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# Liquid crystals in biology I. Historical, biological and medical aspects

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# Liquid crystals in biology
# I. Historical, biological and medical aspects

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In our present state of knowledge, it is useful to assume that all matter, in the solar galaxy at least, is composed of atoms and subatomic particles which function independently or interact in accordance with the laws of physics to form molecules, coacervates or other aggregates. For practical purposes, these states of matter are recognizable in the three-dimensional terrestrial world as solids, liquids and gases. This differentiation suffices also for molecular studies but, to understand the properties of mobile organic and especially of living matter fundamentally, it is necessary to investigate and conceptualize how immaterial electromagnetic and electrostatic processes produce changes in state, phase and entropy compatible with self-replication, molecular memory and vitality This possibility exists in the properties of the liquid crystal (LC) as a mesophase in thermal and optical phase transitions, i.e. as an enantiomorphic intermediate form of matter which can form complex, self-replicating, ordered structures and macromolecules, easily recognizable in everyday TV visual displays, electronic communication devices and computers. It is suggested that, in prebiotic terrestrial situations, matter possessing these properties of the LC was a precursor in the evolution of living from inanimate matter and, in the lyotropic form, in the processes of life thereafter.



## 1. Introduction

The present review in two parts includes references to recent research reported at the 19th International Liquid Crystal Conference (ILCC), held in Edinburgh in July 2002, chaired by Professor George W. Gray and organized by the Royal Society of Chemistry, with support from other organizations. The general scene was presented in plenary lectures by P.-G. de Gennes (Paris), H. Takezoe (Tokyo), W. A. Crossland (Cambridge, England), C. Tschierske (Halle-Wittenberg, Germany), R. W. Pastor (Washington, DC) and D. J. Broer (Eindhoven, the Netherlands). The Conference comprised close on 1000 presentations of original work as lectures, poster displays, short communications and demonstrations. Although the majority of these related to the dominant field of the thermotropic mesophase, there were about 200 which applied directly or indirectly to the properties and uses of lyotropic LCs and amphiphilic systems, as described in the abstracts (ILCC Numbers) and in pending publications. Part I of this review tries to link these and other recent advances to the historic mainstream of the lyotropic LC, and to new applications in biology and medicine. Some items, including details of nucleic acids, have been omitted. These will be included along with consideration of the origins of life in part II.

## 2. Prebiotic history

Through time in planet earth, changes in states of matter, ranging from inorganic to organic and from mineral to carbonaceous, produced eventually a gaseous atmosphere, a hydrosphere and a terrasphere containing minerals overlaid with surfactant gels and clays [1]. These changes explain developments up to but not including the biosphere in which life, as we know it [2] might have evolved 3–4 billion years ago, probably in the hydrosphere, in pools containing electrolytes, carbohydrates, amino acids and lipoids in solution, or as aggregates in colloidal dispersions of self-replicating substances eligible for attention as precursors of protoplasm [3, 4].

This concept is reasonable, but there is not a shred of proof that it is anything more than that. There is nothing to add until, millions of years later, primitive Man learned how to use colloids, dispersions and dough for baking, cooking, healing, brewing, painting, cementing and other activities. Civilized Man paid tribute to this unique endowment by using dispersed pigments to paint images of animals and himself for several millennia during which domestic and formal Science began to replace empiricism and superstition. But it was not until about 1850 that

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organic and physical chemistry began to provide the wherewithal to identify and experiment with substances relevant to living processes.

The discovery of oxygen in 1789 by Lavoisier in France established chemistry as a discipline. A Scottish botanist Brown described in 1829 the random movements linked to his name which revealed simultaneously the innate energy of particles and the vitality of primitive forms of life in the hitherto unknown world of microscopic activity. In 1905, the year of his first paper on Relativity, Einstein applied the reasoning of statistical mechanics to develop a Theory of Brownian Movement (his theory was verified by a French physicist J.-B. Perin who was awarded a Nobel Prize in 1926). Meanwhile, van't Hoff, Emil Fischer and others had discovered enzymes and stereochemistry and, with A. I. Oparin in Russia, forged a link with biology. This led in the 1920s to the biosynthesis of multi-carbon acids, the understanding of hydrolysis and the differentiation of D- from L-sugars, and L- from D- amino acids. In 1936, Oparin and J. B. S. Haldane of Cambridge formulated a hypothesis that such substances were the unique chemical precursors of complex molecules which were building blocks for living substance. In 1938, R. Signer in Bern (Switzerland) confirmed earlier work by P. Levene at the Rockefeller Institute on the identity of DNA which Avery, MacLeod and McCarty [5] in New York in 1944 identified as the heritable transforming principle of pneumococci, the chemical clue to the storage of information essential for replication of living cells. Meanwhile, N. H. Horowitz at Stanford University, USA, was proposing in 1945 that living matter developed when pre-existing complex substances were broken down to precursors by catalysis. So there was plenty of disagreement between capable scientists, alongside innovation and recognition of the role of environmental, evolutionary and genetic factors. All of this contributed to immense advances. Until 1950, the guiding principle was that of G. W. Beadle and E. I. Tatum [6], also at Stanford, that one gene formed one enzyme for biosynthesis of protein as the essential stuff of life. L. Pauling in 1948 suggested stereochemical complementarity as an explanation of enzyme and antibody specificity. There was no comparable advance in biology, but the other pioneers of biochemistry like Fritz Lipmann at Harvard, Max Perutz and John Kendrew in Cambridge (England), Linus Pauling and Erwin Chargaff in New York, Jean Brachet in Brussels and Norman Davidson in Glasgow had suggested more prebiotic substances and elucidated the molecular structure of RNA and DNA [7].

These advances led almost immediately to others which disclosed possible chemical precursors of biological processes from amorphous and acellular through algal, protozoal and metazoal to plants and animals, all of which were presumed to develop and diversify from a common base of protoplasm, energy, information and instruction stored in chemical codes and perhaps physically at various cellular levels. This, seemingly automatic, genetic process, serves now to locate, or to synthesize and assign precise functions to cellular membranes, sub-cellular constituents like ribosomes and mitochondria, structural and functional macromolecules like nucleotides and polypeptides, enzymes like polymerases and transcriptase, specialised macromolecules like haemoglobin and myoglobin, and so on. With hindsight, the same process throws light on Darwinian selection and highlights questions about creative evolution. What it does not and perhaps cannot explain is how chemistry was transformed into vitality, and how subsequent, unique attributes of life like memory, language and thought develop, function and sometimes inevitably die. Although advances in biochemistry since the 1950s probably exceed all others in this century, they cannot even begin to explain let alone bridge this gap between all of our knowledge to date and the spark of vitality which remains as a challenge, as great as that of the Big Bang, to spirited scientific enquiry.

It has to be acknowledged that the bridge over this gap may well remain as a secret of Nature, well beyond any human faculty. But the quest is continuing at such a pace that it is timely to register more milestones while contemplating afresh what conditions might be deemed appropriate to the miraculous shift in the fathomless past from ordinary substance to protoplasm, from inert to animate matter, and from life to death of a cell, organ or body. The abiding lesson and sociable history of science to date is that, irrespective of the goal, the search for it is productive and equally exciting because of the incidental discoveries and people you encounter on the way.

## 3. Gaps in science

Science aspires to be universal. It doubts dogmas and despises cults but, inevitably, there are gaps, fissures and frontiers between disciplines, for instance that between light, electricity and magnetism until Clerk Maxwell closed it. A recent gap is the failure of organized science until the 1950s to exploit or even recognize the existence of the Liquid Crystal (LC) as an intermediate in changes of state and energy of matter. For furtherance of study as well as for its impressive utility, the LC might now compete with ionized plasma for recognition as a fourth or fifth form of matter, with immediate relevance to this gap and quest. Matter in LC form occurs as an intermediate between ordered but rigid solid crystals when they are heated and melt into disordered amorphous substance before becoming a liquid. The essential property of an LC is that it maintains its characteristic molecularly ordered structure and associated properties while its

original rigidity lessens, and kinetic disorder and entropy increase. The solid crystal structure becomes increasingly mobile and manoeuvrable as an LC. This phenomenon, originally observed as an incidental, colourful attribute of cholesteryl esters by F. Reinitzer in 1888 was studied by O. Lehmann, D. Vorländer, G. Friedel [8] and a few other scientists. It was found to apply to a wider range of organic compounds which became anisotropic when viewed under polarized light while they melted from opaque solid through semisolid states to clear, isotropic liquids. Until then, crystals, melts and melting points had been viewed as defining intermediate and end-point attributes of all substances.

