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Liquid crystals in biology II. Origins and processes of life

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Invited Article

Liquid crystals in biology II. Origins and processes of life

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Part II of this review elaborates a hypothesis presented in Part I (2003 *Liq. Cryst*. **30**, 541) and earlier publications. This hypothesis proposes that specified naturally occurring substances in the aqueous lyotropic mesophase of the liquid crystalline state of matter possess the ability to replicate, polymerize spontaneously and engage in further transitions to form ordered two- and three-dimensional layered, cubic, helical and spherulitic structures resembling those described geochemically, and observed experimentally in elementary forms of living substance. This ability uses energy originating in the kinetics of particles showing Brownian movements, spin and tensions at liquid–liquid and solid–liquid interfaces. Energy is derived also from chemiosmosis, phosphorylation, negative entropy and bonding by covalent and electro-weak forces to build macromolecules in ordered sequences of lipids, peptides and nucleic acids. These can polymerize to form glyco- and lipo-proteins, polynucleotides, anabolic and catalytic enzymes, plausibly and causally associated with the morphogenesis, metabolism and replication of protista, plankton and other primitive forms of life observed in prebiotic pools and surfaces of the cooling litho-hydrosphere of planet earth.

Interactions in these ecological niches would undoubtedly promote biochemical evolution compatible with self-organization of diversified living processes. These phenomena reveal plausible, natural mechanisms for formation of bilayer membranes with ionic channels, and other ordered structures providing spaces in which oxidative reactions and syntheses may proceed. All of this can be linked causally to early steps in animation of matter in accordance with laws governing particle physics and chemistry, templates and the general logic of molecular memories expressed in polynucleotides and proteins. Phenomena indicative of transfer of information, cellular organization, metabolism and transmission of neural signals are identifiable additionally as mechanisms for diversification and evolution. There are, however, credibility gaps in trying to extend this reasoning to sexual reproduction, speciation, competitive survival and ontogeny in the higher plants and metazoa. Various probabilities are discussed in statistical and physico-chemical terms. It is suggested that these could justify the hypothesis in so far as natural processes are sufficient to engender conditions for emergence of primitive life and Darwinian evolution in eco-niches of terrestrial space and measurable time. But there are also, in the unique utilization of energy, exploitation of advantages and adaptive capabilities of organisms at all levels to changing environmental stresses, many indications of teleonomic forces operating in ways for which there is no explanation in accordance with the laws of physical or chemical processes. This might be because understanding of particle physics and wave mechanics is insufficiently deployed in molecular biology, or because the laws of physics are scientifically incomplete in this respect.

1. Introduction

Speculations about the nature of matter and origins of life have been constant features of all inquisitive and philosophically open societies, certainly since Hellenic times and probably much earlier, for instance in the use of lodestones as an aid to marine if not to intellectual navigation by the Chinese in 2000 BC. However, except in the Ptolemaic dynasties (364–36 BC), most of this speculation occurred in a prolonged time warp so lacking in verifiable observations and experiments that it was unproductive scientifically until, in 1543, the Ptolemaic system was superceded by the six books of

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Copernican discoveries and the birth of natural philosophy. The Renaissance then added technical discoveries like the compass, lenses, printing, spectacles, telescopes and mathematical tools. These discoveries, facilitating clearer perceptions and communication in astrophysics and mathematics, created a new cerebral faculty of exact and objective science which has, since then, been trying to oust preconception, myth and superstition as preoccupying motivations in mainstreams of human mentality and activity. The contradictory streams and themes of original thinking flowing since the 17th century from the observations of Kepler, Galileo and Newton found expression and supportive instrumentation in and through the Enlightenment, before being canalized by the mechanical and chemical industries of the eighteenth century. Intellectually, these streams diversified to become channels in the nineteenth century for acceptance of evolution and, in the twentieth, of radioactivity, relativity and other outcomes of electromagnetic and biological discoveries.

Included, and in some respects foremost, in the second half of the twentieth century are immense advances in knowledge about the liquid crystal (LC) state, comprising intermediate anisotropic phases of ordered matter produced by thermo-electro transitions of many organic compounds on passing from the amorphous solid or crystalline substances to isotropic liquids [1]. In these forms, the LC has been defined precisely in thermotropic, phototropic and electromagnetic transitions which have transformed technologies in information science with applications in meters and displays (LCDs), in numerous industries producing plastics and polymers [2, 3], and via television screens in domestic life and everyday work.

The LC also occurs naturally at ambient temperatures as aqueous lyotropic mesophases capable of self-regulation, replication, polymerization and other activities with special relevance to biological processes, as described in Part I of this review. Part II deals now with these processes in more detail and with regard to a possible role for the lyotropic mesophase in the origins of life [4].

2. Experimental studies

Anyone who begins the day by washing his face uses a simple lyotropic mesophase by emulsifying soap in water to remove unwanted debris in fatty secretions of the skin. Chemically, this is possible because soap in all its forms is an amphiphile with its molecules packed in layers with one molecular end soluble in water and the other in fat. On this simple basis, complex organic molecules aggregate in ordered form at interfaces to build two- and three-dimensional mobile structures, held together by electro-weak forces, at transitions from solid crystals or amorphous solids through anisotropic, ordered liquid crystals to disordered isotropic liquids. Substances with this capability are essential biologically as precursors and components of phospholipids, lipoproteins and nucleic acids in colloidal dispersions and particles, membranes, vesicles, contractile fibres, neural networks, synapses and ganglia that have evolved from primitive organisms through billenia into Metazoa and all the way to mankind. To fulfil this role, earlier concepts of the lyotropic mesophase must now expand to include phenomena observed in supermolecular, oligomeric, polymeric and other ordered states produced by amphiphilic and complex phase transitions in systems that are self-organizing and so relevant functionally to the processes of life that they would seem likely to be involved in the origins.

Although the possible relevance of the LC to some aspects of biology was recognized earlier, it was not until the 1950s that it received systematic scientific attention. Phase contrast, interference and electron microscopy, crystallography, spectroscopy and other sophisticated techniques began then to be used to detect and quantify transitions in phase and function of designated biological substances at atomic and molecular as well as at micro- and macroscopic levels. These advances laid foundations for a highly specialized domain of knowledge and craft which added physical and mathematical detail to the emergent discipline of molecular biology. In 1953, this merging of disciplines led to the discovery [5], by groups working internationally and independently as described below, of the ordered pairing of chemical bases and nucleotides as a structural mechanism in living cells of plants and animals for the processing and preservation of chemical information for genetic identity and inheritance. In this adventurous new world of cellular and subcellular events, the lyotropic LC found a place in various other ways at molecular and macromolecular levels but its integral role in the ribosomal biosynthesis of protein remains as a surprisingly unexplored aspect of the extensive domain of RNA and DNA which now dominates genetics, biochemistry and all of biology, and sponsors molecular candidates for the origin of life [4, 6, 7]. Yet, relevant as they are and mandatory as they might be, these phenomena do not reveal any physico-chemical process, chain of events, switch or metaphysical secrets which by themselves could have transformed mineral and minimally organic matter in terrestrial time and space into living substance and creatures. So, as in astrophysics or perhaps even more so, there is need for a new concept. In contemplating this nowadays, we need to be sure that, in the pace and volume of new information, nothing relevant has been trashed or swept under the carpet, and, equally, that

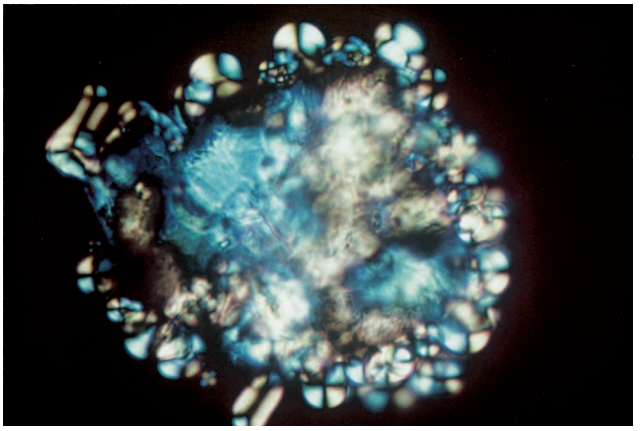

Figure 1. Aqueous suspension of amorphous cholesterol entering a spherulitic mesophase at a peripheral interface with an aliphatic polyoxyethylene ester: ×400, crossed polars.

the scientific search does not disregard domestic realities like, for example, the fact that the simplest lyotropic mesophase is soap and water. In the cosmos and perhaps in organic Nature, almost everything except light is curved, as in space and time, everything on earth and beyond is enveloped in a plasma containing and transmitting energy which we assume to be electromagnetic without knowing how it operates naturally in innumerable transitions of living matter (figure 1).

The foregoing synopsis relates to the LC in the process and maintenance of life as we know it. In atomic, molecular and macromolecular composition there are identities and overlaps between non-living and living matter. The difference and perhaps the greatest of all unsolved scientific mysteries is just how familiar organic compounds, kinetics and transitions in inanimate matter became eligible for promotion to protoplasm in and beyond the Protista, with all the attendant epiphenomena that evolved into organelles, protoplasts, plankton, organisms, plants, animals, insects and innumerable interesting intermediates. This happened only after matter had evolved with no hint of life through the passage and vicissitudes of atmospheric, solar and geophysical events during billions of years. But, at uncertain points in time, components of matter then began to live and evolve towards processes which we now describe as cellularity, replication, metabolism, reproduction, mutation, evolution, inheritance, neuro-muscular competence, consciousness, intellect, creative thought, altruism and acceptance, mainly passive, of the miracle and value of Life itself. At each of these and of innumerable intermediate developments, there are scientific clues as to *what* might have happened *how*, *where* and *when*, not only to Kipling's six honest men, backward through space, time and unexplored strata in which geophysicists tread to find earlier footprints. *Why* remains as hallowed ground in which angels searching for creative evolution [8] do not fear to tread, encouraged perhaps by subdued support from a few of the scientific elite like Maxwell, Kelvin, Einstein and Medawar.

3. The prebiotic world

Given the utter unavailability of observer evidence, it is entirely conjectural but not unreasonable to assume that the gaseous planet earth became a hard inorganic place when it cooled to form a lithosphere of rocks and an oceanic hydrosphere. On this assumption, the main and perhaps the only soft spots in that hard world would be sites where surfaces of rocks were eroded by water and mixed with particulate or powdery deposits to form gravel, sands and clays that could be washed off as particulate suspensions and colloids. The record of this does not have to be written or depicted because it is still obvious and familiar in the natural world in or near which most of us live. Geologists have identified, and elucidated in credible temporal and physico-chemical detail, soft spots conducive to life under preconditions defined expectantly in terms of materials (table 1) and interactions obeying the general laws of physics and chemistry, and the

Table 1. Involving liquid crystals sequences to the animation of matter.

ELEMENTS	Probably all with atomic masses <127 and numbers >56.
INORGANIC	Hydrogen, water, methane, ammonia. Hydroxides of silicon, aluminium; Na^+, K^+, Cl^-.
ORGANIC	Formamide, phosphatides, amino acids, fatty acids, sugars.
PROCESSES	Solution, dispersion, particulation, pigmentation, interfacial.
ENERGY	Electromagnetic strong/weak, Brownian, bipolar, chemiosmotic, phototonic.
TRANSITIONS	Nematic, smectic, tubular, cholesteric, complex.
MORPHOGENESIS	Self-organization, symmetries, tubes, chirality, molecular fit, dendrites, fibrils, lattices, networks, curved cubes, coils, helices, dimers, macromolecules, polymers.
REACTIONS	Amphiphilic, phototropic, anaerobic, ana-/cata-bolic.
PROPERTIES	Flow, viscosity, birefringence, metastability.
ORGANELLES	Liposomes, ribosomes, nucleosomes, mitochondria.
EVOLVING INTO	A Plastids, protista, protoplasts, pico-/nano-plankton; B Protophytes, algae, mycoplasma, bacteria, protozoa.

strictures of conservation of energy, parity and time. In undefined periods, these materials and interactions produced a self-sufficient biosphere in which a hierarchy of eligible substances have collaborated or competed in a manner compatible with Darwinian evolution all the way from protoplasts, protista and plankton to metazoa, mammals and man.

Since evolution of matter is gradual, diverse and has teleonomic features which make sense, it is possible that occurrences of life were inevitable because chemical evolution [9] produced irreversible changes in molecular composition, phase transitions and interactions affecting terrestrial matter, without assistance from any cosmic or non-material influence. But, in Nature, all phenomena including biological processes depend also on the laws of physics which include statistics of probability and quantum mechanics in an electronic free market subject, like all other markets, to Heisenberg's Principle of Uncertainty. This stipulates that, if a physicist, anyone else or an electronic eye knows the velocity of a particle, they cannot ever know its exact position at that instant, and *vice versa*. The counterpart in biology is that there is no proof that a seed is alive until it metabolizes or germinates. These laws also require, in any or all of the four (or more)-dimensional frames of space, time, energy and entropy—edging often on chaos [10]—that order and bonding are conserved or developed in molecular layers, fibres, networks, lattices, helices and more complex aggregates during changes in phase, temperature, kinetic and electromagnetic forces, external pressure, stretching, twisting and folding. This is where the aperiodic lyotropic mesophase of the LC state, for decades in the wings, now comes on stage as a colourful star performer with unique, acrobatic talents and more than one role [11], not excluding that of juggler in the laboratory, imp in the theory and clown in the risky circus of developmental biology.

