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Change of Phase and Change of State in Biological Systems

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Insofar as liquid crystals can exist in lifeless organic substances as well as in living systems, we have to differentiate for the purpose of this lecture between inanimate and animate matter; in other words, we have to ask what, insofar as the liquid crystalline state is involved, is the unique feature of living matter or protoplasm. This question is ultimately part of a much greater riddle but our immediate problem is to see whether the liquid crystalline state is an integral or merely an incidental feature of cellular and extracellular substance in living organisms.

We can start this enquiry with the fairly obvious assumption that life depends upon the formation, presence and exact replication of certain macromolecules, notably of lipids, nucleic acids and proteins (Table 1). While some of these macromolecules can be preserved intact in dehydrated form, we know that biochemical reactivity is demonstrable only when water is present. We can therefore define an elementary or first order of biologic reactions, such as enzymatic hydrolysis and synthesis, gaseous or ion exchange and diffusion, which will proceed in simple or colloid solutions, but it is obvious that the structural conditions necessary for the formation and individual function of discrete cells and tissue cannot be met in solutions or, indeed, in any other single phase of matter. The need for boundaries between cells or tissues, and for more than one kind of subcellular component with a distinctive structural and biochemical individuality, gives rise to the conditions for what we might call the second order of

TABLE 1 Structure and approximate orders of magnitude of organic bio-molecules

Molecular Wt.	Structure	Maximum dimension†	Lyotropic phase	Examples
50–1000	Simple molecules, monomers and dimers	10 Å	Simple solutions, colloids, gels, micellar dispersions, ternary mesophase	Amino acids, small peptides lipids
1000–100,000	Small polymers (chain or cyclic)	5 × 10 × 500 Å	Colloids, gels, binary mesophase	Antibiotic peptides, DNA, RNA subunits
10,000–100,000	Folded or coiled polymers	50 Å³	Colloids	Globular proteins, enzymes
50,000–1,000,000	Homogeneous agglomerates	> 100 Å³	Gels Binary mesophase	Hemoglobins Collagen
> 1,000,000	Heterog agg.	> 200 Å³	Quaternary mesophase, gels	Lipoproteins Nucleoproteins Mucopeptides

† See ref. 57.

biologic reactions, namely those proceeding between reactants in different phases. The simplest examples of second order reactions are seen in osmosis, surface tension and phase-transformation, which is where the liquid crystal enters the picture.

Phase-dependent Reactions

If two substances in different physical states or phases are going to react with each other, the number of molecules reacting is a function of the intimacy of contact or mixing. This is well-shown by the action of a hydrolytic esterase upon a water-insoluble substrate.[1] Dispersion of the substrate, by increasing the interfacial surface, accelerates and increases hydrolysis by several orders of magnitude. This is a common mechanism in extracellular reactions such as digestion, absorption and transportation of triglycerides but, for intracellular reactions, the dispersion of reactants must be stabilized. This brings us to a third order of biologic reactions, in which the essential condition is the establishment of some order and dynamic equilibrium between the phases of the reactants, as in the distribution of subcellular particles such as ribosomes and lysosomes. Myoglobin in its natural state in muscle provides a palpable example of how phase is linked to function: excitation, mediated by high-energy phosphates, alters the arrangement of elongated molecules with corresponding increase in viscosity which accounts for contraction. This system is, in fact, a comparatively simple lyotropic mesophase which Elliot[2] is describing in more detail. The biophysical condition governing dispersion of similar substances is discussed by Mishra, Ambrose, and others in a paper presented at the Liquid Crystal Conference.

A fourth order of reaction, and a big further step in biologic organization, occurs when more than one type of molecule is included in one or more of the phases in a preferred orientatin. This is seen typically in cell membranes in which phospholipid and protein are the essential components. One of the simplest approaches to this is to bring phospholipid in its disordered semi-solid state, as in lecithin, into contact with water, in which case a

mobile smectic mesophase forms spontaneously with water molecules between the ordered bimolecular layers of phospholipid. If sodium or calcium salt solution is substituted for water, the ordered structure becomes firmer to form a membrane at the boundary, through which certain ions can travel. The hydrophilic polar groups can bind considerable volumes of water but the stability and mobility of the whole system is dependent upon the degree of saturation and number of carbon atoms in the lipophilic hydrocarbon chains as well as upon temperature. Biophysical models highly relevant to this situation have been studied in detail by Derviehian,[3] Luzzati,[4] Lawrence,[5] Mauro,[6] and their colleagues. These dependencies must influence profoundly the formation and function of even the simplest bio-membranes, especially in poikilothermic forms of life. The composition of natural lecithins, formed bio-synthetically, varies according to the fatty acids available for the two side chains but it is of significance that even the long-chain saturated palmitoyl and stearatoyl phosphatides are mesomorphic at temperatures up to about 41 °C, a temperature above which organized cellular function comes to a stop in all metazoa and most protozoa. Some of the implications of variations in the structure of lipids and lyotropic mesomorphism of lecithins have been thoroughly reviewed recently by Chapman[7] and his colleagues. In the solid or semisolid state, the conductivity of natural lecithins is relatively high, too high for instance to register a difference after the injection of electrons[8] but, in the lyrotropic mesophase, Thomas[9] has shown that the conductivity is extremely low, suggesting that the micellar aggregates are large and uncharged. Electron micrographs of membranes in intact or fragmented cells fixed in potassium permanganate and stained with osmium tetroxide usually show two electron-dense zones corresponding presumably[10] to the reaction sites of the osmium with unsaturated bonds in the lipid tails of the innermost bimolecular layer. This appearance is consistent with the X-ray diffraction pictures of Stockenius,[10] Baer,[11] Finean,[12] Robertson,[13] Napolitano,[14] and their colleagues which show spacings of a bimolecular leaflet with water between the lipid molecules. Finean,

however, has shown[15] that saturated bio-lipids such as cephalin give electron-dense zones after treatment with osmium; this finding, later confirmed by Stockenius[16] suggests that the osmium-positive zones represent a reaction with the lipophilic polar ends of the molecules.

