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Liquid Crystals in Biological Systems

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There are several good theoretical reasons why matter in the liquid crystalline state should play a part in the structure of living tissue.

1. Many naturally-occurring substances exhibit paracrystalline behavior.
2. The most complex and some of the most reactive forms, the so-called cholesteric, are mainly or exclusively of natural origin.
3. No other formation of organic molecules possesses a comparable pattern of ordered structure in a state of flow.

This is not to say that liquid crystals must, therefore, be involved in living processes. It simply means that theory would fit fact, if facts were available.

Biologically, the facts are available and are, in my view, singularly convincing though few biologists have paid heed and even those few have received scant recognition among the physicists and physical chemists who are expert in this field. In the words of Brown and Shaw,[1] reviewing this whole field in 1957, "Only a few investigators have made any effort to apply the knowledge". Let us see what these select few have found.

Oddly enough, the first description of what was obviously a mesophase came from a biologist, an Austrian botanist Reinitzer who, in 1888, observed transition from a solid to a turbid and then a clear fluid in cholesteryl benzoate heated from 145° to 179°. It was at least a year later that Lehman[2] described the crystalline appearance of the turbid phase and invented the term Liquid Crystal. Thereafter, there is a long gap though passing references to the

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possible importance of liquid crystals in biology were made by Rinne,[3] and by Bernal during the discussion held by the Faraday Society in 1933.[4] Meanwhile in 1922, Friedel[5] had classified the mesophase from the physico-chemical viewpoint, and flow birefringence had been noted in muscle;[6] but years elapsed before these important aspects of molecular organization attracted comment from a biologist,[7] Jospeh Needham, who was one of the pioneers of functional anatomy of tissue. In 1941, liquid crystalline material was described in solutions of the protein of tobacco mosaic virus.[8] Spherocrystals were identified in tissue by various workers between 1946 and 1958. The possibility that these might denote the presence of liquid crystals in tissue was mentioned by Pearse[9] and Engstrom and Finean[10] whose brilliant work on the structure of nervous tissue had disclosed the presence of two components in optical opposition in the concentric multilayers of lipid or lipoprotein forming the myelin sheath. The similarity between the behavior of myelinic figures from nervous tissue emulsified in water and other lipoid structures with hydrophilic surfaces had already been studied in considerable detail by Nageotte[11] who was well aware of the possible importance of liquid crystals as elements of structure though he did not demonstrate their presence in intact tissue. From a study of the structure of Hemoglobin S, Perutz *et al.*[12] concluded that it could assume liquid crystalline form in the red cell. Robinson,[13] in 1956, showed conclusively that the polypeptide poly-Y-benzyl-L-glutamate existed in organic solvents as a cholesteric liquid crystalline spiral.

Thus far, the presence in biological material of components which might or might not have been paracrystalline had been noted mainly as incidental findings in studies of extracted or processed material though spherocrystals, probably of cholesteric origin had been described in living cells.[14–16] A definite claim that liquid crystals entered into the structure of living cells and tissues was made in 1959[17] when it was shown that complex lipids present in the adrenal cortex, ovaries, myelin and also in atheromatous arteries existed at body temperature in a characteristic mesophase. This observation arose mainly out of the examination of fresh tissue, for con-

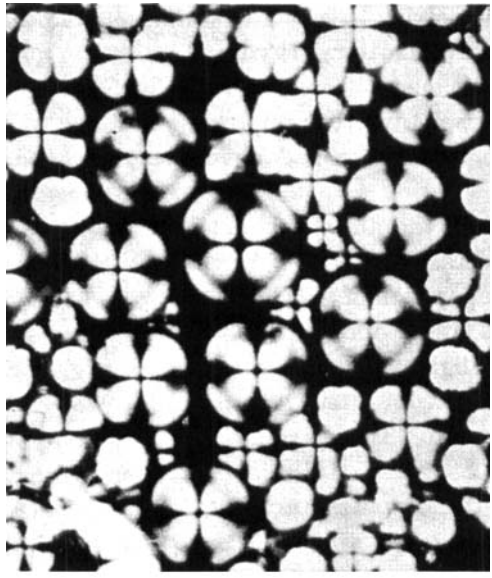
ventional methods of processing tissue for examination, depending as they do upon coagulative fixation, dehydration, organic solvents and other manipulations, usually destroy all the features of a mesophase and probably many other important features as well. This may be why the mesophase had never been identified in classical histology.