Preservation of molecular order with mobility is not entirely unique. Atoms of metals like copper slide over each other in wires and plates, but these are elements bound by interatomic forces. The occurrence of like order in mobile, reversible transformations of specified organic substances in an intermediate, ordered mesomorphic, paracrystalline state—the LC—was largely overlooked or denied by chemists and physicists until 1933, when the Faraday Society in London organized a meeting at which J. D. Bernal [9] described crystallographic studies and suggested a role for the LC in life processes. There was no immediate follow-through but, in the 1950s, G. W. Gray in Hull, England, ruffled the chemical orthodoxy with his studies [10] of thermotropic mesophases in organic compounds. Since then, Gray has conducted an Odyssey of systematic exploration [10] of molecular properties and synthesis of organic compounds possessing these properties, and gradually expanded his laboratory from a prefabricated hut into a Centre of Liquid Crystals at the University of Hull. During this period, Glenn Brown [11] and a few others aroused interest in the USA by organizing the first ever international conference on LCs at Kent State University in Ohio in 1965. The American Chemical Society reacted in 1967 by publishing results of a meeting held in 1965 on Advances in Chemistry [12] in Atlantic City. Brown then obtained funds from the State of Ohio to found an Institute of Liquid Crystals, attract scientists from elsewhere and promote experimentation. A second conference was held in Kent, Ohio in 1968, a third in Berlin in 1970, and in 1968 there was also a Gordon Conference in New Hampshire. Industries in the USA, Japan and Germany expressed interest, and research expanded.

Even so, the LC was still regarded as a curiosity of little importance scientifically and none commercially until, in the late 1960s, the highly variable and reversible acquisition of anisotropy and spectacular colour changes in compounds with a thermotropic mesophase began to be utilized industrially in optics and eventually computerized programmes for displaying charts and images on instrumental monitors and television screens. Since then, commercial exploitation has been enormous, especially in the pan-Asiatic intellectual arc stretching from New Zealand through the Phillipines to South Korea, China and Japan. This is unstoppable, as is a parallel expansion of scientific experimentation and invention arising partly from new research but stemming still from the pre-1950 researches of pioneers like Lehman and Friedel who classified appearances in polarized light of substances in the mesophase as being thread-like and twisted (nematic), layered (smectic) or helical (cholesteric). These appearances reflected the underlying molecular structure and overall arrangement: end-to-end, parallel, tilted, twisted, layered or complex. Studies of optical and thermodynamic properties were followed by analyses of crystallographic configurations and proton resonance. These provided insight into precise molecular structure, interatomic distances, linkages, orientation and interactions in the changes of phase of the increasing range of eligible substances [13] synthesized and studied by researchers like Gray, Brown, Chatelain, Sackmann and Saupe, and by companies such as Merck, Hoffmann-La Roche, IBM and the Radio Corporation of America. There was also a lively interest in Japan in possible industrial applications of these new discoveries, and this is reflected now in the dominance of this and other resourceful Asiatic countries in utilizing the optical, thermal, electrical and colourful properties of an increasing range of substances possessing an inducible mesophase. The general public, globally and suddenly, became aware on their TV and computer screens of revolutionary discoveries in physics and chemistry which almost all physicists and chemists had ignored.

To some extent, this was because substances with a thermotropic mesophase were the perquisite of a few eccentric chemists. However, in the 1920s, some of the eccentrics who were interested in non-organic solvents had noticed spontaneous changes of phase and LC properties at interfaces between certain water insoluble lipids and water, especially if the lipid was surface active or if surfactants were introduced. These observations put biophysics and, in particular, the study of amphiphiles, into business.

## 4. Amphiphiles and the lyotropic mesophase

In 1910, this subject had been initiated in the College de France by a biologist, J. Nageotte [14], who from then until 1937 described in detail '*La morphologie des gels lipoides, myeline, cristaux liquides et vacuoles*'. He drew attention to similarities with the myelin of nervous tissue, at first in binary and then in more complex amphiphile systems composed of lecithin, fatty acids, water and other solvents. Experimental studies with lipids were conducted, also in France, by F. Grandjean (1916–18), in America by J. Langmuir (1916), in England

by J. W. MacBain, and in Germany by W. Ostwald and H. Zocher who proposed in 1927 a continuum theory, contending that the formation of a mesophase required elongated molecules held together by van der Waals or other weak forces rather than covalent bonds [15]. In the 1930s, the chemistry of binary, tertiary and quaternary lyotropic phases was pursued in Sheffield by A. S. C. Lawrence [16]. But it was a physicist and pioneer in X-ray crystallography, J. D. Bernal [9], who in 1933 introduced a molecular level of precision into this field by observing that the alpha-helical diffraction pattern of synthetic polypeptides examined by this technique corresponded to a LC appearance in fibrous proteins. This tentative entry of physics into biology should have prompted further study of a possible role of the LC in structural components of living tissues, and led there and then to a better understanding, but the coming of world war two in 1939 brought most of what was going on to a standstill while European scientists transferred their energy to more explosive topics.

Interest revived in 1946 when Dervichian [18] defined the lyotropic mesophase, and amphiphilic states began to be investigated systematically by physical chemists and biologists. The same behaviour was observed in multimolecular aggregates with boundaries in which lipophilic hydrocarbon molecules were orientated alongside polar hydrophilic molecules [19]. In familiar substances like soaps, these are arranged in solid three-dimensional lamellae but this changes with solvency and rise of temperature as, in practice, all housewives knew and as scientists were beginning to know. It became obvious that alternating lipoid and hydrophilic molecules could be arranged, or could arrange themselves in like-to-like fashion, either in clusters (micelles) held by short range intermediate forces or, as they grow in size and develop radial or more complex arrangements, by long range order in two or three dimensions. Alternatively, bilayers could form or could be constructed as interfacial, limiting membranes around more complex mesophases when additional components were introduced.

It was at first believed that long range systems attained equilibrium in accordance with other transitions [20], but Winsor [21] showed that this applied only to models in which the components entered a mesophase which remained fluid in a gel or lamellar phase. A true LC would retain mobility while being continuously deformed by pull or push. If this kind of mesophase is then allowed or designed to solidify on a surface exposed to air, it hardens permanently, as in emulsion paints. Artists working with egg tempera and gouache have applied this feature of LC theory empirically in their glowing portraits for centuries but, as A. S. C. Lawrence used to say, it was strange that decorators failed to commercialize the same property in emulsion paints and plastics until quite recently. Many other utilities like this are now commonplace but there are many more awaiting application or development in science, domestic situations, industry and especially in biomedical prospects, some of which are described below.

Scientifically, theories about amphiphiles derive from recognition of the fused fluid phases that are thin and membranous, colloidal or gelatinous, stable over a given range of temperature, birefringent, preserving orientation in the parallel, planar or radial dimension, and mobile. Amphiphile layers in interfacial aqueous zones have the capacity for further physical interactions which are either hydrophilic and electrostatic or lipophilic and electrokinetic. According to Winsor [13, 21], electrostatic forces determine the solvent power of the water, while electrokinetic activity governs lipophilic solubility and interactions between molecules in or entering the layers. The system is therefore already ordered, chemically and physically, but responsive to external changes while remaining stabilized structurally by weak forces of attraction between non-polar molecules.

In the early days of these lyotropic systems, most of the experiments were performed with colloids and suspensions in preparations examined by polarizing optical microscopy. Crude measurements were made optically by inserting micrometers, thermal platforms and wave-plates to measure shifts and orientation. Crystallography and electron microscopy, which required drying, were of limited value. Precise meaurements were not feasible until spectrometric, optical, thermal and electrified platforms suitable for experimentation with aqueous preparations were developed. The lyotropic mesophase then became respectable as an eligible field for advanced biophysical study of the nature of the ordered state, of changes in phase and of conditions required for initiation, stabilization and utilization of the LC in biological systems. Despite this promising biophysical overture, there was still a curious delay in perceiving professional opportunities and practical applications in contemporary biochemical and medical sciences until, from 1953 onward, everything changed, with the work of Watson, Crick and others, as described below.