In studies such as those quoted above, membranes are usually treated and visualized as static layers. It must be remembered that, in its natural state, a membrane is an essential component of a living cell or subcellular element. As such, it requires fluid and oxygen. It has a limited thermal range and usually perishes if subjected to mechanical disintegration, high vacuum, dehydration or embedding. It does not normally encounter substances such as potassium permanganate, gluteraldehyde, formalin, osmium tetroxide, lead or uranium acetate. Yet most of our histological and ultrastructural information is obtained from cells or cellular components processed under these conditions not so much by physicists or chemists, who do not pretend to understand cells but by biologists, who do. We must remind ourselves that a living cell is very much greater than the sum of its parts and that, if fractionated or fixed, it cannot be put together again: it is Humpty-Dumpty, translated from the nursery into the laboratory.

To understand what makes the biologic clock tick, more specifically, what is the difference between a cellophane membrane and a cell-membrane, we must therefore interpret structural information strictly in accordance with functional happenings. For instance, it has been known for years that some surface-active substances, such as bile salts, detergents and saponin, cause lysis of erythrocytes. A similar effect is produced by sonication, after which the erythrocyte envelopes can be separated, more or less intact, from the liberated hemoglobin and electrolyte solution. Dourmashkin *et al.*[17] showed that the saponin effect was due to the production of pores in the red cell membrane; Bangham and Horne[18] and Glanert *et al.*[19] showed that saponin had a similar action on phospholipids. Working with experimental models simulating biophysical conditions, Luzzati and his colleagues,[20] Cass and Finkelstein,[20] and Derviehian *et al.*[3] have shown that

various ordered molecular arrangements are compatible with the properties and structure found in membranes. If such studies are interpreted along with more recent electron microscopy,[13,14,10] the case for the " Unit " membrane as a uniform bimolecular structure disappears, to be replaced by the concept of a membrane as a systematic packing of micelles in various arrangements e.g. hexagonal, with long water channels forming pores of 4 Å diameter or multiple layers in which the hydrophilic groups may be at opposite poles or in apposition. Even where a " Unit " or Danielli-Davson model is applicable,[22] it seems highly likely that the outer hydrophilic groups are dissolved in or conjugated with water-soluble parts of protein or carbohydrate molecules.

This wider concept is in accordance with other experimental evidence[23] showing that membranes are affected in appearance and function by agents such as phenol, resorcinol and tannic acid, which disturb hydrogen bonds; by anionic or cationic surface-active compounds or polymers, which alter charge or water content; and by fat-soluble substances which upset phase-equilibrium and dissolve in the cell substance. Studies on bacteria by Gale[24] showed that cell membranes regulated electron transport and the selective passage of amino-acids into the cell substance. Since then, it has been established that, in free-living cells at least, the limiting cytoplasmic membrane regulates not only osmotic tension and permeability which might be expected of a relatively simple smectic lattice but also enzymatic work[25] and biosynthesis of complex molecular such as lipopolys accharides at the cell surface,[26] which necessitates a much more complex structure. From these and many other biochemical or biophysical studies it is becoming clear that the concept of a " Unit " membrane or bimolecular leaflet serves best as a basic model which illustrates one form of alternation of hydrophilic and hydrophobic components in the ultrastructure. To convert this into a living membrane, it is necessary to add to this structure differently–ordered systems of protein, carbohydrate, electrolytes and possibly other more reactive components which, if they are to fulfill a structural as well as a recurring functional role, must be part of the same ordered, lyotropic mesophase.

In natural membranes, the mesophase is ternary or quaternary, with another lipid, protein or glycoprotein as additional components.[22] This complicates the structure considerably, even in model systems, raising the molecular weight of the elementary micelle from below 100,000 to above 1,000,000. Conductivity and viscosity are also altered but, with suitable proportions, a stable equilibrium and elastic resistance to deformity can be reached. This is the outstanding property of biostructural units, that they can maintain the molecular orientation of their functional components during changes in pressure, mobility, ionic strength, and other forces. To meet these conditions, the most stable membranes are those in which protein is the third component, forming an ordered trilaminar protein–lipid–water sandwich consisting of two electron dense layers each about 20 Å thick and an intermediate zone of 35 Å. This is the usual concept of the " Unit membrane " composed of closely-packed hexagonal micellar subunits with intervening pores of 4 Å diameter. The packing of the subunits, and hence the permeability of the membrane, is altered by addition of surfactant toxins (Fig. 1) or amphiphiles; the former by opening the pores, as saponin does with red cells[17] and artificial phospholipid membranes;[18,19] the latter by forming further complex mesoforms which exhibit a kind of self-replicating growth in experimental models. The work of Wallach[27] on Ehrlich ascites carcinoma cells and of Palade[28] on various other cells shows that membranes studied by the usual techniques of separation are liable to contain fragments of endoplasmic reticulum and ribosomes; differential centrifugation causes the " membrane " to separate into diverse vesicles suggesting that the cell surface may not be a " membrane " at all but a mosaic of discrete macromolecules, which is what would be expected if the cell surface is regarded as an interfacial mesophase formed as described above. This would cast doubt on the whole concept of the " unit membrane " but it should be remembered that the supposed structural layers revealed by electron micrography, even on separated membranes[26–28] are not necessarily free from cytoplasmic elements and, even if they are, the electron-dense layers are those which