Techniques applicable to the study of fresh tissue for this purpose have been described in detail elsewhere.[18] Essentially, they amount to a rapid isolation or separation of appropriate tissues, body fluids or biological models at 20–37° for immediate microscopic examination under polarized light. Some cells or tissues (e.g. adrenal cortex, corpus luteum) can be easily examined in preparations squashed under a cover-slip. To determine topographic relationships, sections are cut at 5–10 μ thickness after embedding in polyethylene glycol, without denaturation. Physico-chemical properties are established in parallel by chromatographic, ultracentrifugal and other methods.

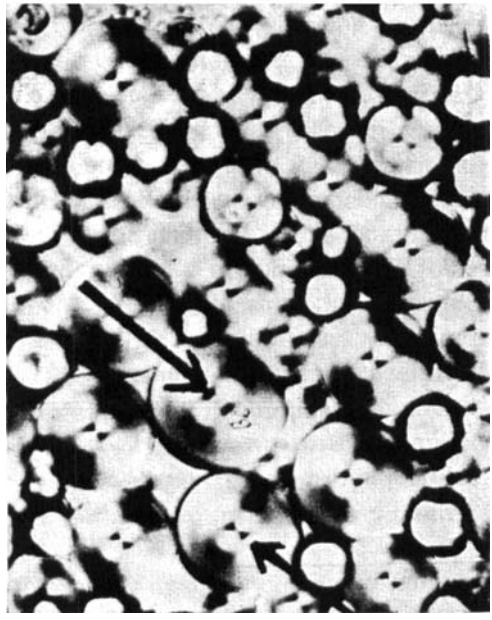
Examined in this way, the following tissues exhibit protein, lipoid or lipoprotein complexes in liquid crystalline form:

Ovary. In health, lipid is confined almost entirely to the corpus luteum which contains birefringent fat-soluble materials including masses of minute spherulitic globules with polarization crosses (Fig. 1). Chromatographic examination with iso-octane as solvent identifies this as a complex steroid structure with free and esterified cholesterol as main components (Fig. 2). Triglycerides and phospholipids are also present. Degradation on standing brings an increase in overall birefringence, but a loss of spherulites, and an increase in coarse sudanophilia, presumably due to unmasking of glycerides and fatty acids in the complex. The Schultz and Lieberman reactions indicate that more than one steroid is present and it is likely that ketosteroid hormones are present in the mesophase, especially since steroid-3B-01-dehydrogenase[19] has been identified in corpus luteum cells, probably representing a terminal step in progesterone synthesis.

Adrenal cortex. The presence of spherocrystals in this organ has been known since 1948.[20] As in the corpus luteum of the ovary,



Crossed polars × 400



Quarter wave-plate inserted.

Figure 1. Spherulites in mesophase from Corpus luteum of human ovary.

these are part of a lipoid complex which exists in the mesophase at body temperature. The complex contains various steroids which fluoresce in UVL and give varying color reactions with sulphuric acid when dehydrated. We cannot at present say with certainty what these various steroids are. From biochemical and biological