## 5. The advent of molecular biology

Whereas great discoveries can arise by accident or inspiration, transitional advances in science occur when a person with expertise in one field crosses into another. This is what happened in the mid 19th century when Darwin moved from medical studies to natural history, Pasteur from chemistry to microbiology and, in general terms, the exact sciences from then onward into biology. This began with an interest in signals that compounds like urea, D-sugars and L-amino acids, physical states like crystals, colloids and brownian movement, and biological

entities like cells, tissues and membranes were subjects requiring interdisciplinary attention [20, 22]. The outcome in the 1950s was an inspiring sequence of experiments, commitments, accidents and discoveries which came quickly to a climax with the happy coalition between Francis Crick, a physicist turned biologist, and James Watson, a biologist who jumped into physics. With encouragement from Sir Lawrence Bragg, Sir Alexander Todd and John Kendrew in Cambridge, and immense help from parallel efforts by Maurice Wilkins, Rosalind Franklin and R. G. Gosling in London, they joined talents to show in 1953 that nucleotides, previously recognized and systematically classified by Erwin Chargaff [23] in New York among others, polymerized in a double helix to form polynucleotides which turned out to be the chemical key to the synthesis of protein, the basis of genetic inheritance and much else [24, 25]. The fact that the operational molecules RNA and DNA behaved as LCs in this process was mentioned subsequently by Max Perutz [26] but not recognized at the time as being relevant to the structure of DNA and the interaction of transfer RNA with amino acids in ribosomes.

Less spectacularly, other lyotropic systems also contribute. Experiments before 1950 had established the structural and functional properties of amphiphiles in biology. From the 1950s onward, it was recognized that lipophilic/hydrophobic and hydrophilic/lipophobic molecules are juxtaposed in ordered smectic layers, nematic fibres and helices into complex structures comparable to the carbohydrates, proteins and lipoids of cellular, subcellular and membranous components of living cells and tissues. Lawrence, Dervichian, Ambrose, Winsor, Palade, Stewart and others described various complex amphiphile systems, and noted how the boundaries between phases moved and vibrated like the bilayer membranes of living cells. Simple simulations of natural bilayers lacked the ability to allow preferred ions of true crystalloids to pass through interstices in the bilayer in either direction or to stabilize levels of electrolytes and functional solutes within the cell. Sophisticated models in which fatty acids, cholesterol and surfactants were introduced into bilayers and lamellae were developed to overcome these impediments, by Winsor, Chapman and others, notably Luzzatti [27] and his collaborators. Fluidity, retention of water, polar solubilities, osmosis and other properties were investigated in relation to changes of phase and structure. Similarities with natural membranes were demonstrated, but the selective regulation of entry and exit of electrolytes was never achieved. It has to be acknowledged (see below) that many of these experiments were performed under conditions of temperature and concentration inappropriate for living cells and organisms.

In investigations of other models, Stockenius [28], Finean [29] and Robertson [30] showed similarities between myelin in natural neural structures and aqueous dispersions of phospholipids and fatty acids. The principal outcome was the construction of a greater variety of myelinic figures which preserved internal orientation and flexibility during physical and chemical strain, and formed strands and tubes resembling neural myelin. It seemed likely that lyotropic systems like these might be involved in biological processes but, until recently, there were few convincing examples and no obvious utility.

In the early 1960s, work on chemical aspects of the organic mesophase was extended by Meier, Zocher and Saupe *et al*. to include optical and thermodynamic measurements of energy expenditure during changes in phase. But most of the work on lyotropic systems was descriptive and qualitative, as described above. The considerable potential of information awaiting discovery in the properties and reactivity of amphiphiles, and therefore in applications to biology was virtually untapped. This changed with the researches initiated by de Gennes [31] who, with colleagues in the Orsay Group in France, investigated the weak distortions in nematics occurring in long range order. He used an idealized system modelled on a single crystal formed by uniaxial nematic molecules aligned in a direction defined by two parameters, the director or unit vector **n**, and an order parameter $S$. Nematics in this system had intrinsic elasticity which could be splayed, bent or twisted, each with a measurable elastic constant $k = \text{energy/length}$, expressed as units of force in dynes and changeable in orientation by weak electric currents with signals that were optically measurable.

In de Gennes's view, the macrospopic properties of this system conformed to the Continuum Theory originally proposed by H. Zocher in Germany in 1933 and elaborated subsequently [32]. It is comparable in the domain of LCs to the physics of elasticity in solids and, although applied mainly to organic nematics, it had relevance to swarms and flow of matter in lyotropic systems. There were however intrinsic defects in models based on this theory and in experimental work because of constraints imposed by the glassware and materials in the apparatus which affected boundaries, surfaces and flow. These defects, described by Friedel and Kleman [33] as disclination points and lines, were however informative in terms of the topology and free energy of the system, whether nematic, smectic or cholesteric. Other important technical constraints affecting the interpretation of these findings in the lyotropic mesophase are described below.

Members of the Orsay Group [34] in France expanded this work into a general study of the energy of order and disorder in lyotropic systems. The next main advance came again from de Gennes [35] in his work on dynamic effects in nematics: scalar pressures, velocity, viscosity, laminar and shear flow in changes of state. He formulated

equations to calculate friction coefficients, rotations, scattering of light and relaxation of nuclear spin. It is beyond the remit of this article and the competence of the writer to discuss these equations critically but it can be said that, in general, they confirm the static and continuum theories of phase change, and extend attention to dynamic changes observed in liquid crystals in electromagnetic and ultrasonic fields. Better understanding of the physics and mathematics of these effects opened doors in the 1970s to further studies relating them to lyotropic and then to biological systems, especially to experimental studies of the structure and function of myosin in mammalian muscle. De Gennes has, moreover, recently extended practical application of this with his studies of the possible role of LCs in movements of artificial muscles [35, 36] which are now approaching utility.

In other fields, advances in theoretical and practical biophysics were continuing with the work of Brahms and van Holde [37] on DNA and Perlmann [38] at the Rockefeller on pepsin. Studies of discotic LCs, first identified by Chandrasekar [39] in Bangalore in 1977, disclosed new formations of discotic molecules with optical properties which extended the range of information that could be conveyed in displays. More recently, as reported in Edinburgh, Cooke *et al.* [40] in Leeds, England have made columnar blocks separated by lamellae, also with the molecular structure of a discogen, and Sergan *et al.* [41] at Kent State University have applied this knowledge to lyotropic systems to improve contrast in nematic displays. Ye and Sato [42] at Akito University in Japan have developed lightweight lenses with focal lengths that can be controlled by small changes in electric voltage through the LC. To get a better view of surface relief structures and gradient profiles, Seo *et al.* [43] in Hallym University, S. Korea, described a series of experiments with nematic LC systems to develop two-dimensional microlens arrays with switching power 1000 times faster than conventional nematic systems.

The twisting power of delicate nematic systems deserves more attention. Seed *et al.* [44] at Kent State University have synthesized colourless, soluble, nematic compounds with increased helical twisting power which are photochemically and thermally stable. This has improved displays of cholesteric colours as predicted by Friedel in 1922 [8] and should be useful as a yardstick for measuring the effects of twist in lyotropic and biological systems. Ma *et al.* [45] in the LC Institute of Tokyo and elsewhere in Japan have developed mechanical rotor models for LC systems based on an idea that the molecular core acts like a rotor and the terminal groups like bearings, as in a machine, to explain phase change and thermal properties of LCs. At Kumamoto Univerity, also in Japan, Ihara *et al.* [46] have also used electron microscopy, NMR and IR spectroscopy to investigate the aggregation of lipid in gels by H-bonding interactions for delivery and controlled release of drugs, and immobilization of enzymes.

The foregoing text has given historical and some more recent examples of observed activity and of the theoretical role of the LC in biological processes. Additional biomedical examples are provided by the present author's experimental work at the Medical Research Council Laboratories at Carshalton in London, UK, and in the Research Laboratories of the School of Public Health at the University of North Carolina, Chapel Hill, NC. This contribution [47–50 *et seq*] to lyotropic aspects of the LC is now reviewed.

## 6. Experimental studies by the writer

### 6.1. *Preliminary observations on the cholesteric mesophase in human and animal tissues*

This work began in 1955 when the author happened to be studying physiological clearing (lipolysis) of triglycerides and lipoproteins in patients, and then in rats and rabbits experimentally [47]. The usual (alimentary) lipaemia of triglycerides and high density alpha-lipoprotein that followed a fatty meal could be cleared rapidly by physiological lipase. But the low density bands ($> 1.0 < 1.04$ g ml) of beta-lipoproteins in patients or rabbits with hyperlipaemia were not cleared, either by physiological lipase or by lipoprotein lipase or heparin or other sulphated dextrans. In exploring reasons for this difference, and also a metabolic difference between rabbits and rats, it was found [48, 49] that cholesterol in the beta-lipoproteins of lowest density in plasma, and in pathological atheromatous deposits in arteries was anisotropic at body temperatures (36–38°C) but amorphous or crystalline at room temperature or below.