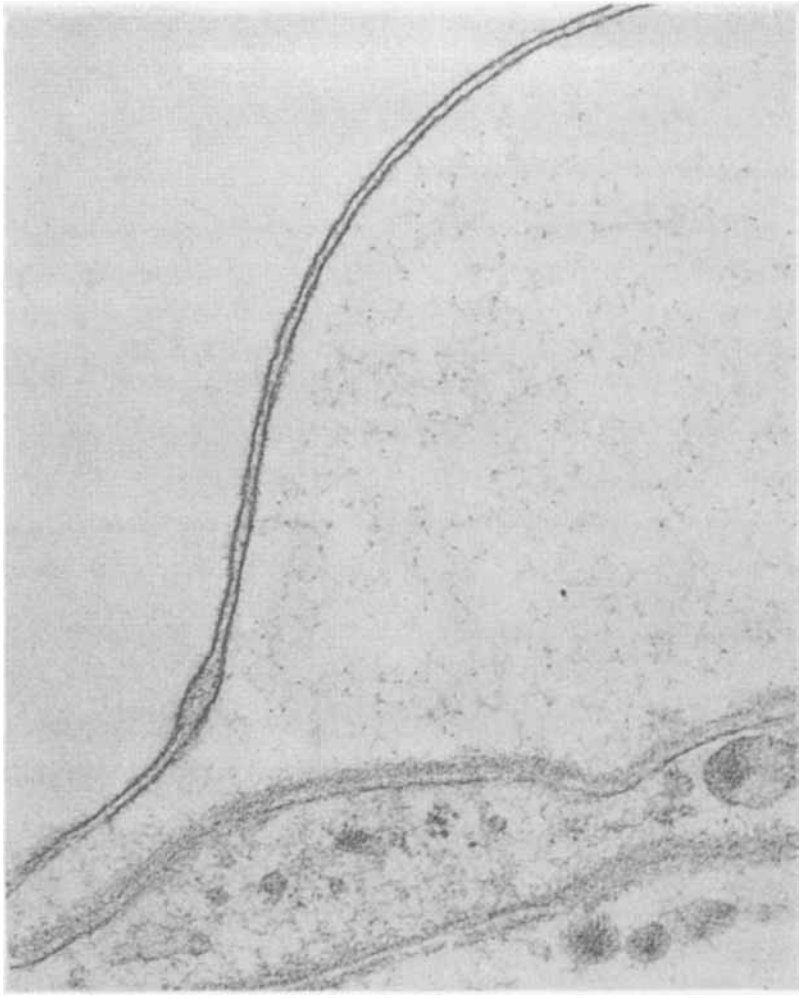

Figure 1. Passage of Ferritin particles (55 Å) through "Unit membrane" of pulmonary alveolar cell (X95,000) in rabbit after administration of toxin from *B. anthracis*. (Experiment and electron micrograph by Dr. F. Dalldorf at Univ. of North Carolina.)

happen to bind with osmium or metallic ions used in the preparative process (Figs. 1 and 6). In the living cell the membrane must be one kind of structure if the cell is free (blood cells, protozoa), another kind of structure if it is an interface in a protoplasmic

continuum (embryonic blastocyst) and yet another if it is a free ectodermal or entodermal surface, as in epithelium and endothelium. But, whatever the situation of the cell, we know that water, protein and lipid at least are essential for the integrity of the surface structure; yet the viewing techniques used for study of ultrastructure depend basically upon dehydration, which removes the first essential component, upon fixation or fractionation, which denature the second and, often, upon treatment with organic solvents, which extracts the third. It is not surprising that there are gaps in our knowledge about membranes. This difficulty is avoided by more scrupulous techniques for examining cells in their natural state *in situ* instead of *in vitro* e.g. in experiments designed to test functional responses, as in Fig. 1.

Change of Phase

It is obvious that change of phase, from solid to gas and at numerous intermediate points, is a necessary and continuous part of all vital cellular and extra-cellular processes. We are not concerned here, fortunately, with the liquid-gas transformation but this does not remove from us the immense task of contemplating innumerable possibilities in solid–liquid and liquid–liquid phase change in living systems; the only provisos are that such changes must involve ordered substances present in or related to protoplasmic constituents and that the energy produced or consumed by any one change must be within the relatively narrow limits compatible with Life, whatever that is in physico-chemical terms. It is simpler to meet those conditions by studying examples than by further generalization.

Phase Change in Physiologic Lipids

The absorption, transformation, and utilization of various forms fat in warm-blooded animals illustrates the extent and complexity of phase-change in biologic systems. Fat is ingested by human beings as a solid (e.g. yolk of a hard-boiled egg), semi-solid

(butter) or liquid (salad oil) either in fairly pure form, as in the examples quoted or, more often, as an emulsion (milk) or stable semi-solid mixture (as in many cooked or processed foods). For absorption in the intestine, dispersion is necessary but not solubilisation; in this respect, fat behaves differently to protein, carbohydrate, and all other essential nutrients, which are absorbed in solution. The first phase change is therefore from a semi-solid to an emulsion or micellar solution in a non-ordered or low-order form. Absorption via lacteals into lymph and thence into the blood stream involves another phase change, namely the formation of the " Blood dust " or chylomicrons of alimentary lipemia. Again, this is an easily-reversible non-ordered micellar solution, the formation of which is governed by the conditions favoring equilibrium of this phase at 37 °C. Normally, chylomicrons (Table 2) consist mainly of triglyceride which are either dispersed as such directly into tissue cells or hydrolysed by " Clearing factor " comprising a lipase and its activator.[29] If this mechanism operates normally, the bulk of the expendible circulating fat is removed for anabolic storage or is consumed metabolically.[30] The need for conversion to an ordered system arises only when it has to be incorporated integrally into the structure of cells and tissues; abnormally, the ordered phase can occur in the blood stream or in the wrong tissues and thereby lead to disease. Indeed, in many ways, the growth and vitality of a cell depends upon regulation of the composition and distribution of the ordered phase while degeneration and senesance are represented by the reverse, i.e. lack of this regulation which, to some extent, appears to be a purely physico-chemical process. In many tissues, fat is stored as a mixed solution of varying proportions of saturated triglycerides; such tissues usually have no function beyond that of an adipose protective cushion or insulator; hence ordered structure, which is found in biologic situations essentially where structure is combined with function at the molecular level, is absent in adipose storage cushions. Curiously, the same chemical forms of fat which are disordered in one situation enter readily into ordered structures in other situations; presumably, the force

TABLE 2 Approximate percentage Composition (W/W) of Lipid Fractions in Plasma

Fraction	Protein	Phospholipid	Cholesterol†	Triglyceride	Free fatty acid	Average Density
Chylomicrons	2	7	7	80–84	4–0	< 0.94
β-lipoprotein (lowest density)	10	20	20	50	Trace	0.94–1
β-lipoproteins (other fractions)	20	20	50	10	Trace	1.03
α-lipoproteins	50	20	20	10	Trace	> 1.063

† Including esters.

