EVOLUTIONARY ORIGIN OF LIFE SCENARIOS: PARADOX OF THE PLASMA MEMBRANE

RICHARD D. LUMSDEN, PH.D. AND NOEL D. FRANCIS
DIVISION OF BIOLOGICAL AND PHYSICAL SCIENCES
THE MASTER'S COLLEGE
SANTA CLARITA, CA 91322
AND
DEPARTMENT OF BIOLOGY
INSTITUTE FOR CREATION RESEARCH
SANTEE, CA 92071

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ABSTRACT

The structural and functional properties of the cell surface (plasma) membrane (a.k.a. *plasmalemma*) relevant to evolutionary paradigms for biogenesis are reviewed. Such a partition would have been essential for the transition from abiotic chemistry to biochemistry, the physicochemical organization of the protocytosol, and the subsequent evolutionary differentiation of first life forms. However, an incremental evolutionary origin for the plasmalemma is gainsaid by these very requirements. The most plausible type of first membrane, a purely lipid bilayer, would have provided the necessary containment principle, but because of its demonstrable impermeability to requisite inorganic and organic molecules, the same membrane would have simultaneously precluded further development, even maintenance, of the emerging protocells. As an alternative primordial membrane model, protein microsieves are addressed and found wanting to the concentrative requirements of biologically relevant synthetic chemistry and its organization. Existing plasma membranes are composites of lipids *and* proteins, where certain of the proteins function as permeation channels and transport catalysts ("carriers" or "permeases"). However, the prospect of a selectively permeable lipoprotein membrane assembling *de novo* by purely natural processes is contraindicated not only by the egregious improbabilities but also by a number of definable physicochemical constraints. Meanwhile, to a creationist model of cell (ergo life's) origin, the integrated structural and functional complexity of extant plasma membranes provides yet further evidence of purposeful design. Not the least of this evidence would be the determinative informational principles on which membrane permeabilities are based.

INTRODUCTION

As Dutrochet [10] correctly surmised in 1824, cells are the *piece fundamentale* of organisms, i.e., the most elementary unit of structure that can manifest and sustain life. Thus, ever since Darwin [9], an explanation of the origin of cells has been incumbent on any evolutionary consideration of life's origin and subsequent diversification [35].

By definition, a cell is constructed minimally of an *endogenous* membrane (*cf.* viral envelops) enclosing a genome and the mechanisms attendant its replication. In the present, all cells originate from pre-existing cells (per Rudolph Virchow [51], *ominis cellula e cellula*). According to the creationist view, such has always been the case, the first cells having been created as units of the organisms that constituted the *baramins* (as defined by Marsh [30] and Wise [59]). The originally created kinds would of course have included prokaryotic organisms (bacteria), contemplated otherwise as the first cell type to evolve from abiotic processes, unicellular eukaryotes (protozoans, algae), and fungi, as well as multicellular plants and animals. Virchow's precept [51], articulated in 1858 as a refutation of the concept of spontaneous generation of life from non-life, remains altogether consistent with the contemporary observations of operational science. Meanwhile, the postulate of life's *abiotic* origin (Figure 1) - via spontaneous chemical formations, self-organization, and membraneous precipitation [61] - remains undemonstrated. While this is hardly disqualifying, considering its status as a one-time event in the unobservable past, it remains that as an exercise in forensics the premises are not based on a great deal of hard evidence. Indeed, for the most part the *data* from abiogenic chemistry have identified the mechanisms by which life could *not* have evolved! The thesis of chemical evolution confronts credibility when there are important - even essential - biochemical compounds