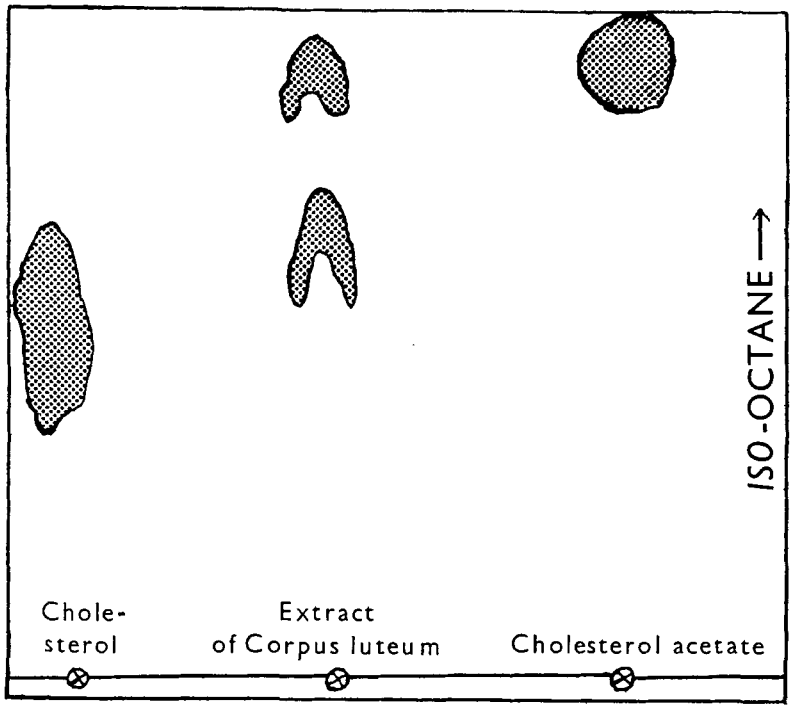


Figure 2. Chromatographic examination of steroid component of lipoid mesophase in corpus luteum (human). (Reproduced from the *Journal of Pathology and Bacteriology* (1961) **81**, p. 388 by kind permission of the Editor.)

assays it is probable that some of them have unsaturated polycyclic rings, that some are active hormones and that various cholesterol precursors are present. It is certain that cholesterol itself is present and may be both a precursor and a degradation product of hormone synthesis which takes place in these cells. The mesophase in this

situation therefore represents a vital element in what we might call structural metabolism.

Nervous tissue. For many years past, the term "Myelinate" has been applied to lipid droplets in fresh preparations of white matter obtained from the brain, spinal cord and peripheral nerves,[11] viewed microscopically under white light. These droplets assume various shapes according to the temperature and physical conditions of the system under which they are observed. Under polarized light, varying degrees of birefringence are formed and polarization

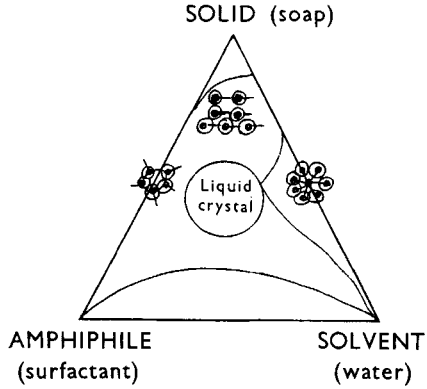


Figure 3. Ternary system providing model for lyotropic mesophase (after Lawrence).

crosses with positive and negative signs. The structure of the tissue as a whole is extremely complex but Finean's studies[10] based on X-ray diffraction and electron microscopy shows that there is concentric layering with several lipid components, some of which are uniquely formed in nervous tissue, like the so-called cerebrosides. The whole complex may be regarded as a highly specialized form of natural lipoprotein, containing ionic substances (fatty acids, phosphatides) linked to protein by their polar groups and non-ionic substances (glycerides and cholesterols) which may be more loosely bound by Van der Waals forces. Experimentally, similar figures can be formed by partial miscibility in water and detergent of certain lipoid substances insoluble in water alone. Physically,

the myelinate figure is a tube, and all that is required for the formation of a stable lipoprotein tube is the presence of lipid with a hydrophilic polar group at an interface with water containing protein in solution. This can be seen in its most elementary form by letting a paste of phosphatide swell into water. According to Lawrence,[21] the tubular nature of the interfacial swelling is due to the development of a smectic layer lattice which is able to hold free water in sandwich fashion. This is, in fact, the liquid crystalline ternary phase of a three-phase system (Fig. 3); the other two phases being an isotropic, stable, true solution and a fine emulsion. The essential condition for the myelinate configuration is the formation of a structure behaving like a membrane at the interface. This might well be a fundamental condition of biological organization, for there are interesting analogies in the formation and structures of various other membranes and subcellular structures, such as micelles and mitochondria which might be more accurately termed "Lipochondria" in the light of modern knowledge of their structure.[22–24] In so far as neural myelin is concerned, the essential components of the liquid crystalline structure are phospholipids which are capable of forming membranes in phase-transition,[25] cerebrosides of unique structures, sterols and protein. Finean's work suggests that the ratio of the three lipid components in the layered structure is of the order of 2:1:2.

