic aspects of living systems. The doubly characterized fluidity is marvellously accomplished by the crystal-liquid property of lipids and especially of phospholipids. Liquid crystals stand between the isotropic liquid phase and the strongly organized solid state. Life stands between complete disorder, which is death and complete rigidity, which is death again.

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