4. Conditions required for animation of matter

Not only at primitive levels, much of living matter is a soup or sludge. Even so, it is by far the most highly ordered and self-sufficient of all earthly and perhaps cosmic substance in so far as there is no explanation of how the sequence of events postulated above could start, continue to exist and diversify in natural materials, mainly if not entirely in accordance with classical laws of physics. These laws are now changing because it has to be acknowledged that the stuff in Nature's larder used as food for life needs to have unprecedented plasticity combined with order and charge so that it can conserve energy, replicate and polymerize spontaneously. Such changes obviously favour the transformation of inert organic to animate matter, in which atoms and molecules are held together in highly ordered particulate and fluid structures by covalent bonds and electro-weak forces capable also of using and conserving water, electrolytes, energy, molecular orientation and chirality, as described below. Whereas non-living matter can acquire or lose energy and structure, and continue to exist in ordered crystals or in amorphous, disordered states as solid, liquid or gas, living matter must find and retain energy for internal and external kinetics, transitions and a different kind of immortality. In theory, this requires a reversion of entropy, i.e. recognition and utilization of negative entropy as a factor in transitions, as was foreseen in general terms first by Kelvin, the Curies, Friedel, Haldane, Oparin, Waddington and, more precisely, by Bernal [12], Delbruck and Schrodinger [11] and Orgel [6].

Conditions favouring such transition in the lithosphere from ordered crystalline or amorphous solid states into aperiodic crystals with dynamic internal atomic and molecular order plus plasticity would be most likely to occur in aqueous pools and shores or on surfaces in the emergent biosphere. Such sites would contain prebiotic chemical precursors (table 1) capable of replicating as dendrites, nematic networks and smectic layers. In these mesoforms, precursor materials would be able to rearrange their axes in line with changes in electro-magnetic fields [13], as in a nematic mesophase on an LC computer or TV screen. In the hard, mineral world of a remote, lifeless past, matter in this state more so than other competitive materials would be able to acquire extra molecular adaptability and reactivity from intrinsic negative, phototonic or solar energy while mandating and maintaining order.

5. The asthenosphere: clays and colloids

The eligibility of clay as a soft spot in the rocks of that hard world was suggested on theoretical grounds by Bernal [12]. It is hard to think of anything less lively than raw clay but there are those who find it exciting, notably Cairns-Smith [13] in Glasgow. By closer attention to geochemical detail in the gross and finer structure of clays in different geographic locations, he has described how aluminium silicates and other mineral compounds, amorphous or crystalline, can dissolve very slowly in rain or become otherwise hydrated to form clays in smectically ordered layers on surfaces of Precambrian rocks and sediments. Radiometric data [9] from igneous rocks and sedimentary strata all over the earth have shown similar features along with fossil imprints of micro-organisms in almost all climatic locations. Although the inorganic composition and physical chemistry vary according to the environment of the starting minerals, the general process is a

polymerization of organic monomers of silicic acid to form dimers and complex polymers in stacks, tubes or smectic layers stabilized electrically with anions and cations supplied by magnesium, aluminium or other metallic hydroxides. Cations of organic bases like pyridine and adenine can enter into these layers. In aqueous media over a long period of time, such clays form lattices or two- or three-dimensional networks with repetitions into which surfactants like ethylene glycol intercalate, causing the structure to swell and form higher order cross-polymeric lattices with reaction chambers for enzymatic and catalytic processes, and storage containers for chemical information. If, as is likely, amino and nucleic acids were formed by catalysis, and a wider range of alkaline earths and metallic salts entered these pools, they would evolve into a concentrate of imminently prebiotic matter.

Clays are not the only pabula with capability for fostering such reactions but they are ubiquitous and provide a variety of opportunities for interactions with atmospheric gases and with solutes deposited as viscous layers or dendrites on tectonic plates in the asthenosphere of the earth's crust. Cairns-Smith [14], the most senior of several potters in this humid studio, selected for detailed evolutionary study clays found archaeotypically and abundantly though by no means uniquely in Montmorrillon in France, as examples of prebiotic syntheses of flexible, self-replicating, enantiomeric macro-molecules, and as likely precursors of aqueous phases of ordered, living substance. Arrhenius [15] and his colleagues in the oceanological laboratories of the Scripps Institute in southern California have shown that the molecular ordered lamellae and lattices in these clays are LC mesophases. This makes sense, because otherwise the temporal and chemical information acquired by solidified clay would become set, as it were, in stone and not recoverable.

The beauty as well as the functional advantage of transitions in the lyotropic mesophase is that the information is encoded in mobile molecular orientation available as phenomena which can be viewed dynamically, reversed, played back for optical and electrical measurements, and stored. Goodby and his colleagues [16] have shown how this can be done experimentally with archaic, natural and synthetic substances with molecular and supramolecular topologies that they identify as 'large scale assemblies creating novel self-organizing systems' in the LC state. Unlike some other options, the clay hypothesis can dispense with teleonomic assistance, adjust to mathematical probabilities, obey quantum mechanics and even provide a perch for Maxwell's demon who arguably guards an ionic, intermolecular gateway to animation (see below). Clays would be widely present during the prolonged greenhouse effect from carbon dioxide and humidity in strata of the palaeo-equatorial belt in the early biosphere ~ 3.5 billion years ago which are still available as a natural workbench and resource for geochemical recall, evolution and further experiments.

Working with different models, P.G. de Gennes of the College de France was perhaps the first [17] to perceive two-dimensional nematic phases with polar hydrophilic groups as examples of self-organizing, metastable symmetries in amphiphilic systems. Since then, workers at the Tokyo Institute of Technology [18] have used these and more complex phase changes in cubic systems to simulate steps toward chiral discrimination for separating enantiomers and attracting reactive chelates into the spaces between smectic layers. Such models would provide common ground for studying phenomena like transport and exchanges of ions, reactive metals, catalysts, simple enzymatic activity, formation and sifting of enantiomers in clays and other situations where self-organization of reactive amphiphilic systems would be a necessary prelude to animation of prebiotic matter. The Nineteenth International Liquid Crystal Conference in Edinburgh in July 2002 was, literally, a watershed for merging of concepts and experimental approaches with different materials in this basic problem.

6. Atmospheric and geological information

Carbon dioxide entered the atmosphere about 4 billion years ago. Geologically, some of the rocks of the lithosphere had acquired sediments of carbon in the form of graphite, diamonds and bitumen [6, 9] produced presumably by extreme temperatures and pressures. Dating with ^{13}C suggests that abiotic synthesis of carbonaceous and eventually other organic compounds began then with the fixation of atmospheric nitrogen as well as carbon. Fossil dating shows that sedimentary carbonates stacked up as columns containing microfossils of filamentous bacteria which would have been at that stage anaerobic, and would have included the volcanic archaebacteria and sulphur-dependent organisms whose descendants are identifiable now as the probable starters of anoxic photosynthesis. It is generally agreed that these organisms were the ancestors also of present-day pigmented species which might have spread in the first place as syncytial slimes growing on softening rocks and clays, using carbon as a main or sole source of energy. Such organisms would grow and replicate without membranous boundaries, and use solar energy to develop a chain of oxyphilic photosyntheses yielding substrates capable of inducing further organic syntheses and catalysis, in programmed chemical sequences, to regulate the evolution of protista, pico- and nano-plankton, higher bacteria,

phages and diatomaceous layers which can still be retrieved in bulk, and probably ancestral form in bottom scrapings from deep Norwegian fjords [19].

These processes, in parallel with continuing abiotic reactions in pools of salty organic soups, sedimentary aqueous layers and clays, would inevitably create a wealth of new matter for macromolecular aggregates, polymerization, synthesis, differentiation and replication of carbohydrates, lipids and peptides as ordered biogenic substances. They define plausible chemical routes to animation in situations where evidence of living organisms is detectible geologically as multicentric phenomena in the Archaean and Proterozoic eras of the Precambrian aeon. Contemporary studies of descendants of these organisms show that they must have had enough resource and adaptability to produce energy and enzymes for self-sufficient survival, metabolism and reproduction [20].

7. Mathematical conditions for origination of life

At the other extreme, perhaps to avoid the endless task of revising equations derived theoretically from the concept of the Big Bang, there are scientists who speculate or simply accept that life might just have been an accident, waiting but not expecting or wanting to happen. Salty organic soups, clays and volcanoes are not prominent on their agendae but matter in these locations would seem to include all the plastic substances with such a potential, having been around for so long in structures compatible physically—and naturally—with life in the kindly, temperate biosphere into which planet earth has meanwhile evolved.

For all this to happen to soups, clays or other prebiotic media, it is reasonable to postulate [21] that two numerical factors are required: a probability that a given molecular configuration compatible with conditions ecologically suitable for life exists; and a number of occasions available for this probability to effect the necessary transformation of abiotic precursors from $3\sim4$ billion years ago onward. These occasions undoubtedly existed but are by definition so variable and complex in time and space that there is justification for some reductionism by simplification of concept and variables. For those intimidated or sceptical about such an approach, it is helpful to turn to the well tempered discourses [11] of 1944 by Erwin Schrödinger no less, a mathematical physicist if ever there was one, who starts—how else?—with keynotes of the atomic score required to establish ordered harmony in particulate matter making Brownian noise. Einstein had already paved the way mathematically for such an approach in 1905 when he was busy also writing his first two papers on relativity. His work was extended in 1925 by J.-B. Perrin, and translated into quantum mechanics by Messiah [22] in 1969 and others [23]. Schrödinger's approach could hardly have been simpler: the random diffusion of molecules of potassium permanganate going into solution was reduced to a partial differential equation which accounted for the movements of the molecules in all directions and their irregular dance around equilibria governed by the gradients of heat and concentration. In such a system, including colloid dispersions, limits of error and minimal size are defined by $1/\sqrt{\mathbf{n}}$, where $\mathbf{n}$ is the number of molecules required to form macromolecules capable of reacting internally with others surely a unique example of simplification of the mathematics of physical science (table 2).

Contemporary approaches to quantification of such movements are far from simple, especially those of Kaufmann [10] on random Boolean dispersions, and of others [20–24] dealing with the building of macromolecules. According to Quastler [21], if H is the information content of any macromolecule, then 2^{-H} is the probability of this event in terms of the information content of a transformed macromolecule. Even if the chance of this happening randomly at any one location is low, say 10^{-4}, the odds against are shortened by the length of time available and the ubiquity of eligible substances, so that a probability shorter than infinity can actually be contemplated. Let us imagine that a self-replicating macromolecule with a molecular mass of 100 000 contains atoms, vibrating at ambient temperature, say in one of the clays described above. If this clay were to acquire a boost of energy from an external fluke or internally from birefringence or quantum jumps by electrons, it would not have a problem in storing all of this as surplus information in the memory of the macromolecule by entering an aperiodic crystalline phase with negative entropy.

Macromolecules constructed and conserved in this way would be better able to maintain interatomic spaces, orientation, identity and reactivity under adverse conditions than looser aggregates, and would be likely starters for further structural changes throughout the layers of clay or whatever else, for instance by forming a lattice or three-dimensional, stable network of atoms in a lyotropic mesophase with altered vibrations. This could serve as a matrix or template for copying and replicating new forms of matter. The knock-on effect could conceivably be a new substance, protoplasm, with ability to defy the second law of thermodynamics by finding energy to create a higher level of order by providing a dendritic substrate for living matter. This could become the algal syncytium found everywhere on top of layers of clay, well placed for absorbing energy from sunlight, water and ions from rain and adjacent wetness to increase kinesis, embody carbonaceous material for general

Table 2. Mathematical functions relevant to the origins of life.

CATEGORY	MAIN FEATURES	REFERENCES[a]
ARITHMETIC	Number theory, Godel numbers, computation, algorithms, indeterminism, axioms, propositions	Cartwright 1983, Atkins 2003.
BIOLOGY of GROWTH	Specified rates, accelerations and phases of growth; eligible parameters, hidden variables. Hamiltonians transformations of form, energy and entropy	Thompson 1966, Cartwright 1983, Medawar 1985.
CHAOS THEORY	Paradox of random events in deterministic situations; turbulence, friction, vibration; interactions of complex variables	Holden 1986, Ruelle 1979.
CYBERNETIC	Monitoring of expectation/outcome, feedback loops, communications, control systems, automation, signatures	Weinberg 1993, Quastler 1969.
EVOLUTION	Coding sequences of amino acids, polymerization, random drift, molecular clocks, divergence of animals from plants, morphogenesis, time scales for change	Pauling 1948, Kimura 1983, Monod 1970.
FLOW REACTOR SYSTEMS	Autocatalysis, parity, isomeric preference	Frank 1953.
FRACTALS	Spatial geometry of biological matter, attractor processes; structured irregularities in natural substances, measurement and classification of surfaces, signatures	Mandelbrot 1982, Holden 1986, Quastler 1969.
GAMES	Enigmas, problem solving and better/worse outcomes and pay-offs, cooperation, opposition, wagers	Maynard-Smith 1982, Stewart (I) 1997.
GENETICS	Molecular coding and transcription, gene frequencies, reproductive invariance; regulation of biochemical synthesis, repressors, mutations	Crick 1968, Beadle & Tatum, 1941, Monod 1970.
HIGGS MECHANISM	Vector bosons, electro-weak forces, gauge symmetries	Sundaresan 2001.
NUMBER THEORY	Arithmetic, Godel numbers, computation, Hamiltonians, algorithms, indeterminism	Cartwright 1983, Atkins 2003.
STOCHASTIC	Random transformations of cells, binomial and Poisson distributions, causal associations, eco-systems	Rubin 1954, May 1973.
TRANSITIONS	Boolean distributions and networks, parameters and orders of changes in state, transport of ions through membranes	Kaufmann 1993, Chapman 1974.
WAVE FUNCTIONS	Fundamental equations, states of systems, uncertainty principles, quantum mechanics.	Schrödinger 1967, Heisenberg 1962, Planck 1937.

[a]See also: Whitehead 1933; Frank 1953, Sutton 1960, Heisenberg 1962, Messiah 1961, Waddington 1966, 1977; Medawar 1985 and general references.

fertility, formaldehyde (HCHO) as a specific primer for anoxic polymerization of other carbohydrates, and L-amino acids for synthesis of polypeptides, anabolic and catalytic enzymes [9, 10, 16, 20].