Muscle. Birefringence in muscle fibres has been known since about 1930.[6] This is present during flow of the long molecules of myosin which form the contractile fibres. Contraction is accompanied by high-energy phosphate exchanges occurring simultaneously with changes in birefringence and ATP'ase activity. One protein component, actomyosin, becomes more viscous and birefringent with increase in ionic strength and forms a polymer (G actin→F actin) in the presence of ATP.[26] The structural change and the energy consumption of contraction are therefore associated with an alteration in the orientation of elongated protein molecules in a paracrystalline state. Since this is a subject in which the energy involved in a twitch of even a single fibre can be estimated, it would seem to be profitable to investigate the significance of the accom-

panying changes[27] in molecular orientation of the polypeptides in the myosin (Fig. 4).

Connective tissue. The fat in connective tissue which accounts for most of the obvious fat in the body—corpulent or otherwise—is composed of glycerides, which have no mesophase though they may enter in solution into other lipoid or lipoprotein mesophase in certain abnormal deposits. The protein framework is mainly collagen, one form of which (α-collagen) consists of two helices winding round a common axis.[28]

Nucleoprotein. The work of Conmar Robinson,[13] more than any other, has thrown light on the twisted liquid crystalline structure which we now know to apply to solutions of certain polypeptides,

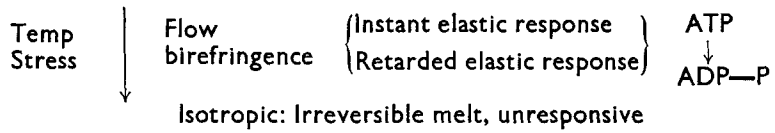


Figure 4. Known structural and biochemical changes in muscle under stress.

DNA and transfer RNA.[29] The existence of paracrystalline structure in nucleoprotein was first pointed out around 1955[22, 23] from experimental studies with extracted material. If I interpret Robinson's work correctly, he has defined precisely the steric arrangement and linkages in these twisted chains, and this is the only example I know in which the full molecular structure of a liquid crystal in its presumed biological state has been described.

Liquid Crystals in Diseased Tissue

As a physician, my interest in this subject is concerned mainly with the investigation of causal factors responsible for arterial degeneration.

A healthy artery has a smooth internal wall (the intima) free from any irregularities other than those governed by the anatomy of a healthy body. This may be the state of the vessels in a healthy youngster but quite literally, the rough soon begins to disturb the

smooth. Degenerative changes can begin quite early in life and, in men of 20–30, it is quite usual to find deposits of atheroma on and within the intima of several of the more important arteries. Atheroma is Greek for gruel, but the gruel is lipid, not carbohydrate and the arteries at this stage are softened, not hardened. It is well known to morbid anatomists that, in an atheromatous vessel at autopsy, the gruel is composed of fatty substances containing typical cholesterol crystals. Oddly enough, no one seems to have thought of looking at these deposits at body temperature, before crystallization and other post-mortem changes occurred. When this is done[18] you find that the atheroma material consists largely of lipoid in a mesophase with a characteristic spherulitic structure (Fig. 5). For reasons which will be obvious later, this is again a lipoid complex with cholesterol as only one component. It is difficult to obtain fresh human material for this kind of study. Tissue obtained post-mortem is often unsatisfactory and many of my observations were made at St. Mary's Hospital in London, in collaboration with Professor C. Rob, on arteries resected surgically. In such vessels one finds that a pure lipoid mesophase exists only in the lesions in the intima (Fig. 6). These are the recent lesions; the older lesions penetrate the vessel wall by various means, excite a foreign body reaction and cause extensive secondary degeneration of the elastic and muscular layers of the vessel. This has now become a point of some controversy in which I find these views are at variance with some of the traditionalists[32, 33] who maintain that lipoid deposition is a purely secondary phenomenon.

Fortunately, the subject can be approached from other angles. There are certain conditions in which the blood becomes persistently and abnormally lipaemic; not the kind of lipaemia which follows a meal of eggs and bacon, which is a triglyceride lipaemia, but a state of essential hyperlipaemia in which the characteristic lipoid is a lipoprotein of low density (S_f 12–20). A similar condition can be produced in rabbits very quickly by feeding them with cholesterol dissolved in arachis oil. The lipoprotein is suspended as a colloid in the plasma, and shows spherulites in films raised by centrifugal flotation. If the colloid equilibrium of the plasma is then altered by