TABLE 3 Properties of Biologic Mesophase in Human Tissues

	Smectic	Cholesteric		
	Erythrocyte membrane	Lipoprotein	Myelin	Atheroma
Mesocthermal range °C	4–42	– 8–42	0–42	0–42
Sonication	Very unstable	Unstable	Unstable	Unstable
Dehydration	Unstable	Unstable	Unstable	Unstable
75,000 g	Unstable	Stable	Stable	Stable
150,000 g	Unstable	Unstable	Unstable	Unstable
Y-radiation	Very unstable	Stable	Stable	Stable
Lipolytic enzymes	Very unstable	Unstable	Unstable	Stable
Surfactants	Very unstable	Unstable	Unstable	Relatively Stable
Glycerol	Stable	Stable	Stable	Stable
Organic Solvents	Unstable	Unstable	Unstable	Unstable
S_f	—	12–200	0–20	12–20
Unit of structure	Hexagon	Spherule	Cylinder	Spherule
Gross structure	Lamellae	Tubules†	Lamellae	Helices

† Dependent upon cholesterol content—low density β-lipoprotein (S_f 12–20) may be viewed by interference microscopy as symmetrical, dimpled spherulites with optical sign positive, suggestive of a helical structure.

and information feed-back governing this is at least more complex but not necessarily more metaphysical than the forces of phase equilibria. Yet if the phase equilibrium is disturbed by a simple physical agency (Table 3) sufficient to loosen the weak bonds maintaining the ordered liquid crystalline state, the result physically is the same as alteration in phase, but, biologically, it is disaster or death of that cell or tissue. To try to define death is outside the terms of reference of this contribution but, in physicochemical terms it can be said that death of a cell or tissue can be simply a loosening of weak intermolecular forces and of the ordered state, unaccompanied by any fundamental chemical transformation. In this fashion, a cell may die from exposure to relatively mild stresses while surviving what are, in physical terms, severe stresses: thus, red cells and many bacteria are

" killed " by immersion in water though they may survive exposure to more complex, reactive chemicals or to other physical agencies.[31] If we regard the cell membrane as being a laminar smectic structure of phospholipid and protein, it is easy to understand how water can have this devastating effect, simply by being attracted to the hydrophilic polar groups of the phospholipid beyond the capacity of the internal lattice.

The most fundamental role of fat, therefore, is its incorporation in ordered structures responsible for the integrity of cells, from the membrane inwards, including such essential components as mitochondria, Golgi apparatus, and various functional micelles. The systems concerned in these subcellular particles are all lyotropic as described by Ekwall,[32] Ambrose (in a paper presented at the Liquid Crystal Conference) and others so it is obvious that there must be differences in the types of lipid needed in cold-blooded and warm-blooded species. Cold-blooded animals in general use fats which are semi-solid or liquid within the thermal ambience of their environment; lowering the temperature slows transportation and utilization of their cellular and depot fat, hence in winter they have to cut down oxygen consumption or perish. The range of lipids eligible for cellular function under these restricted conditions is limited, hence the metabolic potential and genetic diversification of cold-blooded species is correspondingly limited. In warm-blooded species a much greater range of complex fats can be accommodated and utilized, hence the greater adaptability and higher evolutionary progress of birds and mammals compared with fish and reptiles. Cerebral function especially calls for a great variety of lipid-mediated intracellular reactions and it is therefore no surprise to find that the higher levels of cerebration occur only in species such as anthropoids, canines, and porpoises which are not only warm blooded but have stable, highly-regulated temperature maintenance and an omnivorous tendency leading them to consume naturally a wide range of long-chain glycerides which provide side chains for phosphatides, cholesterol esters, cerebrosides, and other polar fats whose orientation and reactivity are dependent upon the lipophilic component of their molecules. Finean[12,15] has shown

that myelin contains a fixed ratio of phospholipid, cerebroside and cholesterol; but these are all complex lipids which, in tissue, are esterified with fatty acids of varying chain length. The consistency and thermal stability of the ordered phase in tissue therefore depends also upon the fatty acid composition which in turn is influenced by exogenous sources of lipid available during formative growth together with hepatic conversion of the available lipds into required lipids. A great deal is known about the metabolic transformations of lipid biochemically in this connection but very little about the phase transitions necessary for transportation and utilization at the cellular level. The biophysics of growth and development is one of the most neglected areas of fundamental research in cell biology but studies like those of Mishra (in a paper presented at the Liquid Crystal Conference) and Chapman[7] point to new ways in which this problem can be approached.

The Mesophase in Neural Tissue: Narcosis, anesthesia, and hallucination

In view of the predominance of complex lipids in nerve cells and their supportive structures, it is not surprising that most of the substances known to cause alterations in neural function are lipid-soluble. The Overton-Meyer[33] theory postulates that the anesthetic effect of a chemical is proportional to its fat solubility and will commence, independently of chemical structure, when that substance reaches a certain concentration in the integral lipid of the nerve cell. According to their anatomic type and location, nerve cells vary in susceptibility which is influenced also by molecular size and free energy of absorption of the chemical in the lipid phase.[34,35] An alternative hypothesis, advanced by Pauling[36] and Miller[37] is that narcotic and anesthetic effects depend upon the ability of the chemical to form hydrates stabilized on the side-chains of proteins in the aqueous phase of the cell-substance. Bangham and his colleagues[38] found that chloroform and ether caused increased loss of K^+ ions from an artificial