In exploration of reasons for this difference, it was found that cholesterol in the beta-lipoproteins in plasma was physically and microscopically the same as that in atheromatous deposits in arteries from limbs which were examined on a warm slide immediately after surgical excision (by Professor Charles Rob at St Mary's Hospital, London, for replacement by grafts). It was anisotropic and showed spherulites with crosses (figure 1) at body temperatures, but was amorphous or crystalline at room temperature or below. In other human tissues or colloids examined post-operatively or post-mortem, it was found that similar spherulites were present in normal ovaries, testes, semen, myelin of brain and spinal cord, and also in abnormal deposits in diseased kidneys and gallstones. When the anisotropic substance was separated, it was found that the main component was, chemically and chromatographically, free and esterified cholesterol [50], except in the ovary where it was present in a complex of steroid hormones and metabolites which were molecularly closely related. These properties corresponded to

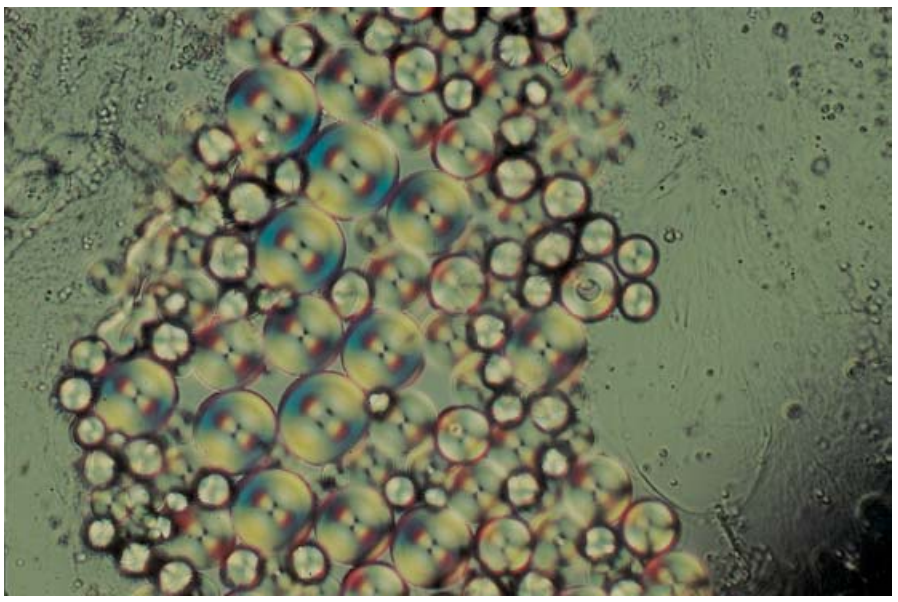

Figure 1. Spherulitic liquid crystals of cholesterol from atheromatous deposit in an artery resected surgically for replacement by a graft. Polarized light, 1/4 wave plate, $\times 1000$.

a general tendency in polypeptides, polynucleotides, haemoglobin and other biologically active macromolecules to form a helical mesophase [22, 29, 34, 51].

The author then consulted Professor J. D. Bernal at Birkbeck College, London, and showed him samples and photographs. He examined a representative sample of anisotropic material from an artery by crystallography, and confirmed it as alpha-helical LC. The visit was welcomed by Bernal who said that he had been waiting for years for a biologist or physician to identify, produce and study the LC in living cells and tissues and, indeed, in the origins of life. He produced models of myoglobin and haemoglobin which Professor Max Perutz was constructing [26], gave an insider view of the new world of RNA (see Part II), emphasized the need for investigation of the molecular structure of gluten in wheat among other biological macromolecules, and drew attention to the helical polypeptide liquid crystalline chains already synthesized by Conmar Robinson in the Courtauld Research Laboratories, London, as a prelude to manufacturing rayon and other synthetic fibres to replace silk and cotton thread and fabric [17]. (Thereafter, polyesters developed in the textile research laboratories of J & P Coats in Paisley, Scotland, replaced the polypeptides as they were more resistant to wetting and decay.) According to Japanese scientists [52], silk is a biopolymer formed when a viscous aqueous solution of two proteins, fibroin and sericin, is spun in the tubular gland of the silkworm (*Bombyx mori*) into a fine, smooth, transparent nematic liquid crystal which is then polished by muscles, and can be pulled out from the cut end of the gland. This process can be modified further by sophisticated spinning and variation in levels of calcium ions. The textile technology in fact resembles the biotechnology of the silkworm.

### 6.2. *Systematic experimental work*

Further experiments were then designed to define the properties of the pathogenic mesophase in human arteries. Low density lipoprotein with hydrated density ($> 1.0 < 1.04$ g ml) was separated from serum by ultracentrifugation at 79 420 *g*. LCs were identified as a cholesteric phase in a layer of anisotropic spherulites showing crosses in polarized light and a characteristic shift under a quarter-wave plate (figure 2). They were re-suspended ultrasonically with and without fibrinogen in normal plasma for pulsation against isolated segments of rabbits' aortas in a perfusion chamber at 37°C. Plasma from non-lipaemic rats, rabbits, humans and simulated lipaemic colloids were used as controls. It was found that a cholesteric mesophase developed in the arterial lining only when low density lipoprotein was pulsated. Deposition was larger in the presence of fibrinogen as a matrix, as in atherosclerosis in humans, but the physical form of lipoprotein (i.e. low density with high cholesterol content, as in figure 2) at pulsation pressure of 120–140 mm Hg was the critical factor. In control rabbits, pulsation with normal plasma or plasma-containing high density cholesterol in lipoprotein gave no change [53].

Apart from its general distribution in membranes and fibres, a similar lyotropic mesophase was identified in various cells, tissues and organs, including normal human ovaries, testes, semen, brain and spinal cord, and also in

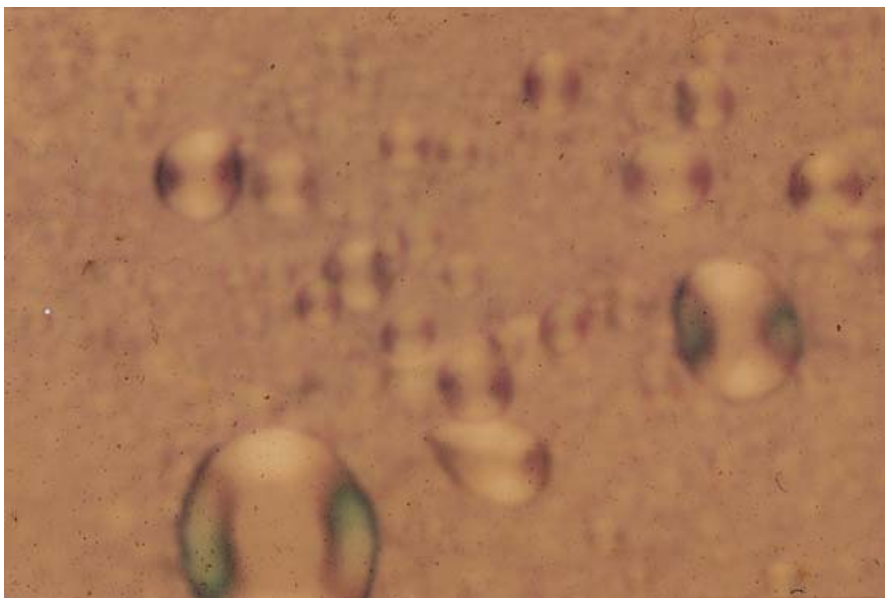

Figure 2. Spherulitic liquid crystals in low density layer of hyperlipaemic plasma. Polarized light. 1/4 wave plate, ×1000.

abnormal deposits in diseased kidneys and gall bladder. Cholesterol was usually present in free and esterified form except in the ovary and adrenal where it was a precursor or member of a complex of molecularly-related steroid hormones.

## 7. Interpretation of the biochemical role of cholesterol

Cholesterol is a normal and necessary component of many forms of living matter. The findings reported above suggest that, although it can be present in LC form in various human and animal tissues, it can alter subtly in phase and cause pathological changes in arteries when deposited as a lyotropic mesophase from low density lipoprotein circulating in blood, because beta-lipoprotein LCs in this form cannot be cleared by lipoprotein lipase or physiological lipolysis [53]. Solid forms undergo phase change to a complex helical mesophase at the interface with phospholipids and long chain surfactants.