for which an adequate prebiotic synthesis has not been elucidated (e.g., pyrimidine nucleosides, with the explanation that primordial nucleic acids did not contain pyrimidine bases!) or, as Stanley Miller notes [32, p. 16] "... (experimental) conditions are so forced (e.g., by use of anhdrous solvents) or ...(reactant)... concentrations are so high (e.g., 10 M formaldehyde) that the syntheses could not be expected to have occurred extensively (if at all) on the primitive earth". Then we have as "evidences" syntheses achieved only with altogether improbable reactant analogues (e.g., purine and pyrimidine phosphoroimidazolides); products frequently arising with biologically incorrect bonds (e.g., RNA polynucleotides with highly unstable 2-5 rather than 3-5 phosphodiester bonds), random (racemic) chirality, in yields of at best trace quantities, and so on. Could it be that such statements as "... the problems of the origin of life stand out as one where the greatest advances are *still to be made*" [32, p. 25, emphasis added] are no more than whistling in the cemetery where the fate of abiogenic theories is concerned? Is the idea of chemical evolution, as Yockey [60, p. 286] concludes, "latter day alchemism"? The postulate of life from non-life is nonetheless essential to the non-theistic materialist view that life - past and present - is a purely physicochemical phenomenon resulting from purely naturalistic physical processes; that the present biosphere has developed through descent with modification from a singular primordial life form; ergo that all life forms are organically related, one with nature. But if the purely physicochemical explanation fails for the beginings, can it hold for the remainder of the putative evolutionary process?

The most widely promulgated origin of life paradigms (e.g., [8, 17 - 19]) and critiques of them [6, 19, 24, 36, 39, 42, 48, 55, 56, 60] have focused predominantly on the abiotic chemistry of protein and polynucleotide formation, the development of biologically productive energy transducing mechanisms, and molecular replication. Nonetheless, for both definitional and operational reasons, the evolutionary progression from primordial molecules to cell structure and abiotic chemistry to biochemistry also requires the containment and organizational principles afforded by a membrane interposed between the physical environment and evolving protoplasm.

MEMBRANE STRUCTURE AND PROPERTIES

Abiogenesis and the requirement for a membraneous partition

Morowitz [33] has appropriately emphasized the critical role of membrane structure in abiogenesis, indeed as a step necessarily *preceding* the emergence of functional genomes and metabolic pathways. Others, e.g. Darnell [8], posit membrane structure as a *terminal* step in protocellular evolution. This school of thought derives from recognition of the permeability barrier membranes would present while serving otherwise as facillitators of organization. In the opinion of Eigen et al. [12, p. 107] "Organization into cells was surely postponed as long as possible ... transporting things across (membranes) ... are tasks accomplished today by the most refined ... processes. Achieving analogous results in a prebiotic soup must have required fundamental innovations." Yet others (e.g., de Duve [10]) are more ambivalent about the timing of this event, but all are agreed on its significance.

It is posited [10] that sooner or later evolutionary progress would have become dependent on Darwinian selection. The precipitation of a membrane sequestering a genome (or molecules with that potential) would advantage the emergence and retention of co-issuants (enzymes, etc.). This higher order of structural complexity would have contributed immeasurably to the survival and propagation of that genome in its "competition" with otherwise similarly fortuitious chemical events not so sequestered in the pre-biotic realm, where innovative products would be lost to the surroundings. And is it not of such stuff that evolution is all about?

Thermodynamic considerations

Definably living systems operate at a distance far from thermodynamic equilibrium. They are pressured by degradative physicochemical events toward the equilibrium state. This follows from the Second Law generalization that all nonequilibrium structures at temperatures >0°Kelvin (see the Third Law) are subject to thermal, ergo organizational, decay. While cellular biology can survive desiccation and ultra-cryotemperatures, it does so in a dormant state. For the most part, biological systems operate in an aqueous (intracellular) environment at temperatures normally >0°C (exceptions, if that, include some arctic, antarctic and alpine forms). Much of the abiotic synthetic chemistry touted to date requires relatively high temperatures, i.e. >150°C (significant exceptions include the polymerization of nucleotides, optimal at ca. -21°C [32, 36]), certainly where reaction rates would be concerned (e.g., thermal peptides per [18], lipids per [22]). Accordingly, most origin of life scenarios are predicated on chemical evolution in a warm aqueous mileu [33], though the simulated syntheses are more often than not best achieved with *dry* heat. Indeed, some of the currently popular versions envision a geo- or hydro-thermically hot environment (e.g., submarine vents, fumaroles, steaming limnic springs, etc.) However, high ambient temperatures would have presented some formidible problems, especially with the synthesis and stability of biopolymers [32]. Moreover, where the essential molecular components are largely water soluble or hydrophilic, their distribution in an aqueous environment tends to be, or become, homogeneous in time. Hydrolytic reaction and dispersive diffusional rates would be exacerbated by high ambient temperatures (i.e., "hot soups"). But to effect coherent systems, the tendency must be concentrative, and if coherency is to be maintained, there must