membrane-like phospholipid mesophase. A similar effect is observed with straight chain *n*-alkyl alcohols ($n = 4$–8) and, since it is known that anesthetic and narcotic effects are proportionately associated with loss of K^+ ions, this finding suggests that a change in the function of the cell might arise in the first place by the amphiphilic lamellar phospholipid in the membrane and in the cell taking up the fat soluble substance and, in so doing, losing electrolyte from the intermediate aqueous layers. New knowledge about phase-solubility has therefore extended the classical concept that anesthetics like ether, chloroform and cyclopropane owe their action to their fat solubility, that is to say to their ability to enter the lipid mesophase of neural cells and somehow disturb the state of metabolic and physical equilibrium which we call consciousness. Alcohol acts similarly but more subtly and it seems that the growing list of sedatives, tranquilizers and hallucinogens might also provoke their peculiar effects by entering the lipid mesophase in brain cells. It is tempting to speculate that if a fraction of the money spent in producing, consuming, and regulating these lipotropic wonders was spent in supporting research into the subject under discussion here today, we would all be wiser about intracerebral events, and probably healthier and wealthier as well. The poison may of course come from within as well as without: derangement of the nervous system is a conspicuous feature of various lipodystrophies and of metabolic toxemias like diabetes and uremia.

Changes in phase during excitation of neural tissue are accompanied by and probably dependent on parallel changes in electric potential and protein synthesis. Electrical stimulation of brain cells produces the following sequence of changes:[39–42]

1. Formation of messenger RNA and synthesis of new protein.
2. Transfer of synaptic protein including acetyl cholinesterase from ribosome to synapse.
3. Utilization of residual, undegraded *m*-RNA for lipid synthesis.

These changes involve ordered movement of macromolecules in

a paracrystalline state within brain cells which are, by their nature, responsible for storage as well as transmission of chemical information about the sequences and steric structure of the lipid, protein and lipoprotein molecules upon which neural function including memory, depend. In the grey matter of the brain, the lipids have therefore, as would be expected, a much higher turnover rate than in myelin[43] which has only about one-fifth of the molar proportion of polyunsaturated fatty acids. This may be a way of saying that cerebral activity, quantitatively if not qualitatively, is linked to integration and turnover of relatively unstable unsaturated lipids in complex phosphatide or lipoprotein macromolecules; or perhaps it is just a pretentious way of recalling the empirical wisdom of our grandmothers who insisted that eating fish was good for brain-work. Either way, in these observations[44] we can see a molecular basis for events in the nerve cell, for transmission of impulses, for reflexes, and for storage of chemical information; we may logically ask therefore if, biochemically and biophysically, we are at a point where we might soon begin to decipher the " Engrams in the brain "[45] by glimpsing molecular transformations which might underly faculties like perception, memory, and cognition. If this is a correct interpretation of the facts, the intellectual and pharmacologic implications are profound, almost frightening.

Mechanism of Integration of Complex Lipids

Complex water-insoluble substances necessary for vital processes are useful only when they are solubilized or dispersed. This requires numerous sets of continuous phase transitions, few of which are recognized and even fewer studied. For many years, attention has been focused upon cholesterol because of its importance in arterial degeneration, also because it is an essential component of many cells and body fluids. Since cholesterol is hydrophobic but not particularly lipophilic, and yet is essential in living tissue, its behavior illustrates well some of the problems in physiologic phase transformations.

Solubilization and Dispersion of Cholesterol

In the solid state, cholesterol displays considerable pleomorphism and is not readily dispersed in water or electrolytic solutions under conditions which could occur naturally. In the micro-crystalline state, dispersion is practicable but the production of micro-crystals requires relatively drastic unphysiological procedures. Yet, since cholesterol is integrated into all cells and most body fluids in non-particulate form, some very efficient methods of micro-dispersion and phase-change must be operative biologically. To understand how, it is necessary to find out how cholesterol in the solid state can be dispersed or solubilized in aqueous model systems, to study the form in which cholesterol and its esters exist in cells, tissue and body fluids, and by experimental methods, to see if a cholesteric mesophase can be produced in tissue from solid state or non-ordered precursor dispersions.

Solubilization of Cholesterol in the Solid State

This has been studied with a variety of amphiphiles and surfactants. Complete solubilization under conditions resembling the physiologic state in the human biliary tract has been achieved in quaternary aqueous systems containing bile salt and lecithin.[46] Solubilization can also be effected in ternary systems with lecithin as the amphiphile, in which case a complex, stable mesophase develops at certain critical concentrations. In

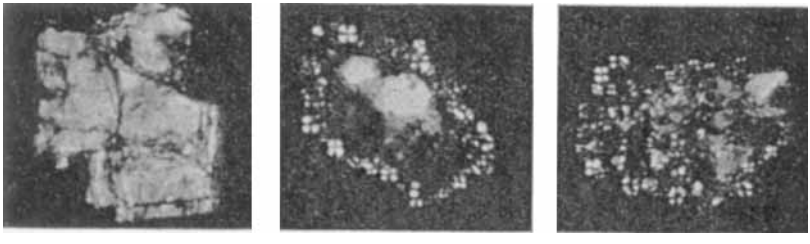

Figure 2. Conversion of a cholesterol crystal from the solid state (1) to a liquid crystal (2 and 3) by a polyoxyethylene amphiphile in water.

aqueous systems, polyoxyethylene esters of sorbitol (Tweens) exhibit a pseudo-membranous smectic mesophase. Addition of crystalline cholesterol results in a more complex mesophase[47] in which the transition of cholesterol from solid crystal to liquid