In the 1950s, J. and J. Cornforth showed that cholesterol is synthesized in the human body, as in most vertebrates, from acetate precursors and solubilized with surface active conjugates in the liver in the free form or as esters. It is also absorbed from foods but, being insoluble in water, has to be solubilized for absorption from the intestine, along with triglycerides and other fats, directly into lymph in intestinal micro-channels (lacteals) where, except in herbivores, it appears in the blood in isotropic solution with triglyceride in microscopic particles (chylomicrons). These are conjugated with proteins in the liver as lipoproteins, varying in three grades of density [54], according to the proportions of triglyceride components: very low density (VLD), low density (LD) beta-lipopoteins and high density (HD) alpha-lipoprotein. The levels of each form are regulated [55] by four apo-lipoprotein genes (APO-ABC and E) on chromosome 19, which also identifies receptor cells and tissues in need of cholesterol in these various forms in which chirality, goodness of molecular fit and density are important. Until middle age, in health, HDL circulates partly esterified as alpha-lipoprotein. Any excess is excreted in bile.

Levels and distribution of the different forms vary with sex, hormonal constitution, geography and ethnic groups, and change with age. In women, there is in pregnancy a natural rise of alpha-cholesterol which circulates as such until the menopause when it may become LDL and form gallstones. In men, any excess is more likely to be converted into LD or VLD beta-lipoprotein, which may be deposited in and constrict coronary arteries to cause heart disease from age forty onwards though, in children or adults with renal or cardiac defects, deposition of beta-cholesterol occurs much sooner. The levels of the different forms of cholesterol in blood and the ratio of the free to esterified forms serve therefore as sensitive markers of cardio-vascular fitness and hormonal balance: for instance, the VLDL increases in diabetes and if thyroid function is defective. It is possible to identify and treat persons at risk of arterial and cardiac disease accordingly. In changing from LDL to VLDL, cholesterol becomes a helical liquid crystal regarded as 'bad' cholesterol because it then causes atherosclerosis and coronary heart disease.

In the liver, cholesterol is metabolized, solubilized by bile salts and acids; it is then concentrated in the gall bladder and excreted in bile. But it solidifies pathologically as gall stones or is deposited in the lining of the gall bladder as described above, especially in a disease well known to surgeons as cholesterosis or strawberry gall bladder because the cholesterol glistens

as pinpoints in the vascular membrane. In 1954, Isaksson described the additional dissolving power of lecithin in the presence of bile acids. This laid the chemical foundation for work described 10 years later by Small, Bourges and Dervichian on the quaternary lecithin-bile salt-cholesterol-water system in the gall bladder which pointed the way to medical treatment now available for dissolving gall stones [56].

In solid crystalline and amorphous form, cholesterol can be converted into the anisotropic LC form by exposure in an aqueous suspension to an interface with long chain polyoxyethylene esters and other surface active agents (figure 3). Esters of cholesterol with fatty acids show striking changes in interference patterns and colour with minor changes in temperature and pressure, varying with whichever fatty acid is used. Solutions of these in triglyceride or in plastic films can be applied to the skin to detect fine temperature differences in subcutaneous tissues and organs, for instance in an underlying inflammatory lesion or neoplasm, and for analysing molecular morphology of surfaces. Recent work in Osaka by Teramoto *et al.* [57] has identified LCs in helical coils in cellulose derivatives and as a general property of semi-flexible polymers They have quantified the basic molecular parameters with measurements of cylindrical contour ($L$) persistence length ($q$) and pitch ($P$) to give twisting power $q(t) = 2\pi/P$. This thinking leads to a theory for $P$ which includes attention to the nematic order parameter $S$, the chiral properties and perturbations in semi-flexible polymers which are of relevance to the behaviour of cholesterol physiologically (figure 3).

In mammals, endogenous and exogenous cholesterol can be reconstructed in the free, esterified and conjugated HD and LD lipoprotein forms. These are identifiable in similar LC form in other organs where they are converted with differing molecular signatures in various specialized cells into hormones in the adrenal cortex (cortisone) and sex glands (testosterone, oestrogens and progesterone) which contribute to their action naturally and as derivatives in contraceptive pills. There is also a relationship with another steroid, ergosterol, a precursor of vitamin D, in the nutrition of bone and skin, with growth and conversely with ageing. Experimental work [58] in the Phillipines suggests that estradiol, one form of oestrogen, also has a role in ageing and neuroprotection, which might relate to its presence as an LC in the charged batteries of mitochondria. In other situations, the LC is similarly identifiable in helical, membranous and myelinic structural components of the central and peripheral nervous systems, probably in all Vertebrates and Insects. In Man, there is an ultimate anatomical and functional complexity in cerebral cells and connections arising from further biophysical interconversions regulated by a gene CYP17 on chromosome 10 [55].

The general properties of LC forms of cholesterol (see the table) include birefringence, ordered aggregation to form spherulites and helical structures (figure 4) and resistance to mechanical stresses, high energy radiation and natural lipolysis [59]. It is tempting to relate these properties to evolutionary advantages, not only in mammalian biology but also in the dazzling LC displays in the chitinous protein in wings of beetles and other insects which change colour with light and movement—first noted by Conmar Robinson in the course of his work on polypeptides [17]. Tsiourvas and his colleagues [60] in Greece have shown surface effects with a variety of dendrimers with amphiphilic and ionic properties,

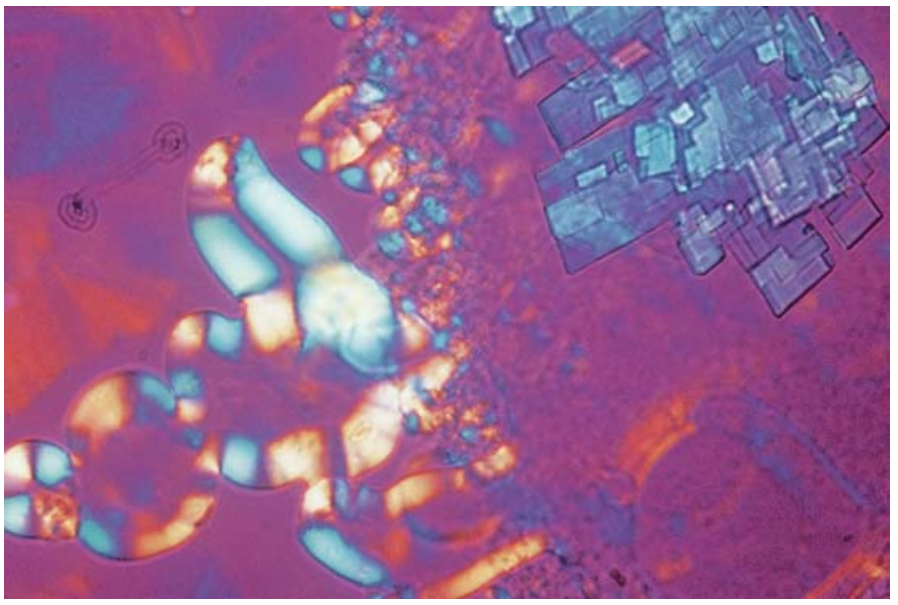

Figure 3. Conversion of crystalline cholesterol to liquid crystalline mesophase at interface with long chain polyoxyethylene ester. Polarized light, 1/4 wave plate, $\times 1000$.

Table 1. Properties of a cholesteric lipoprotein mesophase in human and animal tissues[a].

| | |
|---|---|
| *Physical properties*: | |
| Optical sign | birefringent, positive in |
| Mesothermal range | 35–42°C |
| −20°C | gradual disintegration |
| −185°C | immediate disintegration. |
| Ultrasonic vibration (2 min) | no change |
| (20 min at 0°C) | disintegration |
| Dehydration | partial disintegration. |
| Ultracentrifugation (70 000 *g*) | stable |
| $\gamma$-radiation (5 Mrads) | stable |
| Linear accelarator (4 meV) | stable |
| *Chemical properties*: | |
| Lipolysis (lipoprotein lipase) | stable |
| (Triglyceride lipase) | stable |
| Esterase | stable |
| Glycerol | stable |
| Water and electrolyte solutions | stable, with growth |
| Lipophilic dyes | minimal solubility |
| Organic solvents | unstable |
| Aliphatic surfactants | gradually unstable |

[a] See text for details.

prepared by protonation of alkylated derivatives of cholesterol. These and other properties, as described below, suggest a role for LCs as stable and possibly protective structural substances in the primitive evolution of dendrimeric networks as sensors of change on external and intermediate surfaces of living tissues [61, 62].