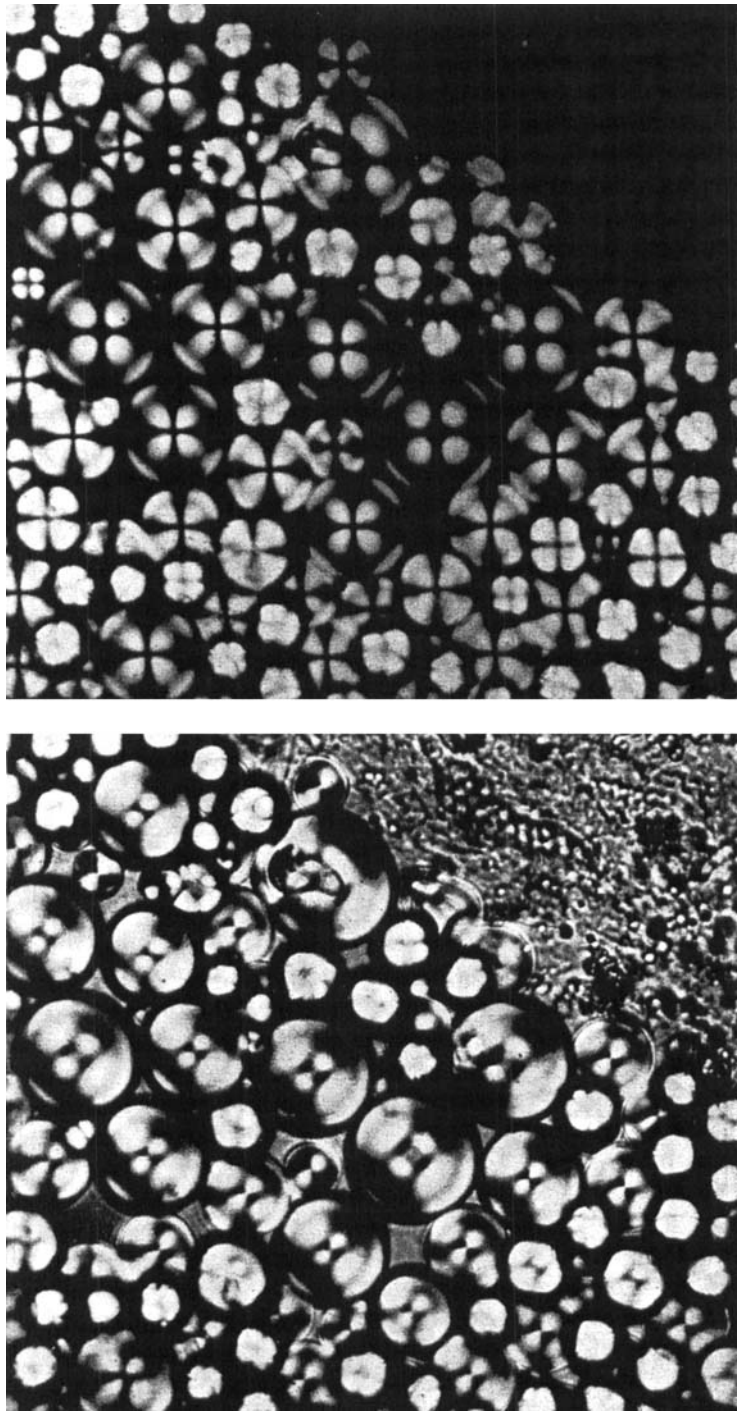


Figure 5.

injecting intravenously a macromolecular polysaccharide like dextran sulphate or heparin, the lipoprotein complex separates out in the low density layer as a uniform mesophase. Examined at 37° C, the spherulites of this mesophase are fluid but preserve their