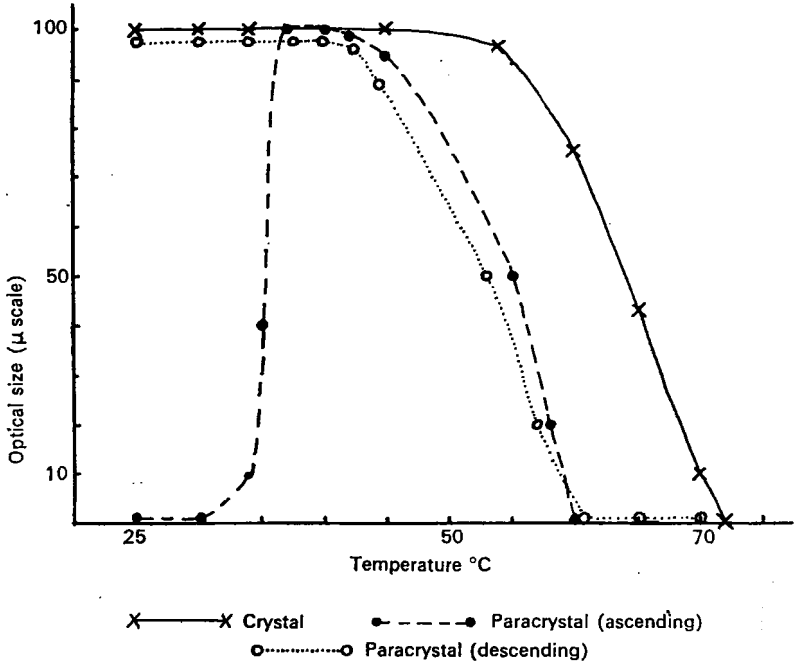

Figure 3. Lyotropic mesophase transformation of cholesterol in ternary system with water and Tween 80-effect of temperature.

crystal can be followed (Fig. 2). This transition occurs within a limited thermal range on warming but, on cooling, the mesophase remains stable at a lower temperature (Fig. 3), which explains the ability of the natural cholesteric mesophase to withstand temperatures below the freezing point of water. In these amphiphilic systems, cholesterol enters the mesophase only in the unsubstituted state: cholesterol esters, irrespectively of the chain length of the fatty acid tail, do not form ordered smectic layers like the corresponding phosphatide esters; on the other hand, the fatty acid chain length of the ester used as amphiphile does

influence the order and stability of unsubstituted cholesterol in mesophase, which suggests that the packing of cholesterol molecules occurs in a steric pattern entirely different from that of phospholipids in bilayers. Further differences in the behavior of cholesterolesters in mesophase are indicated in the NMR studies of Cutler (this conference) and in the permeability studies of Finkelstein and Cass.[55]

Cholesterol in Physiologic and Pathologic States

Admirand and Small[48] have shown that the solubility of cholesterol in human bile is determined by the relative concentration of bile salts (cholates) and lecithin in the quaternary aqueous system. In some pathological states, they found that the concentration of cholesterol was increased beyond the point of solubilization, leading to the deposition of cholesterol and the formation of gall-stones. Their findings amount to an attractively simple biophysical explanation of the occurrence of some forms of gall-bladder disease especially in conditions of cholestasis or when the excess of cholesterol is attributable to metabolic cholesterosis.

In view of the relatively high solubility of cholesterol and its esters in other lipids, it may be asked why aqueous solubilization is necessary. The answer seems to be that certain physiologic processes are dependent upon aqueous or partially aqueous phases, notably those involving transport of ions or protein or enzymatic hydrolysis. Thus cholesterol in erythrocytes, serum and lipoprotein complexes is either in a dispersed phase or in the liquid crystalline state, in which form it can be identified intra- or extra-cellularly in a variety of situations, including leucocytes, nervous tissue, adrenal cortex, corpus luteum, and low density β-lipoprotein fractions of plasma.[49,50]

Of particular interest in relation to the ageing process in humans is the partitioning of cholesterol in plasma lipoproteins.[31,50] The total concentration tends to increase in middle age but it is the distribution rather than the total amount which shows the most

striking change. In youth, cholesterol and its esters are carried in the α-lipoprotein fraction. In men from the mid-twenties onward and in women after the menopause there is a sharp increase in β-lipoprotein[51] which carries a relatively higher concentration of all forms of lipid and is therefore of lower density. About 50% of the cholesterol is esterified in the lipid phase; the remainder forms an ordered macromolecular complex with protein. Normally, excess lipid is split hydrolytically by physiological liposes (" clearing factor ") into water soluble products[50] but this process is less effective against the low-density lipoproteins in which alteration of phase-equilibrium leads to deposition of cholesterol in a characteristic mesophase.[50] Thus the clearing mechanism fails when it is most needed and cholesterol remains in the blood stream or enters the tissues (Fig. 4). Experimental work[52,53] in which cholesterol in its β-lipoprotein mesophase was deposited in the intimal lining of aortas from young rabbits (Fig. 5) by pulsation at normal rabbit blood pressure, confirms the viewpoint that disturbance of phase-equilibria in this way is a strong biophysical factor in the pathogenesis of atherosclerosis (Fig. 6) and therefore of ageing. In similar fashion, the relative immunity of women to atherosclerosis and its consequences during their reproductive years finds a biophysical explanation in the fact that cholesterol is deviated to or held in ovarian cells (Corpora lutea) [49] where it is a precursor in hormonal synthesis; the hormone concerned, estrogen, is effective in lowering abnormal β-cholesterol in men[45] and seems to do so in proportions to its capacity to cause femininisation. In so far as estrogen or a related ovarian hormone exerts this limiting effect upon the transfer of excess cholesterol to the low-density phase of the lipoproteins in plasma, one of the regulatory mechanisms of ageing appears therefore to reside in the reproductive organs—a circumstance which harmonizes nicely with the natural philosophic assumption that, at the cellular as well as at the behavioral level, ageing intensifies as reproductive energy diminishes. Consistent with this hypothesis is the finding that, in diseases associated with signs of premature ageing like untreated diabetes, hypothyroidism