## 8. Artefacts

In all of these scientific examinations of living cells, tissues and organs, especially by microscopy and crystallography, it is essential to respect, and never overlook credibility gaps occurring because of fundamental differences between living and non-living substance which impose technical limitations upon our approaches [62]. Living cells require hydration whereas histology, staining and electron microscopy usually require dehydration. Cytoplasm is colloidal and fluid, and essential components like mitochondria may be transparent. This can be overcome to some extent by phase contrast or interference microscopy, but visualization of detail in living cells is usually impossible because of Brownian movement and, at less than $\times 1000$ magnification, by the wavelength of incident light. This means that visualization of subcellular structures is impossible without fixation by agents like formaldehyde which denature protoplasm. The same restrictions apply to UV ultramicroscopy ($\times \sim 3000$) and X-ray crystallography which map inter-atomic distances, not shape. Conventional electron microscopy ($\times \sim 10\,000$) requires fixation by gluteraldehyde, uranium acetate or other coagulants and solvents never encountered in Nature, which denature proteins, dissolve lipids, expand macromolecules and create artefacts, especially in the loose lyotropic substance of the brain (see Neurobiology, below). What is seen, reported and often believed is what appears in the eye of the beholder—which is a good reason for insisting on confirmation by a second, more experienced beholder in the medical diagnosis of cancer and in differentiating a cellular vesicle or subcellular particle from a provirus.

Cells and tissues should therefore be studied as far as possible in their natural state rather than by artefactual techniques [62]. George Palade endorsed this when the writer raised the question at the second Gordon

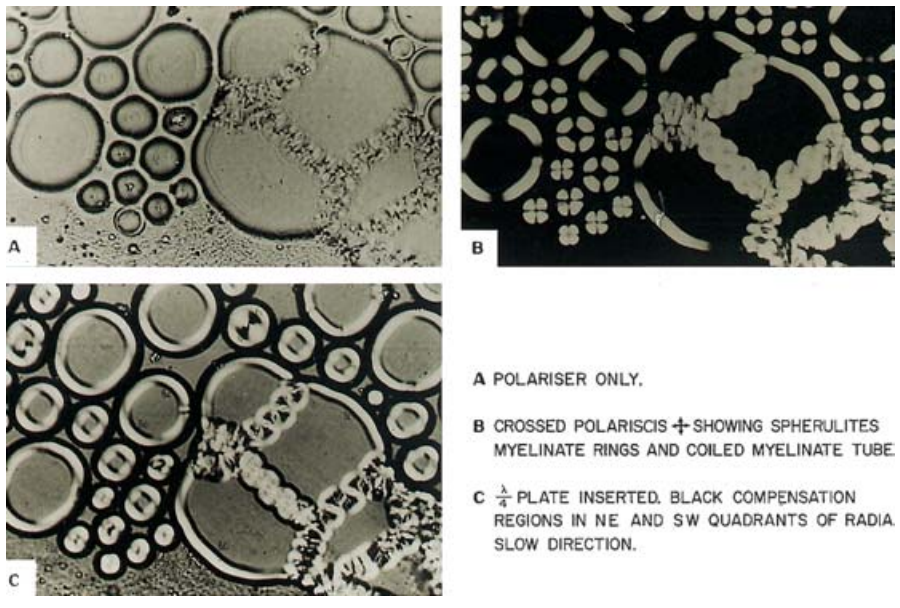


Figure 4. Spherulites of cholesterol growing in helical, liquid crystalline form.

Conference in 1968. As the range of technology increases, so do the possibilities of artefacts and misinterpretation. When the Laws of Physics are applied to biological matter, it is important to remember that they include quantum mechanics, the Principle of Uncertainty and relativism. The brilliant insights into the world of RNA, recently featured by the magazine Nature (11 July 2002), depend often on observations of ribosomes of *Tetrahymena*, a coelenterate, among other convenient laboratory animals such as *Drosophila* and inbred, transgenic mice It is true and wonderful that the coil of life might be botanically, zoologically and biochemically universal but, until we are sure of this, similar cautions apply The helical structure of DNA was not recognized until it was hydrated by Wilkins and Franklin. Recognition of these constraints in experimental technology, especially when applied in therapeutic and genetic interventions, is one of the most important contributions of LC technology to contemporary scientific discipline.

## 9. Membranes

The semipermeable plasma membrane developed by Danielli and Davson at University College in London in the 1940s was used by Mitchell in a series of studies (1961–79) on chains of redox electron carriers and the potential held by the concentration of ions on each side. He showed that the flow of electrons through this chain caused an efflux of hydrogen ions which activated the (reversible) oxidative phosphorylation of conversion of ADP to ATP and changes in permeability of the cell membrane. Mitchell thought that this applied also within the cell to mitochondria, and linked the oxidative reaction to anisotropy [63]. This work has been repeated by others since 1961 and upheld as a general explanation of the role of ATP in the transfer of $K^+$ among other ions, and in oxidative activity in cytoplasmic membranes ranging from those in primitive bacteria to organelles in plant and animal cells. The change in potential across the membrane seems to be driven by the ATP and difference in $K^+$ ion levels. With corrections for differences in energy, this would seem to apply also to $Na^+$, $Cl^-$and other elcctrolytes crossing membranes of thermophilic and other bacteria growing as syncytia without a cell wall. Mitchell's work is now accepted as a Theory of Chemiosmosis with wide relevance in bioenergetics for which he was awarded a Nobel Prize in 1991. During this period, Harold [64] extended the study of membranes to morphogenesis and general bioenergetics.

## 10. Neurobiology

Neural cells and tissues form a complex system in all animals which is biochemically more dependent than any other on proteins, phospholipids and complex macromolecules. The LC is an essential but insufficiently recognized participant in this organization which, from peripheral fibrils to the brain, resembles a lyotropic system in which colloids, gels, syncytia, membranes, dendrimers and cells function individually and collectively, in lipoid and aqueous phases via amphiphiles and electrical and hormonal transmitters, to initiate and coordinate reflexes, hormones, voluntary movement, cerebration and all vital centres. The resource and potential of this system throughout the animal kingdom are enormous and still evolving but, by reason of its intricacy and delicacy, it is more fragile than any other living system. A disorder in a minute locus or part of a gene, or of the neurones and neural network responsible for processing incoming and outgoing messages, can be disastrous.

This fragility of the central nervous system is apparent, especially in mammals, in the history of a wide range of disorders which are only now beginning to be understood because of advances in genetics and neuroscience. For instance, recurring outbreaks of Scrapie, a transmissible spongiform encephalopathy (TSE) in certain breeds of sheep have puzzled veterinarians for a century. Scrapie is never transmitted to shepherds or other human contacts but outbreaks of other TSEs with similar features occur in goats and mink farms, and also in wild deer, elks and other ungulates. Seemingly similar TSEs were described sporadically in individual human patients in Europe by Creutzfeldt and then by Jakob in the 1920s. These are now named (not by the discoverers) as Creutzfeldt–Jakob Disease (CJD) which occurs with a rare but stable frequency of 1–2 cases in adults per million population in industrialized countries. The only recorded frequency of a comparable TSE in humans was a disease called kuru in one tribe in New Guinea which was uniquely confined to women and children who, unlike their menfolk, ate the brains of deceased tribesmen as an ancestral rite. Field investigations of this peculiar disorder in the 1970s led Carlton Gadjusek from the US National Institute of Health to propose that it was caused by transmission of a hitherto unknown 'slow' virus with unique viability. He was awarded a Nobel Prize for this discovery which was accepted as a cause of transmissible encephalopathy until 1982 when Prusiner in San Francisco found that a misfolded but transmissible protease-resistant prion protein (PrPSc) replaced normal prion (PrP) in the brains of sheep with scrapie. Prusiner's work [65] displaced Gadjusek's 'slow' virus hypothesis, and also earned a Nobel Prize.

Also in the early 1980s, the British Government accepted scientific advice that milk production would be improved nationally by feeding proteinaceous offal to herbivorous dairy cattle [66]. Their decision revoked empirical wisdom and regulations, and mandated use of untreated offal. This meant that ruminant herbivores

were force-fed with raw, fatty animal protein on a nation-wide scale. The outcome in 1986 was a biological singularity: a massive nation-wide outbreak of spongiform encephalopathy in cattle (BSE), from four cases in 1986 to 7137 confirmed cases in 1989 and 36 682 in 1992. A similar disease had been recognized in minor outbreaks in Portugal and Switzerland. Like scrapie, BSE was readily transmissible within herds and to some other mammals, and was considered to be similar pathologically to a variant form of CJD (vCJD) observed then in humans [67]. The essential lesion is a degeneration of cells and progressive destruction of ordered tissue in the grey cortex and white myelin of the brain, causing gross incoordination, mental disturbance, wasting and death. In these lesions, the malfolded prion (PrP) of normal brain substance is deposited or converted to a protease-resistant isoform similar to that present in the brains of sheep with scrapie (PrPSc) and to that of several other TSEs which cause outbreaks in other ungulates in a few wild locations, in other farm animals and cats, in some primates in captivity but never in dogs and rarely in other carnivores. All of these TSEs can be transmitted, with variable pathological signs, to some strains of laboratory mice, rats and other rodents.by intracerebral inoculation of brain material or, more slowly, by intra-peritoneal injection, or by feeding transgenic mice with much larger quantities of the same material [68].