Pathways from synthesis to catalysis are indirect and uncertain. Kaufmann suggests that, if the probability of a specific catalysis is 10^{-6}, with ten preceding steps, six million potential catalysts must be tried to ensure success. The chance of finding an RNA sequence for protein synthesis in this would then be 10^{-10} which would be an inefficient pathway unless it was accompanied by numerous simultaneous reactions. But, according to Monod [24], all these reactions are indirect, because all known allosteric proteins are oligomeric and are assembled by non-covalent, non-linear, chemically identical subunits which maintain 'a peak of efficiency and coherence' in cellular metabolism and also in regulation of synthesis of allosteric enzymes. Monod sees this 'gratuitous' dual function—i.e. as specific catalyst and transducer of chemical signals—as a complete, working explanation of how the simplest cell can 'transcend physical laws even while obeying them' in the fulfilment of its purpose. But is not the only visible purpose common to all forms of life *immortality* through progeny? The bill for this would be unending, incalculable and well outside the remit of this review.

For the shorter term, it is suggested that the logic of cybernetics and the dynamics of catalysis can be used to postulate that complex macromolecules held together by electro-weak forces, as in an LC, would induce enzymatic response, liberating constituent molecules to reorientate themselves into firmer structures, like peptides in chains or globular bundles. It is not difficult to view such structures as possible precursors of substances like myosin for contractility, lipoproteins for membranes and mucopeptides, or lipopolysaccharides for cell walls, all of which are well recognised (see Part I) as prototypes of living substance in the form of fibrils, tubules, micelles and various organelles in the protista and their successors. If living cells and tissues are examined and dissected, or fall apart, these basic ingredients are readily identified and can in fact be used in plastic surgery to recreate or initiate creation of new tissue. In this respect, the bio-logic of catalysis includes Monod's gratuitous re-synthesis, and *vice versa*, while the bio-mathematics define conditions for regulation of both, for instance, in the exponential rise of enzyme activity which occurs when substrate is added and of asymptotic termination as it decreases.

Since molecular interactions and phase change occur in all of the above systems, it is appropriate also to use differential equations to assess quantities and factors conducive to differentiation and integration. Models for this (table 2) are now amply available in the immense methodology developed for LC systems during the last fifty years, but await applications in the lyotropic mesophase. Kauffmann's work [10], too voluminous to describe here, provides impressive examples, most of which endorse his belief that they will lead to the creation of living matter sooner rather than later. He is not alone in thinking, or fearing, that this will happen.

In the hydrosphere, there would certainly be eco-niches in which dispersed particles would vibrate, aggregate and polymerize. In these natural niches, simple forms of life could develop over a long period of time by direct transformation of acellular protoplasts into organelles and unicellular organisms. If a living cell is observed in the contrived niche of a polarizing, phase-contrast microscope, the contents are seen to be variously particulate and in a state of ceaseless vibration to which the dynamics of Brownian movements must apply. Catalase, oxidase and other metabolic activities can be switched on and off by inhibitors. To understand how this comes about, it has to be assumed that the information required to produce and maintain animation is coded in the internal arrangement of the limiting membrane, vesicles and organelles which become subcellular components (figure 1). On this basis, Quastler and his colleagues [25] estimated that the number of bits required to select one atom and specify its position in living matter within the limits of precision given by thermal vibrations at 37°C is of the order of 25 per atom. In the following 11 years, Quastler published many studies relating information theory to biological events, including the quantitative aspects of converting simple precursors into the molecular components of common bacteria. Using Linschitz's [26] estimate of converting the associated entropy change into probability, and probability into an equivalent number of binary choices, he accepted as baseline an information content of 10^{13} bits. This is unaffected by structural differences because the eukaryotic bacterium used universally as a prototype, *E. coli*, retains full biological activity after cooling nearly to absolute zero, which destroys dynamic coding but not molecular structure.

Independently, but with allowance for this, Morowitz [27] calculated a remarkably close but generally agreed lower information content of 10^{12} bits. All of these esimates tend on the high side, according to Quastler because—and recent deciphering of innumerable genomes confirms this as a general rule in statistical genetics—much of the molecular information in the cellular structure of living creatures is redundant nonsense and can be reduced, in *E. coli* for instance, to 2^{100} or fewer bits without affecting its function. At

the lowest limit of 10^3 binary choices to exclude recycling nonsense, the probability of making such a choice accidentally would be 10^{-300}. For 10^{13} bits, the probability would drop to one in billions. It could be argued that the time available accommodates these odds, but time alone is not enough even in the metaphysics of spontaneous creation. There must also be an eco-niche for self-organization and provision for maintenance of life in terms of particle physics, quantum mechanics and chemistry. Hoyle and Wickramsinghe [28], who do not deny cosmic factors, were joined by Shapiro in regarding these odds as preposterous. Kaufmann [10], otherwise a strong proponent of terrestrial explanations, surprisingly, does not. But the odds against spontaneous generation of life are those of a no-win lottery, and we are assured by a competing eminence that God does not play dice.

If we forget the lottery (and God, according to Niels Bohr), return to earth and recall the authenticated propensities of clay minerals to form a lattice or smectic plate of statistically and morphologically ordered structures, alternative, less improbable possibilities arise. Salty water and soups adjacent to these surfaces would contain or attract additional carbonaceous and nitrogenous substances like carbohydrates and amino acids which could polymerize, replicate and catalyse further reactions [9, 10]. Replacing the lottery with imagination, Cairns-Smith [14] performed a simulation (a thought experiment) in which he used a population of identical billiard balls numbered 1–100, mixed and then replicated them randomly, discarded 100, replicated the remainder, and continued successive halvings and discards randomly through many generations until all the balls bore the same number, of which at any given time only one would be the common ancestor of all of the balls at some later date, surviving by chance without selective elimination. If there is a selective advantage (s) for the higher numbers, the threshold at which it becomes significant is ($s > 1/2N_e$). In an analogue of this experiment in a population with N self-replicating, haploid molecules (A and A′) in which A′ has this selective advantage over A with relative survival fitness $(1+s)$, Kimura [29], an expert in population genetics, used differential equations to calculate average waiting times for elimination of genes for unfitness. In the simplest case, with no externalities, almost all of the unfit genes would be eliminated from the population in about 100 generations. It is difficult to relate such complex mathematics to phenomena but Cairns-Smith [14] uses these and his own simulations to justify a hypothesis that life in this eco-setting might evolve eventually from a common ancestor with a survival advantage in an otherwise uniform population which is dismissed by chance alone from further opportunity to enlist in the competition. There are several alternative mathematical models (table 2) but none with credible causal connections with prebiotic phenomena except in so far as the end products are presumed to be macromolecules: polypeptides, proteins, glycolipids, dynamic particles and precellular genes as common ancestors of living creatures, as envisaged by J.B.S. Haldane in the 1930s as being likely keys for opening Emil Fischer's doorways to living matter.

In our day, it is necessary to envisage electronic keys in the form of electro-weak (i.e. non-magnetic, non-covalent but cohesive) forces required for assembling and holding natural macromolecules and ensembles together in metastable mesophases. It can be assumed that the end products of syntheses and transitions would be aperiodic chains or aggregates of precursors in linear, helical or globular structures which would link or pair with others to form double-stranded, longer and much larger macroscopic states, with negative entropy and lessening of the stability and symmetry conferred by repetitive covalent bonds. In these macromolecules containing hundreds of subunits with lengths or diameters of 10 000 μm and molecular masses of 50 000 or greater, with every atom or groups of atoms playing an individual role [4, 11], something akin to the Higgs mechanism would seem to be required as a hypothesis to explain the working of the cohesive, electro-weak force. This mechanism caters for events in two complex fields $\mathbf{\Phi}(x^\mu) = \mathbf{\Phi}1 + \mathrm{i}\mathbf{\Phi}$ and $\mathbf{\Psi}(x^\mu) = \mathbf{\Psi}_1 + \mathrm{i}\mathbf{\Psi}_2$ which form doublets symbolized as $(\mathbf{\Phi}, \mathbf{\Psi})$ that can rotate into each other in low energy transitions to form helical and complex structures bound by electro-weak forces without losing chemical identity, parity or symmetry, or infringing the quantum mechanics of Group or Gauge Theories of interactions and transformations. In systems like this with two-way flow of substrates and products, the kinetic equations of F.C. Frank for synthesis of asymmetric molecules in open flow reactor systems are also relevant. He showed in studies at Bristol University from 1953 onward that, although parity of enantiomers is stabilized by electro-weak forces, the isomeric D-sugars and L-amino acids essential for synthesis of nucleic acids, glycolipids and peptides are preferentially switched to give either all D- or all L- by small perturbations. This mechanism operating in open flow pools of prebiotic organic matter in a lyotropic mesophase would explain the formation and contribute causally to the animation of aperiodic peptide chains in primitive organisms and form templates for myosin fibres as units of contractile muscle protein. Electro-weak forces are required also for stabilization of polarized lipoid–protein bilayers in membranes, complex three-dimensional structures

within and between cells, polynucleotides as units of information, morphologenesis, cohesion and activity in ribosomes, lysozymes, mitochondria and other vesicles and organelles observed as phenomena of matter in the lyotropic mesophase. The kinetics are compatible with the metastable conditions required for pre- and intracellular transformations at rates exceeding hundreds per second, yielding metabolites with life-spans of milliseconds to thousands of years.

Self-organization by weak intermolecular forces by these mechanisms is probably enough to explain the cohesion of two single-stranded molecules of RNA in the double-stranded helix of DNA, and the acquisition of a membrane from available lipids (see below) to encapsulate nuclear chromatin in a cell. Meiosis and mitosis, giving haploid descendants, would then replicate molecular information for a clone that would survive, or not, according to conditions specified in mathematical models (table 2) encompassing deterministic, stochastic and other routes to animation. The prebiotic locations and structural matrices are still universally available. The relevant chemicals are the aggregates or chains capable of using peptide (–CONH–) linkages to form oligomers which would serve as primers and then templates for polymerization to polypeptides and protein in the protista. Some of these would then become substrates for catalysis to nitrogenous bases that are known to react with each other to form urea and *N*-heterocyclic compounds [9] to form purines and pyrimidines, incorporate metallic elements known to be essential for life, and establish covalent bonds with pentose or hexose sugars [24]. Such a process would open the negative entropy (or deficit financing) option for biosynthesis of aperiodic crystals of polynucleotides and DNA to store information for the ribosomal conveyor-belt that sequences amino acids for anabolic synthesis of proteins, *a priori* or form catalytic products as above, or for disposal with other nitrogenous or carbohydrate residues, and restoration of positive entropy to the system.

8. Protoplasts and micro-organisms

For animation, the systems described above would need to organize themselves into cellular formats preceded by protoplasmic syncytia in which the participating components would meet each other haphazardly. Ready-to-wear syncytia would already exist as smectic and dendrimeric colonies of bacterial protoplasts (*Terrientes*) in various tectonic situations including wet rocks and clays. There would also be *Mycoplasma* [30] including air- or water-borne *Pneumophila* without cell walls, living slimes of *Aerobacter* species and other soil organisms, species of *Pseudomonas* which use carbon as a sole element for growth, aberrant *Coccacea* which can still be produced by ionic and other biochemical interventions, natural acellular swarms of *Proteus* and other eukaryotes which include acellular forms, now reclassified as eukaryotic *Terrientes* because they have organelles containing RNA in helical form, as in bacterial protoplasts. Horowitz [31] in Israel had already identified subunits of lipid and protein by osmotic lysis and density-gradient ultracentrifugation in these. Since then, scientists at NASA [32] have reconstituted plasma membranes containing ATPase from freeze-fractured preparations of these protoplasts, and achieved the first synthesis of a lifelike membrane. Amiento *et al.* [33] then constructed cage-like, interactive vesicles from membrane protein. Other botanists in Heidelberg have described experiments showing bidirectional flow through such membranes, and the pivotal role of golgi apparatus and vesicles in intercellular traffic [34]. The track record of these phenomena goes back to 1935 when Kleinberger-Nobel described [35] the L-forms of life now regarded as pathogenic pleuro-pneumonic-like organisms (PPLO) which grow diffusely within a plasma membrane. Recent work [36] shows that the plasma membrane is thermosensitive and becomes functional when cholesterol and acetyl co-enzyme A are included with phospholipid from exogenous sources. It can then engage in glycolysis and attain a more stable structure. Organisms with these properties were not necessarily the first forms of life but, in the writ of natural science, and in the phenomena reported here, it is hard to identify sources of protoplasm with a more appropriate phylogeny for biogenesis into primitive pre-cellular forms.

There is, of course, much more to life than protoplasm. A blob of this might survive somehow but would be an all-time non-achiever if it did not show more enterprise. It would need to acquire an enclosing membrane to conserve it and allow two-way transport of electrolytes in a permissive time-frame during which it would have to acquire dynamic subcellular units like golgi apparatus, ribosomes, liposomes and mitochondria operating seven to 11 steps of phosphate-mediated energy at a catalytic rate of 100 or more molecules per second [9, 36], and also for replication and macromolecular fitness to evolve further into plankton, slimes, primitive bacteria and whatever might have followed in the prolonged ascent from unicellular to multicellular creatures. The redox, sugar-phosphate and other fundamental precursors of metabolic processes would already exist and seem still to be mainly unchanged.

With unicellular organisms as archetypes, reactive membranes in aqueous lyotropic mesophases were likely starters in these processes, and still are key elements in meta-stable transitions in self-organizing

cells [7]. In archaic forms as in surviving descendants, this began in anaerobic conditions at high and possibly volcanic temperatures [9] in the presence of ionizing radiation. Vestiges exist in the *Archaea*, *Clostridia* and other species possessing thick membranes and lipopolysaccharde cell walls which serve to identify not only the phylogenetic steps that have led to diversity but also the intrinsic capacity to synthesize and metabolize essential substances required for survival and evolution alongside higher forms of life, not least because a knowing minority learned how to threaten them as parasites and pathogens. Few if any of our contemporary zymotic problems have such a long ancestry.