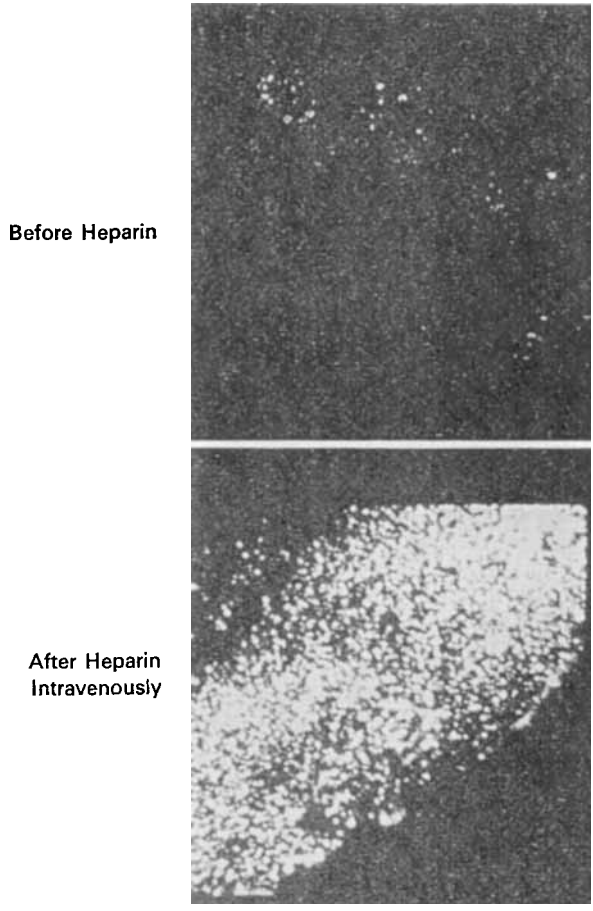

Figure 4. Increase in lipoprotein mesophase in plasma of hypercholesteroemlic rabbit after injection of heparin (X400, polaroid light, low density fraction).

and chronic nephritis, the low-density β-lipoprotein phase is markedly increased and deposition of cholesteric mesophase in arteries and elsewhere very conspicuous.[49]

Non-lipid Paracrystalline Phases

When one or both polar groups of molecules assumes a position of orientation within its solvent by means of mutually attractive

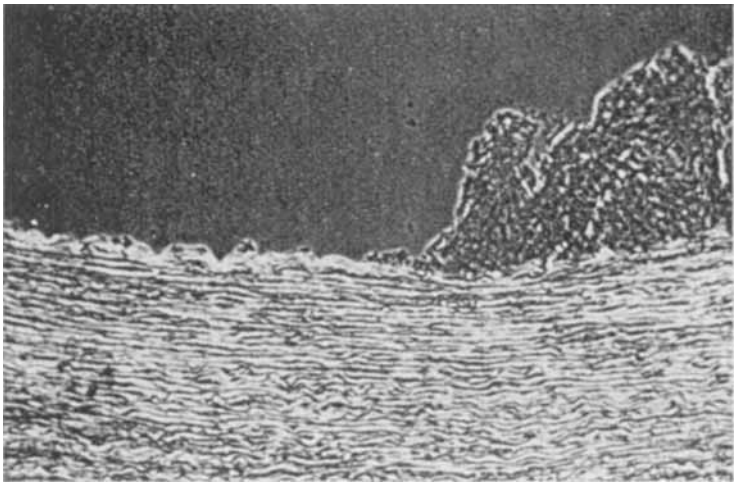

Figure 5. Atheromatous deposit in intima of rabbit aorta produced by pulsating low-density β-lipoprotein fraction of hypercholesterolemic rabbit plasma for 48 hours. (See references 52 and 53). The raised deposit at right of photograph contains a complex mesophase of cholesterol and protein in contrast to the flat intima to the left, which is normal. (Phon-contrast X100).

forces between adjacent molecules, without the intervention of another distinct phase, a relatively stable ordered state is maintained even when the molecules concerned are very large. This is probably the basis for the integrity of the myoglobin molecule in vivo. As Kendrew[56] has pointed out, most polar groups are "quite content" to bond with water or ions in the ambient solution, forming a simple emulsion or colloid. Linkage with a neighboring molecule will occur if this does not involve alteration of configuration, a condition most likely to be met by the exertion of Van der Waals forces between the non-polar groups.[57] This illustrates the possibility that orientation of protein or peptide is not obtained at the expense of chemical bonds responsible for structural integrity of the individual molecules. It is obvious that some peptides and proteins (e.g. serum albumin) can perform their biologic function in simple or colloid solutions in which the degree of swelling produced by low molecular weight substances determines stability and reactivity of the macromolecule in a

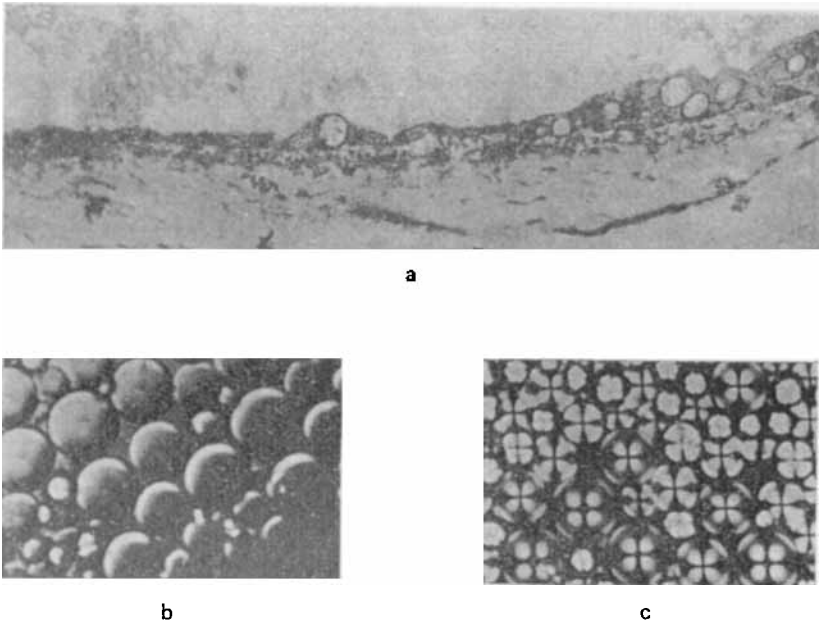

Figure 6. Experimental atherosclerosis in cholesterol-fed rabbit. (a) Electron micrograph by Dr. N. Rodman showing early thickening of aortic intima at right of photograph due to deposition of lipid; (b) surface film of same photographed under phase-contrast to show spherules of lipid with characteristic dimples; (c) same, photographed under polaroid lights howing cholesteric mesophase. In a spherulite, the combination of a cruciate interference figure and " dimpling " indicates a difference in free energy level at one point on the surface and hence a helical rather than radial ordered structure.