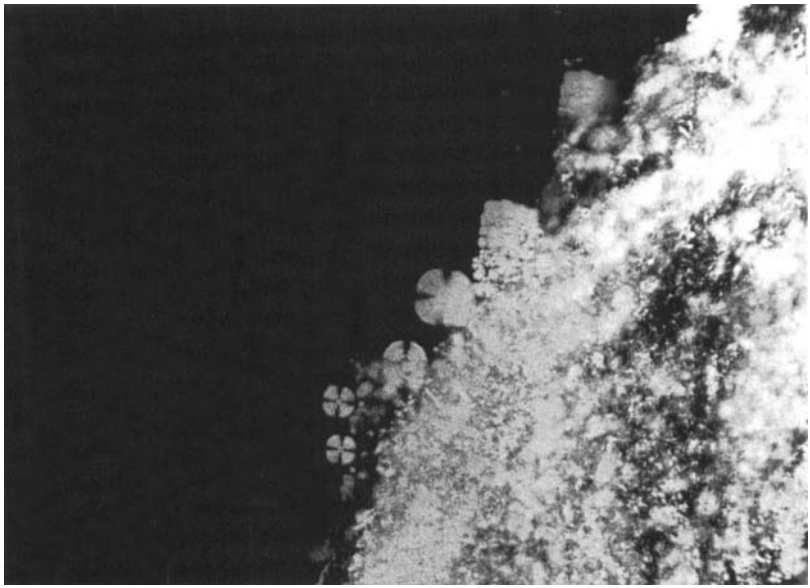


Figure 6. Superficial deposit of atheroma in the intima of an artery resected surgically from a middle-aged man. Lipoid mesophase with spherulites. Polarized light, × 400. (Polyethylene section, unprocessed.)

polarization pattern and present a myelinate appearance. When the temperature is raised to above 40°, the mesophase changes to an isotropic melt. The spherulites in this mesophase, like those in the ovary, and recent atheroma deposits, are optically positive.

Figure 5. Lipoid mesophase from human atheroma. The spherulites are showing secondary rings.

Top figure: Crossed polars 

Bottom figure: Quarter wave plate inserted with slow direction 

It is known that injection of polysaccharide stimulates the action of "clearing factor" in lipaemic plasma. "Clearing factor" is probably a lipase which hydrolyses triglycerides in chylomierons and in the natural lipoproteins of the plasma into free fatty acids.[34] The abnormal lipoprotein and its mesophase are not cleared by this lipase; quite the reverse, in fact.[35] That is to say, low density lipoprotein in this state resists one of the best understood physiological lipolytic mechanisms. Since the mesophase produced in plasma from this lipoprotein is practially identical with that observed in the earliest stages of atheroma, it is hard to escape the conclusion that there is some connection between the two especially since, in males and females, these low density lipoproteins tend to increase at about the ages when the clinical complications of atheroma also begin to occur.

Support for this idea can be found in the atheromatous lesions produced by cholesterol feeding in young rabbits, with smooth youthful vessels. The early lesions are very similar to those in man[18] and sometimes have striking similarity to those which one finds occasionally in children; for, in the presence of certain renal diseases (e.g. nephrosis or diabetes among other conditions), atherosclerosis can begin in childhood. I may say, in passing, that diet-induced atherosclerosis in rabbits is reversible; one wonders if the same may be true in man, in the early stages.

These observations establish the fact that lipoid complexes exist in mesophase in normal and diseased tissue; but they do not provide adequate links between cause and effect, except indirectly. A series of further experiments was therefore designed to fill this gap.

Pulsation of Lipoprotein into Normal Vascular Intima

For this purpose an apparatus was constructed[36] in which lipoid and other material, of known composition, could be pulsated at body temperature against segments of arteries excised from young rabbits, bathed in oxygenated physiological solution.[36, 37] The pressure and tempo of pulsation are adjusted to physiological levels. When normal serum is pulsated against an artery in this way, nothing happens. Fatty serum (i.e. taken from a rabbit fed with

arachis oil or butter) also leaves the intima smooth, as do individual triglycerides in emulsion; fatty lymph, taken from the thoracic duct, is harmless. But serum from a cholesterol-fed rabbit produces small atheromatous lesions, formed of a lipoid mesophase similar to that observed in naturally occurring lesions. These experimental lesions are always small when serum is pulsated but not when plasma is used. The explanation is simple, but points to the presence of an additional causal factor: a large lesion is formed