To understand the evolutionary importance and to control opportunistic pathogenicity in this anthropocentric minority of micro-organisms, it is essential to study phenomena linked causally to their genetic and phenotypic profiles. For instance, genes for the constitutive β-lactamase enzymes which, specifically and uniquely, inactivate natural penicillins, were identified in ancestral strains of bacteria that lived in the Australian outback before the first use of penicillin in the 1940s. The same occurred when derivatives unaffected by this enzyme were synthesized—for that reason—in the 1950s, but it was found in 1961 that a few strains with altered colonial and cellular morphology, and with genetic resistance to the synthetic product, had been isolated before the use of the first effective derivatives [37]. These strains (MRSA) with genomes fully sequenced recently by Hiramatsu and many others [38] in the Department of Bacteriology of Juntendo University, Japan, are now a menace, worldwide.

There are many other examples in microbiology of how micro-organisms can outpace or outwit molecular biology and pharmaceutical ingenuity in solving problems of goodness of fit, evolutionary competence, and morphological and biochemical adaptation, all of which are increasingly demonstrable in genetic and enzymatic resistance to antimicrobial agents. Nucleic acid viruses which have by definition insider information about mammalian genomes have extra ingenuity in these exercises. But any genetic exercise can be accompanied by unforeseeable and cryptic events within or outside the target species. When unnatural interventions are contemplated, there is therefore a need for attention to the long-range effects of any genetic modifications all the way from microbial to plant, animal and especially to human populations, because movements of genes between species no less than within species is an essential part of evolution, and rogue genes can drift or gate-crash into other parties independently.

When syncytia undergo transitions to unicellular organisms, animation probably depends primarily upon the development of interfaces that become cytoplasmic membranes as in the mycoplasmas in which active, selective transport, exchange of ions and replication can proceed. This is demonstrable in the interconversions of syncytia, swarms, protoplasts and cells in laboratory experiments with prokaryotic and eukaryotic bacteria, but what follows is a mystery which defies chemical or physical explanation except in so far as elementary enzymes like catalase and lipase, and ordered structures like protective cell walls, selective internal membranes, energizing particles and replicating nucleotides are identifiable in the evolutionary trail from protista to protozoa. Phages appear also at various stages as adventitious secondary particles whose existence depends entirely upon bacterial cells, just as that of viruses depends upon these and metazoan cells.

9. Viruses as primitive forms of life

Viruses have a shorter pedigree in the tree of life, always as parasitic particles entirely dependent upon preformed, preselected cells in plants and animals for survival, growth and differentiation. They were originally detected and described in the 1860s, like so much else, by Louis Pasteur as particles which could pass through bacterial filters and were capable of causing rabies in mammals and man. The pathogenic role of filterable viruses extended to smallpox, influenza, yellow fever and other major infections of man, mammals, birds, fish and insects but the methodology for identification remained presumptive until the 1920s when ultra-filtrates from patients with influenza were cultured in hens' eggs. Thereafter, particles of infectious chromophilic material were described in exudates from patients with smallpox, vaccinia, varicelliform eruptions and other putative, often minor infections. A major advance occurred in the late 1940s when tobacco mosaic and other ordered structures presumed to be viral were described in electron micrographs, especially when comparable particles were seen in the 1950s by Enders, Salk and others in material and cell cultures obtained during outbreaks with unambiguous diagnoses like poliomyelitis and measles. Much of the guess-work in scientific and diagnostic virology was eliminated by these important advances but doubts remained about the separation of artefacts derived from the mammalian cells and their debris from definitive viruses in the various co-cultures in which viruses were grown. These doubts were largely resolved in 1960 when de Harven [39] at the Rockefeller Institute concentrated classical viruses from infectious material by density-gradient ultracentrifugation in sucrose, and published electron micrographs (figure 2) visualizing viral particles freed in an ordered layer from cellular components. The viruses used were Rous sarcoma and

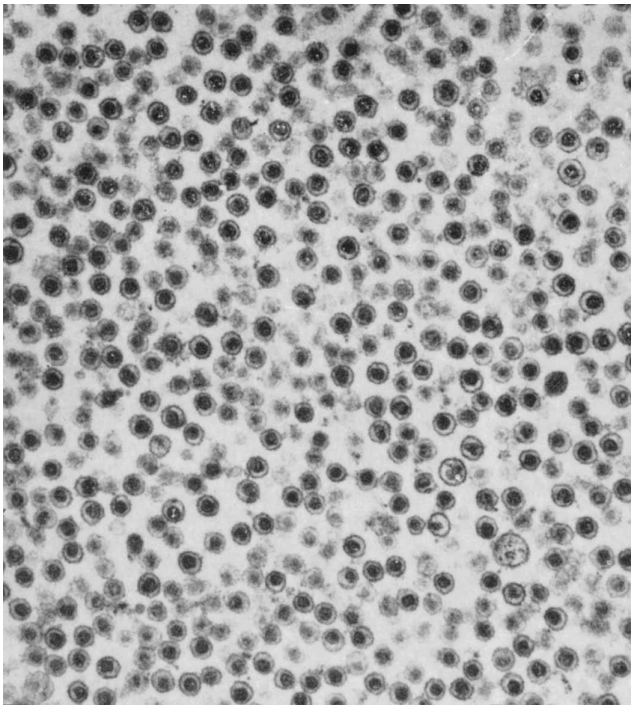

Figure 2. Electron micrograph of a pellet of Friend leukaemia viruses, isolated from the blood plasma of leukaemic mice and purified by double ultrafiltration on millipore filters and by ultracentrifugation: × 19 500. Isolation and purification by E. de Harven [39].

Friend leukaemia and, in cell cultures of these, de Harven also described (figure 3) the vesicles with lipid bilayers and buds associated with, and regarded as signs of intracellular viral activity. In so doing, he established criteria [40] for the direct identification of viruses in pathological material from patients—a caution now often ignored in the increasing dependence upon indirect PCR and other surrogate measurements of viral load for clinical and epidemiological diagnosis of active viral infection.

Viruses are probably the simplest forms of life, surviving and multiplying entirely because of their ability to invade and integrate genomically with other forms of life, and to a capacity to adapt by mutation, with changes in properties and virulence. It is therefore dangerous and highly unscientific to presume their presence by surrogate methods. In this connection, it was strange that Baltimore, Temin and Mitzutani [41] in 1970 ignored de Harven's work along with that of Varmus [42] and others when they claimed that these classic viruses could by themselves synthesize the enzyme reverse transcriptase (RT) and thereby insert their genomes into the DNA of cells of experimentally infected animals. This led to acceptance of RT as a viral enzyme and the award of a Nobel prize, though it was known at the time to be a common, non-specific product of the mammalian cells employed in co-cultures used as inocula. The same oversight, with disregard of corrections, led to revisions in nosology which, officially, reclassified the Rous, Friend and a succession of other agents present in co-cultures containing human lymphocytes as old and new retroviruses collectively as *Retroviridae*. Some of them, like the HTLV series, were discarded as passengers or nonentities but one such (LAV/HTLV3, later renamed HIV-1) was declared in 1984 [43, 44] to be probably the essential cause of an Acquired Immune Deficiency (AIDS) in all its forms in mankind from 1981 onward and archaically in simians [45].

10. Metazoa

With miracles excluded, there is no way in which matter could change spontaneously into Metazoa. In simpler forms, like saprophytic moulds and free-living coelenterates, the particles similar to those in unicellular organisms are organized into layers, columns, globes and tubes of units which differentiate into ordered aggregates capable of adopting specific structural and functional responsibilities in the growth, metabolism and reproduction of the parent cells. In the first few generations of an embryo, all of the cells are similar and might be pluripotent developmentally while the genome remains invariant. The chemical framework necessary for this was available and irreplaceable in lipoid structures (See Part I, and below) which could form interfacial bipolar solubilities with water, and be energized by the movements between layers, within compartments and in transit through intermolecular channels. When cholesteric and other steroid molecules with side chains were incorporated, structure and reactivity were further enhanced by formation of helices which are versatile in providing templates for reproductive and hormonal chemicals, scaffolding for cells and fibrils for neural tissues [4, 7].

Covalent bonding and electro-weak forces, as stated above, are sufficient to hold macromolecules extra- or intracellularly together in LC and other systems but, for organization of cells and intercellular material into tissues as in all metazoa, a firmer and more complex gasket is required for sealing and trafficking. In 1963, Robertson [46] described a single intermediate, microscopically dark interfacial layer of sealant substance formed by fusion of external surfaces of the plasma membranes of cells. Under the new general heading of Tight Junctions [47], this membrane-derived interface operates as a molecular gate and fence between cells. It is formed by oligomerization of protein–lipid components into structural plaques with intervening pores for passage of ions and secretory substances, solute–solvent

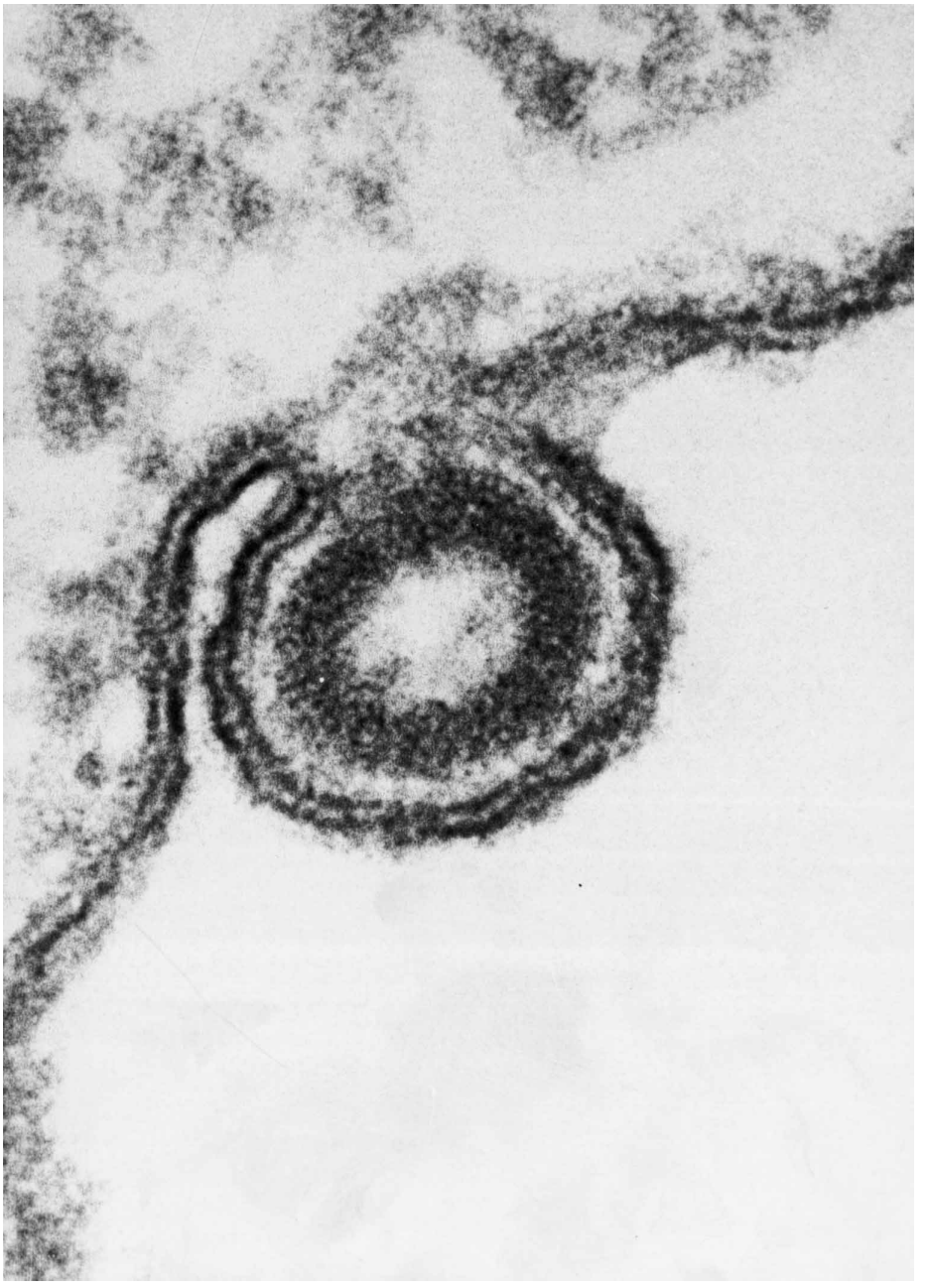

Figure 3. Friend leukaemia cell in a culture studied by electron microscopy, presenting a typical virus particle budding on the cell surface. Note the complete continuity and similarity in structure between the plasma membrane of the infected cell and the envelope of the nascent virus particle, and the lipid bilayer of the vesicle: EM × 554 000. Isolation and EM by E. de Harven [40].

coupling, chemiosmosis and electro-kinetic activity, all of which are essential preludes for the conversion of cellular aggregates into metabolically active, regulated organs. Phospholipids and proteins in the original and modified membranes then become involved in the metabolism of the cell, organ and host as well as in regulation of ionic exchanges. The key process in this is chemiosmosis but, surprisingly, there is no reference to the extensive work on this for which Mitchell [48] was awarded a Nobel prize in 1991. It had already been suggested by de Duve [49] that this process developed when primitive cells moved from primordial pools of concentrated ionic and organic media into more dilute ponds, and developed vesicles for intercellular exchanges and imbibition of particles. This would intensify with the evolution of higher eukaryotes and

multicellular organisms, which could then use membrane material for adhesions and formation of organelles and imbibition of macromolecules and particles. Recently, Connor and Schmid [50] have shown how the plasma membrane became the main regulator of endocytosis and a range of subsidiary mechanisms for cellular metabolism and homeostasis. In fabricating tight junctions between cells, this also enabled them to form epithelial layers for absorption, secretion, ciliary activity and integration into the complex organs required for evolution of the metazoa. These processes would seem to relate to the more primitive plasma membranes described by Karnakig [51] with which fish control the ingress of ions from sea water, also by chemiosmosis.