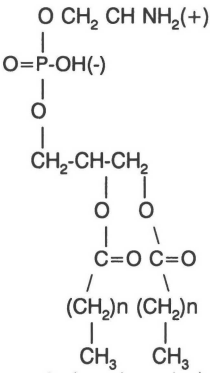
be a countermechanism to diffusion. This ultimately requires a phase separation from the environment. Yet the system must remain interactive with the environment. For growth, replication and any further differentiation to ensue, the system must be able to accrete from the environment the raw materials for synthesis and the requisite energy transductive functions.

Viewed by many evolutionists as rebutting the *pro forma* creationist argument from Second Law thermodynamics [57], there is, in effect, a Fourth Law (or perhaps more accurately an additional theorem), which states that the flow of energy from a source to a sink through an intermediate system "orders" that system [17, 33]; the order so derived is equivalent to increased "complexity" in Morowitz' [33] contextual definition of the term (Figure 2). For reasons detailed elsewhere [17, 33, and references therein] its theoretical conventions are not contraindicated by Morris' [34] predictions of *disarray* consequent such energy flow. And these conventions do have pragmatic significance to the evolutionary paradigm for cell origin. It is, for example, blind physical force - ultrasonics - which converts intially multilaminate liposomes to single membrane-bound vesicles [2, 52] (Figure 6). In any case, requisite the application of "Fourth Law" thermodynamics to proto-biology, the principle of heterogeneity must be operative. Energy flow is a necessary but not a sufficient condition in and of itself for establishing anything like biological organization. Indeed, Morowitz [33, p. 83-84] adds the caveat that "This principle of complexification [per Figure 2] follows strictly from the physical chemical properties of such systems and in no way demands biological organization." He continues, however, "It seems reasonable to conclude ... that such a principle is propaedeutic to the emergence of the biosphere."

Where initial conditions are homogenously aqueous, biphasic partitioning would be accomplished by an essentially non-aqeous intervening boundary. In practical terms, this is defined as a membrane comprised of amphiphilic molecules, i.e., of both hydrophilic and hydrophobic structure.

Composition and structure of amphiphilic membranes

In present day biology, the requirement for amphiphilic membrane structure is met by phospholipids, where their fatty acid hydrocarbon "tails" establish a hydrophobic interior domain and their polar "head" groups (represented by phosphate with or without hydrophilic polar organics) interface with the adjacent water milieu:



A phospholipid (1,2-diacyl-*sn*-glycero-3-phosphoserine)

These properties underlie the macrostructure of biomembranes as continuous lipid bilayers (Figure 3). The general concensus [8, 33] (but see [18] for exceptions) is that phospholipid bilayers, or lipoidal equivalents, also constituted the first plasma membranes. It is contended by proponents of this hypothesis that appropriate lipid precursors, including phospholipids *per se*, could have been present in an early earth "primoridal soup" [22, 33, and references therein]. It should be noted, however, that the very low water solubility of phospholipids makes the formation *de novo* of a phospholipid from water soluble precursors energetically difficult in aqueous media (experimental abiotic phospholipid synthesis involves evaporation in a hot sand or dry clay matrix, and subsequent incubation under desiccation, per Hargreaves et al. [22]). In present day cells, membranes proliferate by expansion of pre-existing membrane structure; phospholipids are synthesized in association with already extant bilayers in which the enzymology is bound or phospholipids synthesized elsewhere are incorporated therein immediately post-synthesis [4]. With very few exceptions, *omnis membrana e membrana*. Nonetheless, once formed - abiotically or otherwise - such lipids do, in fact, spontaneously form bilayered membrane structure [2, 49] (Figures 4-6).