relatively disordered state. Function at the cellular or subcellular level is then limited to interaction of macromolecule and solvent. Elementary order is apparent if the structure is regular, with repeating units (Table 1), as in the muramic and teichoic acid mucopeptides of bacterial cell walls.[58] Small peptide molecules, such as penicillin and cephalosporins, polymerise in solution by internal reaction, between their repeating amino units[59] to form polycrystalline masses with amorphous matrices. This process, which is expedited in mucopeptide or polysaccharide gels, may explain structurally the well-known interference caused by these antibiotics with the orderly synthesis of mucopeptide in the cell

walls of bacteria.[58] The self-condensation of amino acids involved in formation of polypeptides may therefore lead to regular linear polymers with random coils and amorphous structure, extended crystalline chains with hydrogen-bonding between chains or regularly folded chains with intramolecular hydrogen bonds[57]; this lat arrangement accounts for the fully-ordered structure of α-collagen[60] which has two helices winding round a common axis. Proof that such arrangements were liquid crystalline was offered by Robinson[61] who worked with an unusual polypeptide (poly-γ-benzyl glutamate) in organic solvents. Further work with polypeptides and proteins in aqueous systems is required to establish relationship with biopolymerisation and tissue structure, especially to understand the physical basis of biochemical information systems. Crick's central dogma[62] is that, once information has passed into a protein, it cannot get out again; this seems to apply not only to amino acids in protein but also to bases in DNA and RNA. On the other hand, it is known from experiments with inhibitors and modified molecules (e.g. antigens, antibodies, bradykinin, insulin, antibiotics) that a particular biofunction is not necessarily attributable to a unique molecular composition, and also that vital interactions and syntheses can occur in bioreactants without all components taking part (e.g. base-pairing, antigen–antibody combination). This points to the need for a better understanding of " Goodness of fit " in biophysical terms and particularly to possibilities therein of exploring a physical basis for the transmission and storage of information in living systems.

There are many other ways in which an adaptive mesophase plays a combined structural and functional role in biologic systems, from the simplest to the most highly developed.[63–65] In summary, it may be said that a lyotropic mesophase is a constant feature of reactive, intercellular and intracellular substances. Artificially, lyotropic mesophases can be created with various solvents but, in Nature, the main solvents are water or electrolytic solutions. The simplest lyotropic systems depend upon partial solvation of an amphiphile with a water-soluble polar group.

With some substances (e.g. protein), solvation is relatively uniform and phase change is brought about by changes in energy and in viscosity leading, for instance, to contraction of muscle, which is accompanied by alteration in the orientation of myosin molecules. With water as solvent, many substances can form equilibrated phases more or less predictably, according to their molecular structure. Substances insoluble in water can be stabilized in aqueous colloid by the presence of amphiphiles and surfactants in such a way that molecular orientation is preserved during flow, providing a unique basis for biologic reactivity. Intracellular micelles such as lyosomes and mitochondria have this general structure and it seems likely that many vital biochemical reactions and syntheses can proceed only if an orderly sequence and layering of molecules is present in such a way that individual polar groups enjoy maximal opportunities for contact with reactive groups in aqueous solution. This condition applies to many membranes which, in their simplest form, consist of a molecular bilayer between liquid phases. In mitochondria and micelles, the external and intermolecular phases are aqueous colloid but the internal substance is a lipid mesophase whereas, in lyososomes the internal phase is aqueous enzyme protein.

The basis of the biologic mesophase is best illustrated by relatively simple structures such as are described above, and the physico-chemical conditions governing stability can be to some extent assessed with fair accuracy from these systems. Many other structures of much greater complexity are found however in cells, tissues, and biologic liquids in mesophase, including transfer RNA; steroid substances in ovarian and adrenal hormone-secreting cells; hemoglobins *S*; α-helical protein in collagen; myelin; nucleic acid.[47,64] In most systems, the liquid crystalline form has a structural as well as a chemical or physical functional role and there is reason to believe that disturbance of this role can have profound effects upon the vitality of cells and tissues, contributing simultaneously to the mysterious process of ageing and yet, by mechanisms which are not too dissimilar in physico-chemical terms, accounting for the orientation of molecular layers

and for macromolecular aggregations essential for important aspects of growth, differentiation, cellular function, and reproduction. In identifying not only the presence but, more importantly, the functional relevance of the lyotropic mesophase in protoplasm we are surely therefore glimpsing, one of the qualities which distinguishes animate from inanimate matter. If we go further, and accept the notion that life has a physical as well as a metaphysical origin, then we might also, in our subject of study, be seeing a clue to the transformation of the physical and organic world into the biosphere. Such a view would necessitate a departure from established viewpoints in more than one scientific discipline but, equally, it might in so doing reveal if not fill a vacuum which has long existed between physics, chemistry, and biology concerning the modus operandi of specific functional groups and " *signatures* " in molecules.[66] Schrodinger[67] described the small molecule as the " Germ of a Solid." Larger aggregates be described as being either " Dull 3-dimensional repetitions " i.e. solid crystals with established periodicity and no possibility of variation in structure or " Aperiodic solids " in which every atom or group of atoms played an individual role. What he did not visualize is the need for a combination of these properties in structures in which molecular repetition and submolecular reactivity co-exist as, in a gene, they must. The concept of the biologic mesophase provides a biophysical, verifiable hypothesis for the unique reactivity of such structures and also for the origin of simpler structures such as membranes, micelles, and biopolymers whose properties are entirely consistent with the molecular order and energy flow in experimental lyotropic systems.

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