From an evolutionary perspective, these cellular developments in the natural history of the Metazoa open the text of an exciting serial, thrilled periodically with infallible memories and fresh experiences of the variety and activities of living creatures. Their embryology often repeats the morphogenesis and re-enacts or emulates behaviours inherited from previous evolutionary developments, illustrating biologically the scripture and in practicality the experience and lessons of reproduction and maturation. The voluminous phenomena of this saga are masterfully condensed in the writings of Konrad Lorenz [52]. In the context of this review, it may be said that the transition via the lyotropic mesophase from primordial matter to free-living prokaryotes and thence to eukaryotes and multicellular organisms is as good a guide as any to the mechanisms triggering animation which made self-supporting evolution inevitable and unstoppable, in Lamarckian and Lorenzian as well as in Darwinian terms.

11. Information as a function of molecular memory

In this respect, the properties of the LC are essential but not unique. Any substance that can crystallize will have a memory in so far as it can replicate its obligatory ordered state. It does this by remembering the Maxwellian electro-magnetic, thermal and kinetic changes which sponsored its morphogenesis, with conservation of its energy and palaeochemical pedigree. The silicon chip in a personal computer is a mimic of this phenomenon, and is constantly being improved by theory and technology, for instance by introducing other elements for advanced miniaturization and electronic circuitry. The LC phase is different because, although stabilized by similar hydrogen and other chemical bonds, it can alter its shape, charge and reactivity to form loosely bonded molecular partnerships with other substances which make it instantly and visibly responsive to mechanical, electronic and kinetic stimuli. Lyotropic forms are even more active because they have a more varied three-dimensional composition and an aperiodic sequence, and contain spaces within and between layers, fibrils, chains and helices in which water, electrolytes, energizing phosphates and enzymes can be stored and act on substances in transit, like Na^+ and K^+ in and out of cells, and steroid hormones in reproductive and stress-orientated glandular physiology in the animal kingdom.

The morphogenic memory of the natural lyotropic mesophase in this respect is analogous to that of polyester swimwear which adjusts exactly to the anatomy of the wearer when wet and retains the ability to do so after drying out. If the swimwear is spun from a natural fibre like cotton, it will react with the water and shrink, i.e. close the inter-fibrillary spaces, while drying unless it is protected, as in non-shrink garments, with a protective film of synthetic polyester which has a more reliable molecular memory of the original morphology. Simple, shapely and empirically familiar differences between plastic materials are now the technical basis of an enormous industry in synthetic fibres, elastomers, sheets, films, and also in surgical grafts and transplants in which artefacts are mimicking and if need be replacing naturally ordered structures as grafts for tendons, fascia, heart valves and blood vessels with colour and texture to match. None of this explains the origin of life but all of it, based on the lyotropic mesophse of the LC, is now an essential part of everyday life everywhere. Could women cope with life without tights? A technician working with the writer suggested we should transfer our work to the larger economy of feminine fashion by featuring the undoubted anatomic, colourful and emotive potential of the LC.

12. Probiosis and antibiosis

There is much to be learned about life by back-tracking cellular metabolites to their origins. This possibility exists in moulds which produce a variety of metabolites from primitive starter substances. This became apparent to the writer in the late 1940s [53] in the natural history of the β-lactam antimicrobial agents, all of which were extracted as intermediate metabolites from growing cultures of primitive moulds like *Penicillia* and *Cephalosporia* after which the original drugs and their biosynthetic descendants are named. The designation anti-biotic for these metabolites is in fact parochial and biologically incorrect. The antimicrobial powers of the intermediate β-lactams, of the cyclic polypeptides (Polymyxins) and of the small peptide bacitracin are just as likely to be natural if fortuitous *pro*-biotic defenders against competitive bacteria in primitive struggles for existence in nutrient and waste products, for instance in the action of the

cyclic polypeptides on growth of primitive species like *Pseudomonas*. The other intermediates are small peptides. Penicillin is a highly reactive, unusual dipeptide formed by a twisted fusion of the amino acids L-valine and L-cysteine into a thiazolidine and β-lactam ring dimer, 6-aminopenicillanic acid which acquires useful anti-bacterial activity only by addition of benzyl or other side chains [37, 54]. The dipeptide can thereby replace muramic mucopeptide and disorganize the cell wall of many species of eukaryotic bacteria: a good example of accidental goodness of molecular fit—a happy accident remembered by Alexander Fleming in 1929 [55]—and of a natural process which provided an infallible template for subsequent molecular modifications and advances in therapeutics then and in the 1960s [37] when more new derivatives were synthesized by a team of scientists at the Beecham Research Laboratories in England.

In experimental work on these deivatives, it was observed also that, in aqueous solution, they began to polymerize spontaneously with cleavage of sulphydryl linkages to form plastic oligomers and then harder yellow macromolecules with masses up to 50 000, composed of 230 or more molecules of the parental dimer, benzyl penicillin and its degradation products, with paracrystalline properties. In clinical usage, the oligomers conjugated with protein in the blood of patients and experimental animals to cause highly specific immunological and allergic reactions [54]. This polymerization was accelerated when the chain reaction was primed by lysine, dilute formaldehyde or half-saturation with ammonium sulphate to produce a plastic keratin-binding polymer which was also immunogenic and allergenic experimentally. Similar polymerization in commercial fermentations was accompanied by a linkage with mould protein to form different, highly allergenic complexes. For purification and for practical purposes (i.e. prevention of reactions to therapeutic doses of penicillin), this highly allergenic complex could be removed by dialysis and chemical purification [56].

Similar polymerization occurred with biosynthetic derivatives of 6-aminopenicillanic acid, the functional but biologically less active chemical nucleus of all the penicillins, and in chelation with various metals which yielded identifying spectra of colours (figure 4). When these polymers were fractionated by sephadex and analysed by column chromatography, infrared spectrometry and proton resonance, it was found that antibacterial activity depended upon retention of the intact β-lactam ring as evidenced by the stretching vibrations of its already strained carbonyl–benzyl bond [57–59], whereas immunochemical activity and antigenicity depended upon increase in molecular mass, heterogeneity

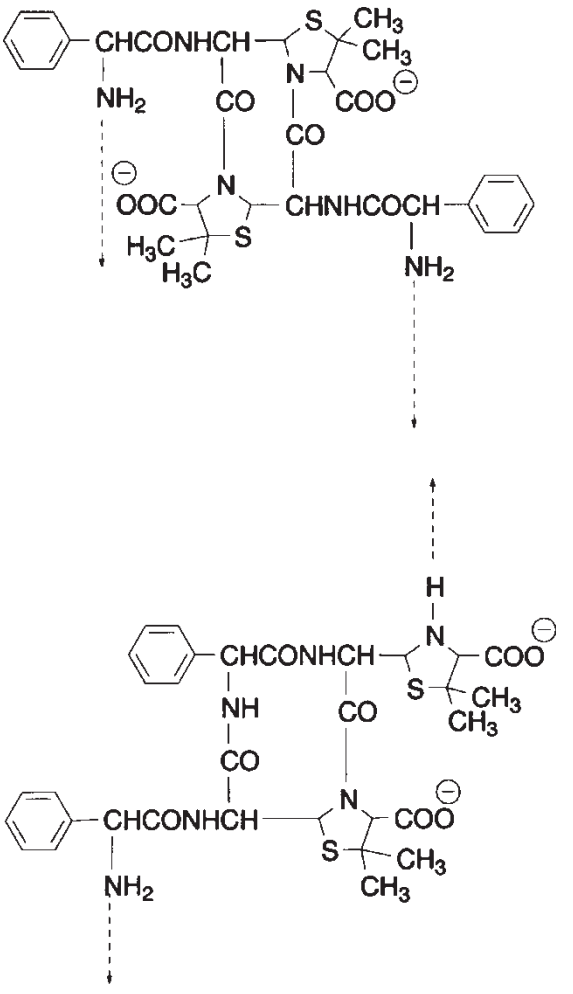

Figure 4. Biopolymerization of ampicillin (α-amino penicillanic acid). Dimers of ampicilloates as in the upper and lower structures, forming units for linear and cyclic polymerization as described in the text.

of the component amino acids and conjugation with other polymers. Polymerization occurred most readily (figure 4) with an L-epimer of 6-aminopenicillanic acid (ampicillin) which was also more immunogenic and wider in antibacterial spectrum because of the greater reactivity of the *N*-benzyl amine group [37]. This derivative was the most active and soon became the most widely used of all antimicrobial drugs. The grammes used for chromatography and biosynthesis in 1960 began to be used therapeutically in tonnes within a few years. The biosynthetic potential of linear and cyclic polymerization, and of the enantiomeric properties in these β-lactam small dipeptides was recognized at the time and in subsequent spectroscopic studies [57–61].

In 2003, Shahjee *et al*. [62] at the National Institute of Immunology in New Delhi showed that the L-isomers of various amino acids acted as osmolytes

which enabled *E. coli* to survive under environmental stresses of heat, cold and raised salinity, whereas the D-equivalents were inhibitory. This property, as observed with a much wider range of bacteria inhibited by ampicillin and its analogues, raises interesting general questions about the differences in biological activity between L- and D-isomers of small molecules.

Polymerizations similar to the above were obtained also [60, 61, 63] with aromatic and other derivatives of 7-aminocephalosporanic acid (cephalosporins), in which the lactam ring is replaced with a dihydrothiazine ring, and with bacitracin, a more complex oligopeptide. The immunological effects were similar to those obtained by conjugation with protein, in which case the cyclic derivatives acted as the prime determinant also of antigenic specificity in a manner similar to that described by Landsteiner in his seminal analysis [64] of the exacting specificity of goodness of molecular fit in serological reactions. Fine differences are also observed in blood group antigens [65] as precisely ordered immunological determinants. These findings provide causal significance for the signature principle of Henry Quastler [21], and its extensive use in immunology, in genetic finger-printing and in metabolic glycosylation of lipoproteins. Similar approaches to ordered biosynthesis have been quantified for RNA, DNA, peptides and profiles for mutants in larger molecular landscapes by Kauffman [10].

13. Moulds

The natural processes by which filamentous moulds yield arrays of metabolites with unique biological properties is revealed to some extent in the recent disclosure of the huge genome of just one species, *Neurospora crassa*, regarded as 'a central organism in the history of 20th century genetics, biochemistry and molecular biology' – quite an achievement for a form of life often despised as a contaminant. But there is no doubt of the importance of these metabolites as agents of chemical evolution for they include enzymes with ana-/cata-bolic activities, carotenoids, oligomeric peptides, steroids, aminoglycosides and other pro- or antibiotic agents. These must have evolved from relatively simple organic precursors in primitive cells growing independently and producing optically active D- and L-isomers, as described by Pasteur. Other moulds grow in swarms of segmented mycelia with undifferentiated cytoplasm in which regulatory nuclear material is diffuse though recognizable experimentally as mutable nucleotide. Productivity can in fact be increased by soft radiation in moulds that construct chains of peptides and other ordered structures in aperiodic sequences from nitrogenous and carbohydrate precursors to produce enzymes, retinoids and of course many major antibiotics. Apart from their mutability, these moulds automatically resist adverse conditions and re-enact their phylogeny by sporulating. They use the Krebs cycle to catalyse preformed compounds to form aldehydes, ethyl alcohol and acetic acid from organic matter, as in their alternate ancestry of brewing and vinification. They employ substrate inhibition for opportunistic regulation of end points, and to activate feedback loops. Entropic disorder is thereby lessened but, amid the biochemical chaos of catalysis and decay, moulds can reverse it to initiate a food chain with intermediate metabolites, diversified morphology, mutability and billennial preservation of their place all the way from roots to flowers and fruits in the tree of life. In other words, primitive as they were and are in the evolution of living substance, these archetypic moulds have evolved an adaptable metabolism with informative chemical pathways for endurance without evolutionary change by cytoplasmic transitions in lyotropic and amphiphilic ordered phases. It is likely [36] that similar pathways have been devised genetically for mediation in lyotropic phases in the Golgi apparatus of the plankton.

In searching for reasons for this in precursors of living substances and for clues to the origins of life, it is helpful to track metabolic processes backwards, for instance with penicillin, the best known and most useful metabolite. Although Alexander Fleming was no chemist, he was a natural scientist who investigated, identified and filed clues which top chemists failed to recognize until a second exploration by Florey, Chain, Heatley and others in Oxford disclosed them [66] and gave us penicillin as the first and pre-eminent member of successive and successful dynasties of antimicrobial agents. Fleming, meanwhile, had identified lysozyme as the first enzyme with a natural capacity to fit and split protective mucoids. Newton and Abraham [67] at Oxford, then purified and crystallized this enzyme, another first and independent but parallel event.

These examples illustrate how oxidation, high energy phosphate cycles, and other familiar abiotic chemical reactions in colloidal and other media can join with polymerization and with autocatalysis of polypeptides to purine and pyrimidine bases, to prime inter-acting and self-regulating probiotic chain reactions using waste products which are themselves supportive of other forms of life. This process, once started, is self-reproducing and continuous but conservative of energy, and is a causal clue to metabolism—and to the successes of the polymerase chain reaction. The prebiotic and immense biological importance of the polynucleotides, is a topical and more popular further example.

14. The nucleotides

In 2003 of all recent years, with republication and reviews in Nature [6] of the memorable triad of original papers [5] by Watson and Crick, Wilkins *et al*., and Franklin and Gosling in 1953 about base-pairing in nucleic acids, it would be redundant to recapitulate the detail of the discovery and the enormous volume of subsequent work. The scientific implications were instantly undeniable but Francis Crick was wrong, or wrongly reported, when he said that they had 'found the secret of life', a claim reiterated recently by Watson [68]. They had ingeniously modelled DNA and RNA respectively as helical files in which the chemical secret of inheritance was assembled and preserved, and they established molecular biology as a major discipline in science. Their papers condensed the results of an immense volume of independent preceding work, most of which was reported and assessed in 1955 in the book [69] by Chargaff and Davidson which is required reading for an understanding of contributory, earlier developments.