The official Inquiry [69] into the outbreak of BSE in the UK concluded in 2001 that it was a bovine form of scrapie, resulting from a mutation of PrPSc occurring 20–30 years previously. This accepts evidence that the pathognomonic change in all of the TSEs is a replacement of normal by abnormal, misfolded, protease-resistant prion similar to PrPSc. Recent investigations however show that, in all the rodent models used for tracking and differentiating TSEs, the PrPs are heterogeneous with a significant ($\times 4$) rise in a different protease-sensitive PrP in an LC complex of phospholipid with cholesterol as encephalopathy develops. Various manipulations in cell cultures, including recombinations of PrPs, failed to produce this conversion though soluble PrPs in micellar form can be produced in bulk. In sheep with scrapie and in mice inoculated with PrPSc, there was no difference in the localization or amount of the associated mRNA. Spongiform softening ('schammig'), now regarded as pathognomonic of BSE, CJD and vCJD, is lacking in all but one of the original cases described by Creutzfeldt and Jakob, and there is a considerable overlap in clinical and pathological features between all forms of CJD and other encephalopathies [70]. This raises doubts about the claim that PrPSc is the exclusive cause of BSE and vCJD, as accepted by the National BSE Inquiry and hence by scientists, veterinarians and governments in most countries.

In this regard, it is appropriate to recall the origin of the outbreak of BSE in the UK and reconsider a previous suggestion [71] to the Inquiry and in recent publications [72] that BSE is primarily a nutritional disorder transmitted not by an infectious agent but by forced feeding of scrapie-contaminated lipoprotein in offal to herbivorous dairy cattle. Proteins can become toxic when misfolded [73] and PrPSc cannot be excluded as a product, valid marker or contributory cause of BSE. But vCJD might be different. All cases to date are genetically homozygous for methionine [69]; there is no evidence of transmission of scrapie or PrPSc, or of TSEs in other ungulates or felines to humans; and sporadic cases have been diagnosed as vCJD in countries where there are no recorded outbreaks of BSE.

Epidemiologically, the outbreak of BSE in the UK was common-source wherever lipo-proteinaceous offal was fed to cattle (which often rejected it). Lipophilic organo-phospates which cause cerebral oedema, affect synapses and diminish appetite, were administered compulsorily during the same period. It is of significance also (a) that there was no BSE in herds of suckler-fed, grass-reared pedigree beef cattle unless there was contact with dairy cattle; (b) that referrals of other forms of human CJD have doubled since 1996; (c) that there is no evidence so far of a significant increase in vCJD; (d) that there is no increased frequency of vCJD in farm workers, veterinarians, handlers in abattoirs or others at highest risk of exposure to affected cattle since 1980; and (e) that, in statistical reports to date, there is no analysis of confounding factors such as drug addiction, alcoholism and neurotropic pesticides which can cause similar symptoms.

The fact that the glycosylation signature of PrPSc is identical in patients with vCJD and animals inoculated with BSE material, and the paradox that most carnivores including dogs are immune to natural and experimental TSEs await explanation. Nevertheless, and even if the incubation period is long, the virtual absence of vCJD in contacts at highest risk in twenty years of exposure to BSE is in itself a reason for dismissing infectious PrPSc as a sole cause unless it can be shown that homogeneity for methionine or other genetic factors denote susceptibility to vCJD, or that the risks of symptomatic BSE and vCJD are compounded by those of other neurotoxic factors, as above.

Irrespectively of the underlying cause or causes, the symptomatology and transmissibility of these and other encephalopathies reveal dysfunction and profound disturbance in the *ordered structure* of the brains of wild and domesticated mammals and of human patients. A better understanding of this could be a step towards effective medication.

## 11. Sensi-neural functions

In the last decade, new technologies have revealed unexpected relationships between chemical, electrical,

optical and mechanical properties of biological membranes which relate to sensi-neural mechanisms. Petrov's work [74] in Sofia on ionic transport in a model of the cochlea suggests a means of amplifying hearing. Betzanos *et al.* [75] relate the sensitivity of bacterial cell membranes to the tilting of adjacent subunits in contractile responses of a visual iris. Sensitive membranes are ubiquitous and essential, though sometimes accidental, components of all forms of life, and it seems likely that observations as far apart as these, with increasingly accurate techniques for measuring and reproducing them, will provide more opportunities for lyotropic systems to rival thermal mesophases in utility. In his update of Darwin, Steve Jones [76] provides many examples of unlikely but meaningful linkages between orphan genes surviving from the distant past.

## 12. Neuropharmacology

Work on cytoplasmic membranes is important pharmacologically in various ways, for instance in identifying receptors for the attachment and intrusion of pathogenic microorganisms into cells, and the access of therapeutic drugs to combat these and other problems in intracellular pathology. Danielli and Davson had drawn attention in 1939 to the fact that many neurotropic substances and drugs (alcohol, volatile nitrites, ether, chloroform, chloral hydrate) were fat-soluble and therefore able to penetrate the lipids of the bilayer. Palamine *et al.* [77] in the Phillipines have recently ascribed the effectiveness of the antidepressant drug chlorpromazine to this property. Other phenothiazines have similar effects.

Changes of phase in neural cells affect transmission of electrical signals through synapses to end-receptors in motor and sense organs. To perform complex tasks, nervous systems must handle innumerable to-and-fro signals simultaneously, converting chemical activity into neurochemical and electrical messages. This is accomplished by networks of cells interconnected by afferent and efferent fibrils, leading inward to the spinal cord and brain from sensory organs, or outward via synapses to muscle plates and other end points capable of registering, remembering and transmitting messages. Within the brain, the interconnections are a nematic network. In the spinal cord and peripheral nerves, they are tubular phospholipids in myelin, as described by Finean [29]. Of the many mechanisms operating, that involving a protein dopamine is of special interest because it is an endogenous neurotransmitter regulated by a gene D4DR in chromosome 11 which affects cerebral function in various ways. If the dopamine is too low, the brain cells gradually lose the power to transfer messages to the voluntary muscles of the body and face which therefore become stiff and immobile The intellect is at first unaffected, so the person knows but cannot alter the affliction. Personality then becomes fixed and depressed. This distressing condition (Parkinson's disease) can be at first relieved by treatment with dopamine but responsiveness may not be sustained. Patterns of symptoms and severity vary, and minor forms of this disorder account for differences in demeanour of persons who are otherwise well. An excess of endogenous dopamine causes various forms of hyperactivity which may extend to risk-behaviour in experimental animals as well as in humans, and has been linked to some forms of schizophrenia. Persons with excess dopamine are at risk of frenzied reactions, delusions and hallucinations if they take stimulant drugs like amphetamine, cocaine and their derivatives, such as Ecstasy—which can also cause deaths from cardiac shock by its effect on brain-stem neurones.

The dopamine system in the brain is a striking example of how mental functions are influenced by changes of state and genetic regulator. Combinations of neurotropic drugs are dangerous and sometimes lethal, especially when used 'recreationally' to obtain or maintain euphoria, because they interact with each other and with cerebral intermediates in various ways. Volatile agents like alcohol, with their solvent effect on cell membrane bilayers, potentiate the action of narcotic drugs not only on behaviour but also on vital centres in the brain stem; volatile nitrites similarly aggravate hallucinogenic effects on higher centres; and there are many other interactions. The euphoric effects expected in recreational use of such drugs may change into dementia and sudden death.

On the positive side, there are many other drugs which are psycho-active in the sense that they relieve depression, whether endogenous or acquired, often at the price of physical and mental side effects, but always with risks of interaction with other drugs. Few are selective, but lithium deserves mention because it acts in elemental form, and only on endogenous depression. The related atoms sodium and potassium, with fewer electrons, cannot penetrate the selective blood–brain barrier and have no such effect. Oxetines, notably fluoxetine, are also relatively free from interactions and side effects. They operate by inhibiting the re-uptake of the neuro-hormone 5-hydroxytryptamine, a substance commonly released physiologically at sites of injury. In this connection, it is important to acknowledge the recognition and memory capacities of LC systems. A good example is described by J.-D. Marty and his colleagues [78] at the Université Paul Sabatier in Toulouse in studies with templates of molecular imprints of crosslinked polymers which retained and reproduced memory while keeping the flexibility of the network. Chiral templates induced a similar structure in the mesophase.