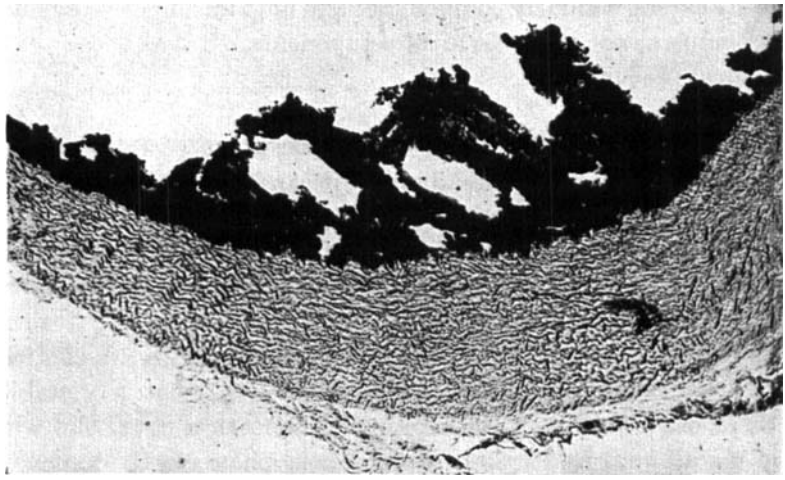


Figure 7. Section of rabbit's aorta after experimental pulsation of plasma containing an excess of low-density lipoprotein. The lesion, stained black with sudan dye, is a deposit of lipoid mesophase in a fibrin matrix.

(Fig. 7) only when fibrin is deposited. This happens when an activator (thromboplastin) in the vessel wall converts the fibrinogen of the plasma into fibrin. This forms a matrix for the soft lipoid, and a bulkier lesion results. This is not the whole story for, in the intact animal, a corrective fibrinolytic mechanism then comes into play, which limits the process. The dynamics of blood flow and the contour of the vessel also influence the location of the lesions. Even so, these experiments provide evidence of a direct causal relationship between abnormal lipoid complexes in the blood and

atheroma, one of the essential points of the relationship being that in both situations the lipoid forms a stable mesophase.

The next step in this investigation was to isolate from normal and abnormally-lipaemic sera the various lipoprotein fractions, by serial ultracentrifugation. By this means, it is clear that the atherogenic component is the lipoprotein of lowest density (1.00–1.04 g/ml hydrated). Mixed with albumin and fibrinogen, in physiological concentrations, this lipoprotein component produces soft atheromatous deposits in a characteristic mesophase. Cholesterol and its eaters, similarly compounded, do not: the effective agent is the complex, not the individual components, vital as these are as chemical precursors.

Preparation of an Artificial Mesophase

From other evidence already cited it can be postulated that the substances most likely to enter into atherogenic lipoid complexes are cholesterol, phospholipid, protein and water.[38] An artificial mesophase can quite easily be prepared from these components in the following way: amorphous cholesterol is dissolved in acetone and added to boiling water; the acetone evaporates, leaving the cholesterol, still amorphous, in fine suspension, relatively stable; a three-way interface is then prepared beneath a cover-slip with aqueous albumin and lecithin; a stable mesophase, partly myelinate partly spherulitic, forms at a critical point in the interface and rapidly grows in complex fashion (Fig. 8). Lecithin can be replaced in this system by some polyoxyethylene esters and other substances, and I am reminded here of the similarity to the ternary liquid crystalline phases which Lawrence has described in full physicochemical detail (Fig.3).

It is important to note that the spherulites observed in the sheets of lipoid mesophase from atheromatous lesions are optically similar to those present in the cells of the corpus luteum, of the ovary, in the experimental lesions produced by pulsating low-density lipoprotein into vascular intima and also in leucocytes sampled after local injections of cholesterol or its esters into living tissue. In all of these situations, cholesterol has been identified as a main component.

Experimentally, however, cholesterol does not form this kind of mesophase unless it reacts with water, protein and a phospholipid or similar polar substance at an interface. Even amorphous cholesterol acts as an amphiphile in this system and a mesophase, probably smectic in nature, develops at the boundary where the phospholipid is forming a lyotropic mesoform with the water and protein. The texture, flow and general reactivity of this depends upon the viscosity which in turn is influenced by temperature, stress, and the stability of the adjacent isotropic solution or emulsion.