The track which led to the discovery of the nucleic acids can be traced back to an event in 1866 in a less academic field in the surgical wards of the Royal Infirmary of Glasgow where Joseph Lister was trying to persuade a reluctant medical profession that the 'laudable pus' in patients with septic wounds was a defensive response to bacterial infection [70]. In 1868, a Swiss chemist, Friedrich Miescher, working in a hospital in Tubingen in Germany, decided to see what was in the septic pus. Using acidified pepsin to digest and ether to extract material from bandages of patients with septic wounds, he and posterity were rewarded by the discovery therein of a substance which he named Nuclein. He then returned to Switzerland where, in extracts from serendipitous spermatozoa of the salmon which in those days swam in the Rhine, he found the same substance and identified it as protamine phosphate. This was renamed nucleoprotein when A. Kossel, Emil Fischer and others working in Germany found, between 1885 and 1901, that it contained new heterocyclic bases which were later shown to degrade into, or to be synthesized from uric acid into a series of purines—adenine, thymine, uracil and cytosine. These accounted for much of the basophilic staining substance already named by Paul Ehrlich, also in Germany, as the chromatin network of jumbled chromosomes in the nuclei of all plant and animal cells in which Jean Brachet and others had meanwhile identified similar acidic complexes of nuclear matter in yeast, thymus gland and other biological materials containing purine and also pyrimidine bases [9, 71]. In 1912, Levene and Jacobs [72] had demonstrated glycosidic bondage between these nucleosides and a sugar deoxypentose to form macromolecules of the four nucleotides, adenylic, guanylic, cytidylic and uridylic acids which could thereby be differentiated into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) a momentous discovery that marks, historically and biochemically, the date and base of the new science of RNA.

In 1933, Brachet in Brussels had located nucleic acids in tissues histochemically, another key finding during the period 1930–38 when there was a surge of independent work by Levene, Kossel, Stacey, Todd and others on xanthines, guanines, oxypurines, polymerases, depolymerases, active degradation products and metabolic intermediates involved in the biosynthesis and metabolism of the nucleic acids. These are described in a book by Davidson [73], working in Glasgow and New York, of developments before 1950 when it became clear, largely from the work of Levene at the Rockefeller Institute in New York City and of Signer in Bern, Switzerland, that both nucleic acids were long, thin, viscous chains of nucleotides linked from the 9-position of a purine or the 5-position of a pyrimidine to the 1-position of a cyclic pentose sugar. Both acids could be hydrolysed at selective sites by purified intestinal nucleases. The larger molecules of DNA had masses exceeding 106, were viscous, stiff and birefringent, and accounted chemically for much of the nuclear material, though the general view remained that the heritable factor was either a tetranucleotide or a long polypeptide chain of nucleoprotein, with DNA as a carrier. The advent of World War II in 1939 brought new research in this field almost to a halt.

Fortunately, some pre-war work on a different track by Griffith at the Lister Institute, London, had been revived by Avery at the Rockefeller Institute in New York City. Griffith had found that non-virulent R (for rough) variants of pathogenic pneumococcal bacteria could be transformed into virulent S (for smooth) forms by adding heat-killed S bacteria to R cultures. With his colleagues Macleod and McCarty, Avery found during the war years that the transforming principle was a high polymer of sodium desoxyribonucleate, a salt of DNA. This finding, published in 1944 [74], stimulated Chargaff [75] at Columbia University and others to reinvestigate the prevailing hypotheses. The discovery of chromatography had enabled Davidson and his colleagues [73] at Glasgow University to identify RNA in mammalian cell cytoplasm and in viruses, while Chargaff and the same Davidson [76] and others were using this technique plus UV spectrophotometry to question and ultimately falsify Levene's tetranucleotide, along with the later polypeptide hypotheses of genetic sequences by showing that the essential nuclear substance was DNA. Although it varied in composition in different species, DNA was

similar in crystallographic images which did not differentiate finer differences in molecular structure and complementarity between sugars and bases. The question of whether nucleic acid or protein was the source of chemical information for cells was answered, for bacterial cells at least, by Hersey and Chase. They showed, in 1952, that phages tagged with radioactive phosphorus and sulphur conveyed only P from nucleic acid into the cell, never S from protein.

Even at the time (1950), and certainly with hindsight, it was meticulous work by Chargaff [69, 75] that set the stage for a big revision by revealing an important dys-symmetry between the ratios of A+T and G+C, ranging from 1.9 to 0.4 and that, since there are species-specific differences in DNAs with molecular masses exceeding 106, it is necessary to allow for huge permutations in sequence and conformation of the long chains—a point which still escapes attention in the new world of NAs when experimental findings in one species are often generalized. Yet, by 1952, Chargaff could and did generalize (correctly) by finding empirically, and stating emphatically his essential Rules that (i) the totals of purines and pyrimidines are equimolecular, A+G=T+C; (ii) A and T are individually equimolecular, A=T; (iii) similarly, G=C; and (iv) that in microbial DNA, uracil (U) replaces thymine (T) in equation (i). These rules, together with analysis by X-ray crystallography and chromatography, showed that DNA molecules were variable chains of bases linked by phospho-diester bonds in spirals which Chargaff [75] described as 'beautiful representatives of non-representational sculpture'. This chemico-aesthetic description of DNA, plus a sketch by Wilkins and Stokes, sufficed until Rosalind Franklin brought it nearer to real life by identifying the relative humidity required to show in her crystallograms that it had a helical molecular structure compatible with the model which Watson and Crick were trying to develop. It was in fact a Liquid Crystal [4].

The flexible plasticity, complementarity and electro-weak energy required to assemble the double helix in the nucleus of a living cell were explainable by the LC structure of DNA, and it seemed likely in 1953 that this would apply also to the mobile role postulated for RNA as messenger for the transfer of molecular information to microsomes. But there was no explanation of the mechanism for biosynthesis of protein until Hoagland, working with the group at Massachusetts General Hospital [77], found in 1955 that the necessary energy was provided by adenosine triphosphate (ATP) binding with a separate enzyme to each of the 20 essential amino acids. This function, mediated by m-RNA and t-RNA, recognized immediately by Crick as the answer to the coding problem and the main mechanism for sequencing, is now generally accepted, though there might still be a need to see just how far the data obtained from experiments in bacteria and rats relate to the more sophisticated systems required for the synthesis and degradation of lipoproteins and other proteinaceous macromolecules in the higher mammals.

The elucidation since 1868 of these processes leading to the biopolymerization and twisting of elongated liquid crystals of DNA in the nuclei of all cells is a story different in almost every way from anything else in Nature. Chemically, DNA stores and prints molecular memory in a way which is obviously non-accidental though it uses the chirality, extraordinary length, complementarity and reserve of energy for conservation and replication of molecular memory. This ensures long-range geometry and invariance in messages via m-RNA in ribosomes for customized tailoring of L-amino acids into proteins; while aperiodicity in short-range cyclical groups adds a potential for mutation, recognition of feed-back from end-products and sensitivity to transient messages. DNA preserves these properties while scrambled in a helical string in the chromatin nuclei and nucleoli of somatic and reproductive cells which can instantly unscramble *in vivo* as *in vitro* into a precise and invariant array of longitudinally paired or unpaired chromosomes. It accommodates nevertheless a substantial residue of chemical nonsense which can degrade into fodder for the new bases required for growth and metabolism. These properties of DNA are greatly enhanced by concentrations of super-helical zones with greater curvature in nucleosome core particles which would appear to provide additional facilities for genetic regulation and transcription. In a recent investigation of this recognized but unexplained difference between oligonucleotide and nucleosomal conformations, Richmond and Davey [78] at the Zurich Institute for Molecular Biology have found that binding with histone doubles the curvature and produces super-helices with a different pitch, more turns, steps and mobility. It is suggested that these changes in nucleosomal DNA have additional implications for sequencing and recognition of proteins, and other genetic functions. Van Valen [79] drew attention to the fact that, although nuclear RNA degrades rapidly, some remains and can be confused with viral RNA or that formed independently from non-cellular sources as products of reactions in other environments, as described by Cairns-Smith [13]. This could be a further example of how self-organization of DNA and RNA is sufficiently economical and efficient, spatially and temporally, without a master plan except in so far as RNA is a template for complementary pairing of bases and sequences. This seems to be enough to guarantee

continuity of species [68] while permitting minor change for ecological adaptation and perhaps recognition of teleonomic information.

The annals library of knowledge opened by the discovery of DNA are more thrilling than any other biological serial to date. But this library now has its own selective Who's Who, and it is difficult to ensure that all contributions from 1950 onward have been recognized or that all major errors have been corrected. One such error was embedded in the central dogma that information from DNA is transcribed via messenger RNA in one direction only. This does not explain the activity of *reverse* transcriptase, or the corrective activity of reverse loops and two-way regulators in superhelices with different conformations, as described above, or the process of biological accountability. The roles of quantum mechanics and negative entropy are also disregarded. Another, uncorrected, error is the assumption that copies of unpurified starters primed in the polymerase chain reaction (PCR) necessarily represent specific identifying components of organisms without purification from co-cultures. Kary Mullis, whose Nobel Prize was awarded for this technology, and the manufacturers of PCR kits, have often expressed reservations about this procedure.

A larger overall impediment which still pervades molecular biology is the failure to recognise—as Darwin, Lister, Meischer, Mendel, Huxley, Waddington, Fleming and Avery surely did in this field—the importance of non-artefactual observations and empiricism, of previous work and of publication somehow, somewhere, of results if they contain information which questions, falsifies, contradicts or replaces prevailing views. This difficulty is one which has often impeded attempts to translate experimental work and reasoning about the lyotropic LC into the mainstream of molecular biology. However, it is now increasingly clear that ordered transitions into aperiodic sequences, chirality and especially electro-weak linkages reflect the operation of a physico-chemical system capable of perceiving and restoring order to situations in which the free-for-all chemistry of impending life seems, in the words of Kaufmann, to edge repeatedly on chaos.

DNA and RNA explain processes of cellular metabolism, co-variant duplication, inheritance, mutation and much else about life but not about its origins because these substances are intermediate or degradation products of preformed protein or other macromolecules, as described by Van Valen above and by Horowitz [32] in earlier work which has also been overlooked. It is of interest that analogues of nucleosides inhibit the multiplication of RNA viruses, and that aminoglycosides and other bases interfere with ribosomal protein synthesis in bacteria, as described in the medical aspects of LCs in Part I of this review. It appears that the enzyme RT enables retroviruses to enter the human and certain primate genomes, and it is known that retroviral RNA of uncertain origin accounts for a small but constant part of the human genome. Viral fingerprints including HIV integrase have been identified in the nucleoli of human cells. It is possible therefore that ancestral RNA viruses from various sources might have become endogenous in animal and human genomes, and can be released during cell division. Whether or not exo- or endogenetic processes have released infectious virions capable of causing new diseases is a question which has been raised in recent years in looking for explanations of the changing pattern of viral diseases, of the sudden onset of Lassa and Ebola fevers in Africa in the 1970s, of AIDS in the USA in 1980 and, in a different pattern, in Africa in the 1990s, and of SARS in China in 2003. This question raises many others which are too specialized for discussion in the scientific brief of the present review, but they are of immense importance in the natural history of microbial infections and in the practice of medicine and public health.

A detail which is important in the context of this review is that RNA, presumed to originate in micro-organisms, can be detected by direct isolation and traced by genetic probes and serology which provide information about their origins in the tree of life and in the spectrum of zoonotic and human diseases. The rate of multiplication is such that mutations will be numerous but experimental work since 1960 has shown that ancestral properties in bacteria as well as viruses are nevertheless conserved, or can reappear with evolutionary advantage, or the reverse. For instance, the strains of multi-resistant *Staphylococcus aureus* (MRSA), which are now notorious as bandits causing cross-infection in hospitals everywhere, retain the MecA gene in their DNA which made them resistant in the first place [37, 38, 80], along with the facility to revert to primaeval syncytial or L-forms [31, 34, 81] in which they and other pathogenic bacteria can survive adverse conditions, with ablation of chromosomal patterns. Protozoa and the lower orders of metazoa do this successfully by retaining the ligneous, embryogenic and sporulating properties of plant life which embody sufficient intracellular information for ordered transitions into compact capsules, spores and cysts for prolonged survival under adverse climatic and other stresses [82–84].

15. Lipoid LC systems

Although many micro-organisms and genetic materials can survive cosmic radiation and adverse conditions like fractionation, freezing and drying in liquid

nitrogen, they are in general unable to tolerate the volcanic temperatures which preceded cooling of the lithosphere. But life would not have occurred without exceptions, now classified as the thermophilic Archae, with thick membranes tolerant of 'extremophilic' temperatures, acidity and ionic concentrations, composed essentially of non-symmetrical tetra-ether glycolipids with sulphur- or methane-dependent metabolism [9, 84]. The methanogenic species *Methanospirillum hungatei* is a natural source of these rugged lipids which have been used as a model for synthesis of analogues by Goodby and his colleagues in the Liquid Crystal Group founded by George Gray at Hull, England. Their work, collaboratively with Plusquellec and others in the Ecole Superior de Chimie at Rennes, in France [85], shows that these compounds, like the natural product, are amphiphilic when aggregated or inserted into membranes. As such, these would appear to be the first forms of a lyotropic mesophase to emerge as living substance in extreme locations which preceded the conversion of planet earth into a temperate biosphere. The chemistry is complex and defines qualities which differentiate the Archae from the Eukaryotes which superceded them in evolving microbial populations. In addition to their chemical tracks which included schedules for glycosylation and chirality, these Archae endowed microbial posterity with other enduring characteristics, such as spores, the tough lipopolysaccharide cell wall of rod-shaped bacteria and the formation of the purple protein *Rhodopsin* which opened further tracks leading through anaerobic to aerobic synthesis in plants and animals and, eventually, to visual purple in the eyes of creatures, including Man, with the priceless gift of dark and colour vision. These and other phenomena in primaeval cells lead on to the recurring question of how the autogenous morphogenic and functional roles of acids and esters of glycerides, phospholipids and cholesterol in prebiotic phases switch themselves, or are switched into animation, cellular growth, differentiation and metabolism with memorizing and precise replication of each morphological and functional change in the main systems of vertebrates and insects.