The relevance of this work to neural memory is obvious, and should be considered in relation to experimental and clinical studies in the 1960s by Robert Heath and others

[79] at Tulane University in New Orleans and the Delta Primate Center, Louisiana, on the action of tetrahydrocannabinol (Cannabis). Abuse of this drug spread explosively in the 1960s. It slows and tranquillizes mental activity, but weakens memory in a manner that relates to the production of endogenous cannabinoids which control both the acquisition and the extinction of aversive memories [80] in the hypothalamus of the primate brain. It seems likely that this neurotropic function of liquid crystalline material in neural tissue should apply also to the role of natural endorphins in the brain, analogous to that of opiates, in relieving pain and memories of pain, as shown in the 1960s by Hans Kosterlitz at the University of Aberdeen. The vast field of neurobiology with its attendant pharmacopoiea is conceivably the most important and certainly the most immediate target for application of knowledge and technology of changes in the ordered states and phases of components of the nervous systems. Physiologically, this applies throughout the animal kingdom, but it is of practical and immediate importance in *Homo sapiens*, in all of his seven Shakespearean ages—plus an intermediate adolescent age of disordered cerebration caused by psycho-active drugs, beginning also in the 1960s and now dangerously diversified by the widening range of mind-altering drugs used for therapy and, casually or addictively, for recreation.

## 13. Antimicrobial drugs

Ribosomes, as main sites of protein synthesis in bacteria as in most forms of life, are targets for attack by aminoglycoside antibiotics (streptomycin, paromomycin, gentamycin, etc), and chloramphenicol. These antibiotics inhibit multiplication of bacteria by interrupting synthesis at the ribosome after the release of amino acids by transfer RNA in a LC phase, and are therefore bacteriostatic. With the aminoglycosides, interruption of the sequence of amino acids leads to substitution and sooner or later to mutants conferring resistance to antibacterial activity which is remembered in the RNA feedback loop. This leads to populations of resistant organisms which can then, independently, convey the resistance genes or cytoplasmic plasmids to other bacterial species. This is less likely to happen with chloramphenicol, also acting at ribosome level, but otherwise the insertion of these bacteriostatic antimicrobials into ribosome subunits can create new populations of infectious organisms which are cross-reactive to all aminoglycosides, and invalidate treatment, for instance, of infections with *Mycobacterium tuberculosis*, even when the mutant strains experience parallel changes leading to loss of virulence. By the same token, this process should yield organisms with natural reduction of virulence, as may be happening now with nucleoside analogues targetted against HIV. Such organisms might be useful as vaccines.

Antibiotics are in general small molecules which are not LCs, but some of them can polymerize spontaneously into ordered macromolecules in their interaction with micro-organisms and in forming biologically active aggregates with mammalian proteins. These interactions sometimes explain their therapeutic action, for instance by inhibition of protein synthesis at ribosomes as above or by displacing ordered structures in membranes, mucopolysaccharide or lipopolysaccharide cell walls of bacteria.

On a different antimicrobial tack, penicillin is a natural beta-lactam antibiotic formed by the mould *Penicillium notatum*. This yields a simple but unusual dipeptide with a thiazolidine ring formed by fusion of the amino acids L-valine and L-cysteine. It has no mesophase but, in aqueous solution and in body fluids, it enters the muramic acid polymer of the cell walls of most bacteria during cell division and, by itself polymerizing therein, destroys the muramic polymers and kills dividing bacteria by lysis of their cell walls. In terms of chirality, it is interesting to note that ampicillin, a dipeptide prepared biosynthetically from 6-aminopenicillanic acid as an alpha-carbon analogue of benzylpenicillin in L+ and D− epimers, has much more antibacteral activity on the L+ epimer as if, biologically, it had to follow the track of the amino acids that would form a natural protein [81]. This chirality, which is not unique, must be an important clue to antimicrobial action. It also affects goodness-of-fit of the beta-lactamase and other enzymes which destroy some penicillins by opening the integral lactam ring or breaking the peptide linkage of the side chain which, otherwise, acts as a bridge to spontaneous polymerization that arrests antimicrobial activity [82].

One of the main objectives of antimicrobial chemotherapy is the development of antibiotics or synthetic drugs which can penetrate not only pathogenic micro-organisms, but also defensive cells, body cavities and colloids harbouring or damaged by these pathogens. Penetration of cells which have a mucopolysaccharide or mucopeptide cell wall outside a selective lipoid membrane is obviously more difficult, but not impossible with selected combinations of drugs with complementary effects, as in those now used for the chemotherapy of leprosy, tuberculosis, malaria and AIDS and in penetration of the selective blood–brain barrier.

Cell-based approaches to therapy were enhanced by the work of Ambrose [83] on membranes through the 1960s at the Chester Beatty Institute in London, relating to the causation and chemotherapy of cancer. There are now possibilities of more sophisticated cell-based interventions arising from work by Hannon [84] on RNA interference (RNAi) and hence on protein-coding genes. Another alternative is the use of negatively charged nucleic

acids for interaction with cationic liposomes as transfection agents. This is practicable but, as with all genetic interventions, carries hazards of other interactions. To overcome this, Francesangeli and colleagues in Italy [85] have used a DNA-phosphatidyl-metallic multilayer complex for self-assembly with liposomes for possible gene therapy. Although there are no unambiguous successes and many failures to date, this is a field where the various incentives for therapeutic interventions related to natural cellular structure and transfer of genetic information is irresistible.

Such information conveyed at molecular levels in genes, mutants, plasmids, mitochondria and other sub-cellular elements can cross species barriers naturally, and is being increasingly used artificially by ingenious techniques such as transfer of LC mesogens, recombination of antigens and transfection for medical research and therapy. There are dangers as well as benefits in this, in that natural linkages may be destroyed or accidentally displaced. But even freak linkages can be productive. For instance, under anaerobic conditions, certain heterotrophic bacteria assimilate carbon dioxide and form chains for phototropic exchanges of energy and release of pigments. The freak counterpart to this, originating perhaps as a primitive form of non-oxygenic photosynthesis, is seen in halobacteria which grow in white light as purple colonies forming rhodopsin, a lipoprotein showing LC behaviour and similar in molecular structure to the visual purple present in the cones of the retinal membrane in the eyes of vertebrates. This substance is activated by light from carotenoid precursors of vitamin A, essential for completeness of colour and night vision. The writer learned about the similarity of these precursors to LC in cones in the retina from Dr Antoinette Pirie at Oxford and commented on it [62] in 1965 but was unable then—as was she—to pursue it. However, it would appear now to be relevant to work reported in January 2002 by Professor Alex Ignatiev and a group of ocular and space scientists at the University of Houston who have developed solar cells which mimic the visual properties of the rods and cones of the retina. Their intention is to use those cells as implants for repair of damaged areas in the retina. The same approach might be useful also for improving sight in persons with diabetes and congenital disorders of the retina, and in other aspects of neurobiology. The remarkable properties of the LC in dendrimeric lamellae, optical lenses and diskettes in other recent reports offer possibilities of considerable extensions of scientific and industrial exploration in this field.

The same applies to new molecular forms. Dipolar, bent-core banana mesogens have received recent widespread attention in studies, some of which are collaborative, in the Martin Luther University in Germany, in the Tokyo Institute of Technology at the University of Colorado at Boulder and elsewhere [86]; so also have discotic forms, elastic layers, self-assemblies and, at last, studies of Brownian movement in simulations of molecular dynamics in amphiphiles [87] and fields with bipolar aggregates. Such developments aroused expert interest and criticism in the plenary and invited lectures, and in poster demonstrations and exhibits at the LC Conference in Edinburgh. In a plenary overview, Dr Hideo Takezoe of the Tokyo Institute of Technology [88] said that new structures should be assessed in terms of chiral recognition of end chains, dipole–dipole interaction and shape. New developments in the lyotropic field included applications of quantum mechanics to interfaces, DNA detection, and the production and use of polyester prostheses for vascular grafts. This work, based largely in Canada and Scotland, is the outcome of the earlier research reported above [17] on synthetic polypeptides which was one of the first demonstrations of the strength and mobility of twisted nematic and helical structures.

The present review highlights some of these developments in the overdue expansion of LC lyotropic systems into biology and medicine, but stops short of the enormous volume of work on nucleotides in the past fifty years on biological information, protein synthesis, genetic conservation and reproduction [4, 5, 7, 23–26, 89, 90]. There is hope that developments in these fields will be the subject of further reviews from the Russian Academy of Sciences. RNA, DNA and their analogues have become a new science in themselves because they seem to combine a molecular basis for conservation with a potential for innovation and change, as in LC systems. Until the 1950s, the status of the LC in living processes was hypothetical, but experimental work since then has shown that it has an integral role in the process, evolution and perhaps the origins of life. The additional attention which this requires will be offered as part II of the present review.

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