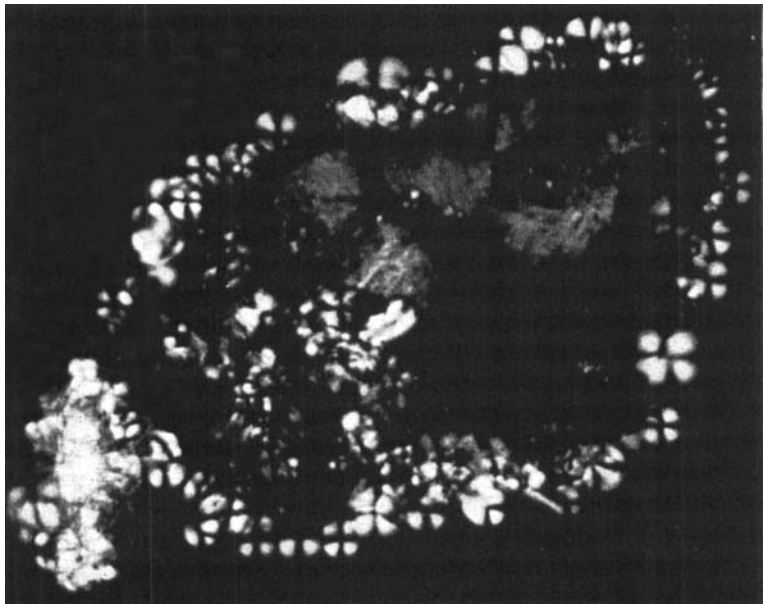


Figure 8. Spherulitic mesophase developing in lyotropic fashion around crystalline cholesterol at a critical dilution of sorbitol polyoxyethylene oleate in water.

The naturally occurring spherulites from tissue show maximum flow with orientation about 37° and enter an isotropic melt about 40°. Sudden cooling destroys them, often irreversibly. At 25° they are evenly spherical and optically positive (Figs. 1 and 5). In aqueous or oily emulsion, they enlarge into rings with interior films

having positive uniaxial interference figures with optical axes perpendicular to the film. In this state they are stable for days and readily exhibit flow with rise in temperature or indirect pressure, the interference figure and color sequence being preserved. They resist the action of lipolytic and non-polar surface active agents but are solubilized by long-chain esters. Acidification, denaturation of the protein component by aqueous formalin, and sudden cooling lead to disintegration into crystalline rhombs of

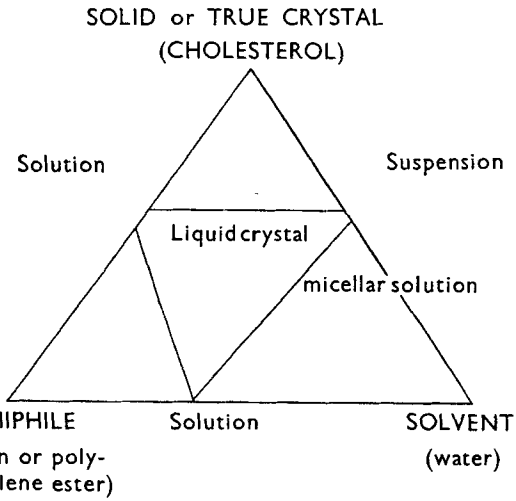


Figure 9. Ternary system forming lyotropic mesophase.

steroid material, fatty acid crystals and triglyceride globules. It seems reasonably certain that this mesophase is a lipoprotein complex containing sterols, probably in a helical arrangement. The unexpected positive optical sign may be due to the presence of other components or to some other complexity in the structure.

Current publications on liquid crystals tend to discuss the biological aspect as "Chaotic"[39] or altogether missing.[40] This is surely untrue in the light of the evidence presented in this communication. Liquid crystals are not only present in tissue; they would appear to play a singularly important role in that their structure is

part and parcel of biochemical function and reactivity, in normal tissue and in at least one major degenerative disease. Even more important is the possibility that liquid crystalline structure exemplifies in its process of development from relatively simple organic precursors, the power of elementary physical growth and what might be a self-replicating organization of macromolecules. In the case of a true crystal and, to some extent, in a thermotropic mesophase in its ideal thermal range, aggregation and growth are periodic, repetitive and indefinite. This is not so with a lyotropic mesophase in which the grouping of molecules is self-limiting, since the statistical physics of stability therein depend upon a balance being struck in one zone, not necessarily three-dimensionally, and thereby precluding the possibility of a similar balance in adjacent zones (Fig. 9). The situation therefore resembles Turing's interesting concept of a stochastic situation in which one fast-working center of substances, brought together by chance, interact to produce a new and more complex substance diffusing outwards, inhibiting other developments in its vicinity and assuming a dimension governed by the conditions of the milieu in which it finds itself. If the liquid crystal does this, then its place in biology is far from new. More likely, it has been present from the start, for living substance could not have developed without it.

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