In the context of lipids and proteins, there are likely explanations of these phenomena in recent large-scale and collaborative experimental work at major centres in Italy, Japan, the UK and the USA. This work is concerned with the role of specialized 'semaphore' proteins (SEMAS) as guides and regulators of signals to endothelial, angiogenic and neural cells. One group [86] in Turin, London, Stanford and Tokyo is concerned with activation and regulation of vascular morphogenesis. Another group [87] in Baltimore and Seattle is reporting overlaps in the semaphorin messages to immune and neural receptors. One protein, SEMA 7A from membranes, is immuno-modulatory, and also concerned with development and guidance through neural axons. A similar mchanism operates via phospholipase in Golgi-mediated translation of messages from the plasma membrane for activation of the specialised *RAS* proteins which regulate cellular growth, differentiation and division. This relates [88] convincingly to other studies on the folding and activities of *Rho* and other regulating proteins, and to the possibility that there are key molecular switches in the plasma membrane and Golgi apparatus for guanosine-mediated (GTP and GDP) cellular energetics, and for completion of protein synthesis, including folding such as occurs in prion proteins [89]. These phenomena are dynamic and, theoretically, might be a basis for oncogenic as well as natural growth for they recall original work by Ambrose [90] in the 1960s on the possible role of cell membranes and surfaces in malignant transformations.

Of many lipids with leading roles in cellular biology, three groups merit special attention in the biosphere because they are conspicuous and appear to be both unique and essential for the development of life, living processes and awareness [4, 7, 91]: a large agenda! These are

(A) Phospholipids with their ability to energize kinetic activity, flow and ionic exchanges within and through interfacial surfaces between aqueous solutions and lipoidal suspensions (figure 5), make phosphatides available for high energy reactions.
(B) Long chain esters of fatty acids in low energy conjugates with proteins which serve as particles, bilayers and chylomicrons for extracellular transport of these substances, and for formation of organelles like mitochondria for high energy intracellular reactions.
(C) Cholesterol in its native form as a pivotal molecule for esters and steroidal derivatives which, in association with fatty acids and surface-active agents (figure 6), form stable, helical complexes with structural and hormonal properties in cells and organs.

In all of these forms [1–4, 46, 84, 85, 91, 92 and appended references], observations and experiments since the 1950s have shown that lipids in diverse lyotropic mesophases combine self-organizing structural with replicative and metabolic functions which are indispensable for prebiotic initiation and maintenance of living processes. This is true not only in the generality of Nature but also, as shown in Part I of this review, in the evolution of afferent receptor, integral processing and efferent transmission of neural and cerebral activity leading certain species of insects

Figure 5. Myelin tubes of phospholipid: polarized light: $\times 200$.

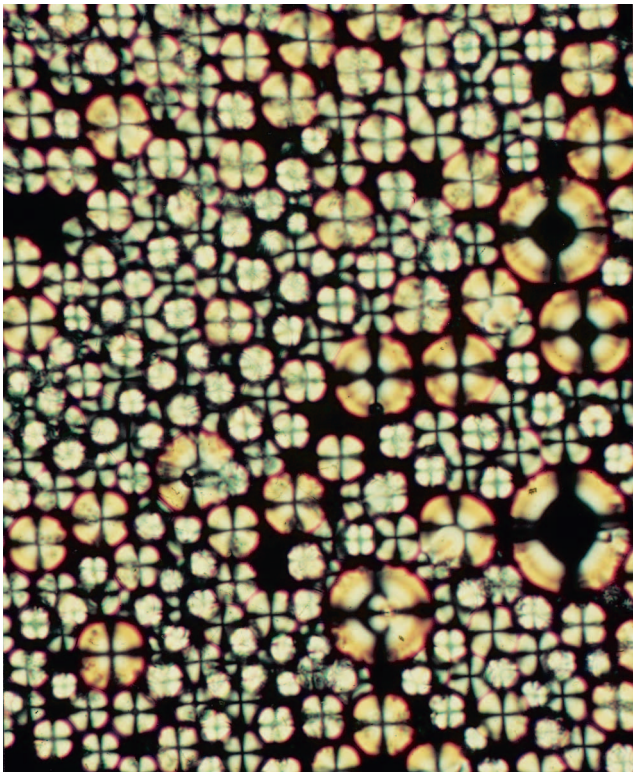

Figure 6. Microspherulites of cholesterol from a helical complex in the corpus luteum of a human ovary, showing polarization crosses and secondary growth: $\times 400$, polarized light.

and mammals eventually to social organization and, in primates and humans, to higher levels of analytical intellect.

Evolution to these levels must have been preceded in various locations in the biosphere by billenia of trials and errors in the laboratories of Nature of happenings which appear to have been, in terms now intelligible in man-made laboratories, largely haphazard and accidental. The prebiotic state contained most if not all of the chemical agents and physical conditions necessary for these natural experiments to proceed and to survive, decline or fall in accordance with the reproducibility and efficiency of their performance in Darwinian terms. These processes would be accelerated with the advent of life which would undoubtedly have offered greater employment opportunities and advancement for entrepreneurial biochemicals with new molecular expertise. Substances and circumstances propitious for surface action, coacervation, formation of peptide linkages, uneven handedness, birefringent glamour, torque, spin and conjugation profiles on the biochemical catwalk would have obvious appeal. Combined with a natural facility for dissociation and re-assembly, these biochemicals would soon emerge as an elite of macromolecules with integral properties and evolutionary potential that would outpace Darwinian selection and arguable criteria of fitness. Nature is profligate, quixotically wasteful, encourages replication, enjoys abundance, and would undoubtedly reward the plethora of attractive macromolecules, especially in new LC forms, with further opportunities to acquire superior status as more interesting stuffs of life. It is not unscientific to predict that news of these evolutionary successes would spread and possibly spin with all the electrons in the biosphere to ensure that the superiority thereby on offer would prevail, with the impetus of a new, convergent force, *animation*, to overcome entropic disorder and set a new pace in terrestrial time and space. The Higgs mechanism could contribute and might be necessary for this to happen [30].

16. Space, time and light

To maintain the pace (or flight) of thought in this study, it is appropriate at this stage to consider the fourth dimension of time and especially the influence of light in the expanding spatial setting of the lyotropic LC in general biology and in its special role in the lipoidal structures of neural systems. As waves or photons, light is a universal source of energy and information which can be stored as photonic structures in ordered arrays of layers, fibres and tubes in prebiotic and in living matter, conferring dynamic properties of change in iridescence, colour and refraction in plants, animals and insects. It has probably evolved as cryptic

nanostructures in protoplasts but it has become conspicuous in the phototonic colours of flowers, aquatic creatures, butterflies and beetles as a mechanism of unending biodiversity for survival and reproduction. Phototonic structures are especially prominent in LC form in spherulites and helical complexes of cholesterol with anomalous optical rotation [93] in chitinous exoskeletons in *Coleoptera*. This helix is unaffected by high levels of ionizing radiation [4] and might be a factor in the survival of insects and of neural tissues subjected to nuclear magnetic imageing. The energy equations of these properties await attention.

In all its forms, the LC has a schedule of ready reactions to other stresses like pressure, electricity, temperature and chemical change. Although conservative of energy and order in spheroidal, helical and other complex morphologies, the LC seems in some situations to depart from the second law of thermodynamics by recreating order and higher levels of energy from disorder and entropy, thereby introducing an unconsidered phenomenon into the Principle of Uncertainty. This is not incompatible with Schrodinger's, Boltzmann's, Einstein's or other mathematical models and equations, and is not trifling. There is in fact a need for additional attention from physicists to consider how these equations apply if they include the electro-weak forces which hold oligomers and macromolecules together in the LC, contemporary plasma and classical aether, perhaps by the Higgs mechanism. In such matters the LC is a scientific epiphenomenon or spoke in the wheel of natural philosophy, like Maxwell's demon. Some of what is said here and in the key references might bring fresh perspectives, especially those available in direct or polarized optical and photonic examination of matter compatible with life (figures 1, 5), and with regard to applications of the Principle of Uncertainty, quantum and wave mechanics to molecular biology.

In the lyotropic mesophase, wave mechanics and transitions may trigger events conducive to animation of matter in steady states. In Einstein's famous equation, the square of the speed of light c in a vacuum is the constant linking energy with mass, m. Transfer of energy E causes transfer of mass, $m = E/c^2$, i.e. both are conserved. If both sides of Einstein's equation are divided by m, the mass of a particle, it becomes $E/m = c^2$ which makes sense because photons are an almost weightless source of energy. In the anisotropic LC, photon energy will be retained internally by birefringence in accordance with enthalpic conditions for heat (h), pressure (p) and volume (v), $h = u + pv$, where u is the internal energy conserved by photonic mechanisms [91]. Particles vibrating in a wave-front will follow a sinusoidal function ($y = a \sin 2\pi \ (t/T - x/\lambda)$), where a is the angle between vectors and t/T is the time period. The frequency of vibration changes with the water content, surface action and h,t,p. If both wave-fronts have the same frequency, there will be a transition into a more ordered state at the critical point in a ternary or quaternary phase, as described by Winsor, Chapman and others [see 3, 4, 10], with restoration of energy. This can be used in a further transition, or it may dissipate and set up a chain reaction similar to a Turing model [94] of a chemical system in a steady state. Planck's Law and the Principle of Uncertainty will apply in both.

Transitions from the steady state in lyotropic systems as above can be measured by phenomena like birefringence, optical interference, Bragg diffraction, mobility, gradient density, enantiomerism and electrical changes, all of which can be identified also in prebiotic organic matter, clays, polymers, lattices and ionic channels. Such transitions must relate also to changes in mass, energy and entropy as above, in the logic of relativity without seeking changes in fundamental laws of physics, though Schrodinger in 1944 [11] and a provocative mathematician at Stanford University, Nancy Cartwright [23] in 1983, thought that this might be necessary, even overdue. Both of them, like most others in this field, ignore Klieber's and other earlier laws (1932–49) which postulated crude but undeniable correlations in log–log plots of quantifiable phenomena like metabolism, body mass, life span and energy. This relationship has been used in 2002 by Rau [95] of the Department of Physics and Astronomy at Loiusana State University to develop a new approach based on the power of biological fluid flow and metabolic rates on differences in geometry and rates of growth. In macrobiology, for instance, this can be applied to trees and elephants' legs and explained mathematically by surface to volume ratios in three dimensions, scaled as $(-1/3)$ instead of the $(-1/4)$ required in Klieber's Law. Cartwright generalizes by including additional parameters and intermediate variables, and concludes that the main laws of physics ignore biological and other realities and causality. She herself ignores Klieber's Law.

17. Neural systems

Phenomenologically, a low order of neural activity is detectible in various responses by unicellular protozoa to physical and chemical stresses. This would seem to be based on the reactivity of lipoid membranes as described above, but in some species the process is more highly developed as in the light-sensitive red eye spot of species of *Euglena* and the responsive flagellae and encystment of *Giardia*, *Paramecium* and many other protozoa in which the memory and signals for response can only come from intracellular molecular changes.

These are effectively polyvalent and reliably invariant—a remarkable example of self-sufficiency in the lower zoological strata which merits more attention to see whether, in this respect, the ancestry and impressive survival of the protozoal underclass has any simple lessons for less viable members of the metazoa upstairs. Many scientists find it enjoyable as well as informative to work with friendly, lively and responsive creatures like *Entamoebae* or notably *Euglena gracilis* which helped Muriel Robertson herself to evolve so graciously as the first female Fellow of the Royal Society of London.

It is inconceivable that life could begin with metazoa, so the movement of some protozoa from the unicellular self-sufficiency of *Euglena* to multicellular pluralism in the metazoa is not a challenge to Darwinian evolution. Biochemical materials, molecular memory and experiential intelligence geared merely to growth, reproduction self-interest and survival can obviously be transferred or copied by direct or genetic contact between groups of cells and creatures without need for reward, outside intervention or independent intelligence. A seed, sperm, ovum or zygote is a package capable of delivering all this information to remote recipients. But how could this development lead onward to intelligence and purpose, even in reptiles and especially in insects, without a genetic or other code-in-waiting to design and transmit operational manuals? How could it construct the psycho-biological organization of the central nervous systems even of insects and the simpler vertebrates? What else would be required for the emergence in humankind of by far the most complex and highly organized attribute of anything in the biosphere?

Embryogenesis and especially neurogenesis is relatively slow in all animals and never instant. The processing of information starts in neurons and follows pathways shown experimentally [87] to be defined by outward growth of axons and guided by specialized proteins (semaphorins) which may also convey information to endothelial and immunological receptors. Passage through synapses is measureable and shows delays, sometimes indicative of imperfection in structure, defects in conduction, or evolutionary mistakes. For instance, the skeletons of the longer dinosaurs have a presacral cavity to house a secondary ganglion—a minor brain—for relay of distal reflexes to its elongated rear end: a belated, ridiculous and unsuccessful evolutionary effort to cope with the unfitness of largeness in Jurassic times. In insects, reptiles and smaller animals, and in all mammals, evolution discovered or devised skeletal rearrangements for security of the ordered but delicate nematic networks of intercommunicating peripheral relays in brains of increasing size in the higher vertebrates; with additional neurons, convolutions and capability, identifiable by differences in proton resonance in the human brain (figure 7). In higher mammals and birds, the neural load is formidable, in humans, it obtains extra space by convolutions and folding of lipoprotein and cerebrosides without much increase in cerebral volume though, biomathematically, it still exceeds explanation by orders of magnitude.

In the vital neuronal centres concerned with the cardio-respiratory control and maintenance of life, and with hearing, balance, proprioception and vision, there had to be shorter routes or circuits to relevant parts of the brains which evolved *pari passu* into anatomic compartments with specialized functions like sensors, auditory vestibules, cerebellum, mid-brain and brain stem. To provide increasing motor skills, sensitivities, responses, cybernetic feed-back and awareness in brains which are still evolving, this requires a vast but compact system of interactive layers, columns and networks of neural cells, fibrils, ganglia and synapses. Mechanically, this system is similar to a fast, sequentially processing computer which can be programmed in its hardware and software, and during use, to the requirements of an individual or company with the important difference that, in the mammalian nervous system, the entire assembly is soft, plastic, intricate, much more subtle and independent, though it also can be programmed.

18. Awareness and consciousness

Many neural processes can be described if not explained in mammals at least in accordance with the laws of physics and chemistry, inherited imperatives, evolution, the wisdom of Konrad Lorenz [52], the growing rivalry of computer technology and even the trenchant criticisms of Nancy Cartwright [23]. There is, however, a major segment which still defies description in these expansive terms of reference. Mysteriously but unequivocally and perhaps exclusively, this is the faculty of intellectual consciousness in humankind. It is a subjective universe which includes power of analytical reasoning, communication, fuzzy logic, objective foresight, altruism, rational creativity and, not least, the slippery feedback slope of internal awareness that includes conscience. All of these and many more faculties constitute a curriculum gifted uniquely to humankind in terrestrial space and chronological time, with options in cosmic time, perhaps in the extra-curricular classes of Albert Einstein and independent reasoning by other gifted scientists. Neuroscience has insights into the chemistry and histo-architecture of the brain and special senses relating to these abilities, especially the actions of an increasing number of neurochemical messengers, intermediates, metabolites and

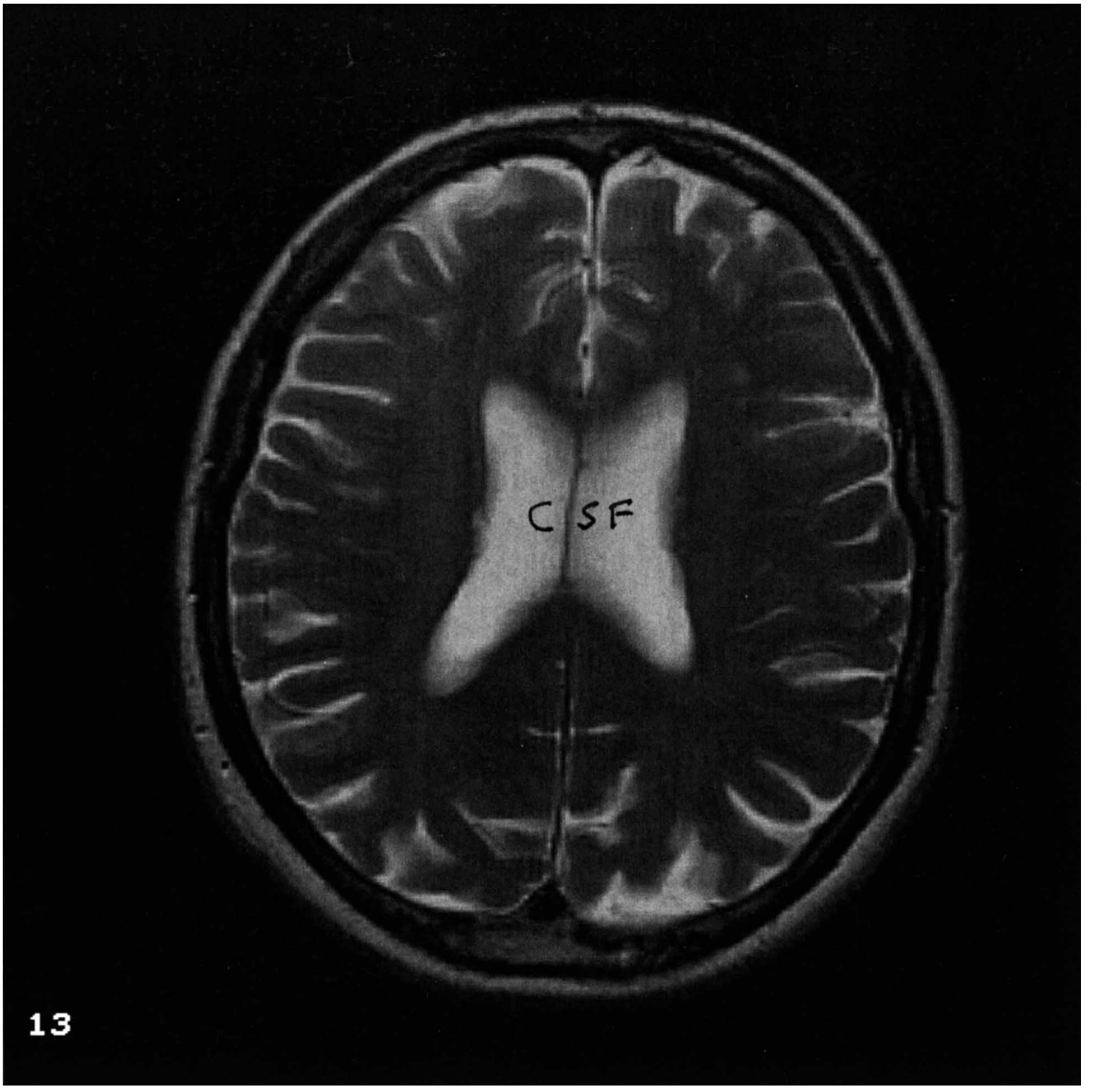

Figure 7. Ordered structure of the normal human brain. Magnetic resonance of a T2-weighted axial image at the level of the lateral ventricles which contain cerebro-spinal fluid (CSF) spread also thinly over the brain surface within the outer cranial bone. The neurons of the cerebral cortex form a superficial layer over the surface and extend into the convolutions, shown as white indentations. The myelinated axes for conduction occupy the remainder of the space (photograph by courtesy of Professor Joanna Wardlaw of the Department of Clinical Neurosciences, University of Edinburgh).

drugs which can occasionally differentiate between normal and disturbed states of neurological and mental function, as described in Part I of this review. But there is no scientific evidence about the essence of consciousness and free will as yet in this enticing and exciting domain [see 11, 24, 34, 95–99] beyond the crude and obvious fact that both can be reduced, modified, twisted, deformed or abolished by nutritional and psychological deprivations, injuries, chemical, microbial and other forms of demonstrable damage, or by pharmacological and behavioural experimentation—which includes child and self-abuse.

The grim logic of this, and of the well-known effects of brain-washing, mind-changing drugs and other interventions in consciousness, are leading in some quarters to acceptance of a new dogma that all of consciousness will eventually be explained, like heredity or hallucination, by adventurous experiments, in neurochemistry, drug-use, genetic manipulations, etc. And it has to be acknowledged that the human brain is so vulnerable to

insult in these ways that the grim possibility cannot be lightly dismissed. Medical science recognizes that a seemingly minor intrusion, recreational drug, haemorrhage or localized injury almost anywhere and anyhow in the brain can have a devastating and permanent effect on consciousness, intellect, speech and movement, and also that, for reasons which are seldom clear, there can sometimes be astonishing recovery of what seemed to be irrevocably lost.

A possibility often overlooked in perceptions of the nature of consciousness is that cerebral memory and the ordered states of neural matter upon which it depends may not obey all the laws of chemistry, physics and biology [11, 23, 24, 28, 98]. This is demonstrable simply in responses to pain which, although instant, are usually well explained by neuro-transmission and pathology. There is no memory of the feeling of pain but there is memory which may be everlasting of any kind of painful or traumatic experience [99]. This can apply to other vertebrates, even fish, and there is no doubting the immediacy and fastidious accuracy of olfactory, ocular, spatial, sonic and perhaps extrasensory memory and recall. But, in mankind, the cerebral memory upon which the huge additional dimension of human consciousness and objectivity depend is deeply imprinted from an early age onward, not only in a highly ordered system for storage of molecular particles but also in an immaterial psyche which enables previous imprints to be repeatedly and accurately retrievable, with an incomparably rapid recall, often without diminution in intensity, colour, smell, image, words and emotion, backward through years of time. This ability enables any human being—and possibly a very few other species like dolphins—to recall abstract memories instantly, with resonance within the temporal space of his, her or its mentality. Although this experience may be so subjective that the person virtually lives again within a psychic memory of the event, the result can be objective in so far as the content of the recall is frequently a verifiable impersonal happening like a newspaper headline or accident or conundrum. Whatever is recalled can then be verified, revised and used from infancy onward as a learning experience, beneficially or otherwise, in innumerable personal, impersonal and exploratory ways in the logic of evolution as well as in everyday behaviour. The recall might be a happy accident remembered, and felicitous, or it might be injurious, or it might, as in computers, be swamped or scrambled by the complexity, or sent to the bin for recycling or extinction, or just get lost.

Intellectually, this power of recall and rescue, irrespectively of content, is a necessary *modus operandi* of cerebral memory in a binomial or possibly Boolean [10] dispersion of signals to and from neurons via nematic networks. This comprises a vast array of abstracted but compelling phenomena like insight, dispassionate love, compassion at one end, and mathematical ability, inventiveness and musical composition at the other, to provide learning and achievements, for good or evil. In this respect, any resemblance between human brains and any computer disappears. What does appear is the possibility that Nature has installed supervisory levels of self-awareness and conscience as a teleonomic scanner to protect consciousness from corruption by trespassers and hackers. In materialistic terms, this is contained entirely within and dependent upon transactions in the intact neural network of neurons and white and grey matter in responsive loci and circuits for reception, storage and conduction of messages, processing, memory and transmission [97, 98]. In the words of Francis Crick, a master of the art of decoding biological messages, all of this is an Astonishing Discovery [100]—mainly of how much more remains to be discovered.

19. Conclusion

Part II of this review enlarges and endorses the scientific and everyday importance of the LC in molecularly ordered, lyotropic phase transitions, structures and hierarchies of natural matter in circumstances which are plausibly and causally relevant to the origins and processes of life in animals and plants. This contributes to the synthesis of lipoid, protein and other macromolecules held together by electro-weak forces to conserve energy in cytoplasmic organelles, and to organize nuclear chromatin and chemistry for cellular metabolism and reproduction. The crucial (*sic*) crystallography by Rosalind Franklin and her colleagues [68] showing that DNA formed a helix conforming to that modelled theoretically by Watson and Crick [5] only within a limited range of humidity was a molecular proof of the hydration by rainfall, conserved moisture and unique properties of water with which Nature, since time immemorial, has made dry seeds germinate, oxygenated the atmosphere, promoted green revolutions everywhere and endowed planet earth with viable creatures and populations. The fact that this was due to a phase transition in which the helical structures of DNA and RNA were identifiable as lyotropic mesophases escaped attention at the time.

In previous papers dating from 1959, based upon observations and experiments with living material, the writer has suggested [4, 7, 56] that the lyotropic mesophase in various forms is always present in, and is indeed required by living matter whenever molecular orientation and low energy spatial reactivity have to be maintained in phase transitions involving flow,

interfacial reactions, tensions, mobility, reversibility and other stresses or, in other words, to provide an atomic and molecular structure capable of maintaining highly ordered but instantly reactive and flexible additional states of matter which show how cells function and damage is repaired in plants and animals. Since then, advances in technology have enlarged research and promoted innovative biomedical applications using these properties. But the fundamental question of how organic matter became and might or must still become variously and permanently animate remains unanswered, despite successive advances in understanding of simple, self-replicating molecular mechanisms in lyotropic lipoid interfaces, bilayers and interfaces, and in nucleotides in the 1950s, chemiosmosis and early concepts of metabolism in the 1960s, and the RNA world in the 1970s. In the 1980s, de Duve [49] and others were reviving earlier concepts that proteins came first, and suggesting that RNA must have come from somewhere to assist amino acids in forming peptides. The present study extends this to other macromolecules. In so doing, it invokes a long-standing divergence between those who believe that life began as a chance combination of atoms [99] or a logical sequence of molecular events unfolding scientifically [10, 21], and the great majority who perceive instead a technical impasse, an intellectual no-mans-land with a need, to assign animation to metaphysics or to GOK; or to God Himself, as Newton and Victorian scientists did and, *faute de mieux*, some still do. The consequence is a void or querulous scholastic distance between disciplines which, in the absence of experiments with natural, biogenic materials and closer collaboration in physics, chemistry and biology [30, 98, 101], persists as tantalization in science fiction that disregards the excitement in real life.

To minimize the fiction, these contingencies are discussed here in terms of *phenomena*, *theoretical explanations* and *causality*. The *phenomena* comprise observations on prebiotic peptides, nucleotides, carbonaceous, lipoid and semi-mineral aggregates like clays which, boosted by photosynthetic, oxidative, phosphorylated and other fundamental chemical reactions, reveal similarities with highly ordered components like membranes, mitochondria, nuclei, genes and dynamic subcellular particles in living matter. The *theory* consists of explanations which raise questions about some of the laws and equations of physics, especially those dealing with energy, entropy, quantum theory and probability. *Causality* is an over-riding imperative, because observations and theory, however interesting, are unimportant if they fail to connect with cause and effect. All three approaches come together successfully in abiding examples: the kinetics of lipoid membranes and synapses; the importance of electro-weak cohesive forces; the invariance of molecular memories in DNA; the reliable automation of RNA in selecting amino acid sequences in ribosomes; reverse transcription of cellular RNA; the energy concentrated—and dangerously vulnerable—in mitochondria and Golgi apparatus; the difference in activity between L+and D- epimers and isomers; the general importance of rotations, chirality and symmetries in ordered structures, the reversal of entropy required for animation of inert matter. All of these, and other phenomena in the text and literature, relate to causalities. In the writer's view, the answer to remaining questions must come from research within this scientific trinity, if and only if it recognizes a wider, arguably cosmic dimension of natural philosophy. Meanwhile, the aforesaid scientific trinity yields information which challenges orthodoxies but respects laws of chemistry and physics that keep it on track technically, with options in the fuzzy logic of the loose or lunatic fringe of biology in which chaos, uncertainty and teleonomy keep entering the forum too often to be ignored.

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