Polypeptides, depending on their amino acid content and sequence, can be amphiphilic, a point stressed by Sidney Fox [18] in his argument for the feasibility of *protein* membranes as "first partitions". Folsome [16] has called attention to the oily film, presumably a hydrophobic polymer, that typically accompanies the formation of amino acids and oligopeptides in the Miller-type experiments. Fox [18, p. 130] asserts that his thermal protein (produced by heating amino acid mixtures to dryness) has "most of the (physical) properties of phospholipid". He moreover describes proteinoid microspheres as exhibiting "osmotic-like" swelling and shrinking, from which the existence of proteinoid membrane structure might also be inferred. Parenthetically, we note that analogous volume changes would be exhibited as well by a hygroscopic protein gel, *cf.* a semi-permeable membrane-enclosed vesicle *per se*.

The coalescence of amphiphiles into membraneous sheets is both predictable and demonstrable. For phospholipids in water, where G is the Gibbs free energy content of the system, DG for the formation of a bilayer structure is an arithmetically negative value (approximately -60 kJ/mole lipid [3] for both "free" phospholipids and those in micellar formations [30, 42]; the self-assembly of such lipids into membranes is therefore thermodynamically favored. It is also empirically observable, per the laboratory construction of liposomes (i.e., vesicles) and "black lipid membranes" (planar sheets) (Figures 4 and 6). The assembly of membrane structure per se from amphiphilic peptides is more inferential. See, nonetheless [50] and Figures 7 and 8. Fox [18] interprets electron microscope images such as Figure 7 as evidence of bilayered membrane structure (analogous to that formed by phosholipids) at the surface of proteinoid microspheres. However, for more variable-controlled systems, protein "membranes" (where such are actually formed) typically have the structure of a meshwork sieve (Figure 8), an interpretation not belied by Figure 7. There is also a porosity attendant the structure of polymeric gels (Figure 9) and even crystaline protein (Figure 10).

Absent other controlling factors, amphiphilic proteins do not form sheets but tend to a globular tertiary thence quaternary structure in an aqeous medium, with the hydrophobic sequences shielded by the hydrophilic. However, predominantly hydrophilic proteins form adsorbate layers onto planar substrates [47], as embodied in the now albeit obsolete model of biological membrane structure envisioned by Danielli and Davson [7] and Robertson [39]. Predominantly hydrophobic proteins become discrete particulate entities of membranes by insertion into their hydrophobic domains [44] per the currently accepted model of biological membranes as fluid (or "liquid crystaline") lipoprotein mosaics. There is also the potential for membrane-like protein comprised structure in sol/gel phenomena [37]. However, polymeric megamolecular gels exhibit a relatively large (micron-range) porosity and therefore relatively non-selective permeability for molecules in the size range (nanometers) of amino acids, nucleosides, sugars, etc.

Amphiphilic lipids thus remain the most plausible entities involved with "first membrane" evolution.

Membrane closure into a vesicle, an event most likely to occur when the membrane is a liquid hydrocarbon (i.e. lipid *cf.* protein-constituted), provides a tri-phasic system: exterior milieu, partition, and interior compartment. According to the evolutionary scenario, the exterior phase is the source of free energy (photonic, electromotive, and/or chemical) and the material precursors to proteins and nucleic acids; the interior becomes the sequestered microenvironment in which biologically determinative chemical reactions can occur; the partition becomes a barrier to dissipative diffusion (efflux) of reactants and products, developing thereby the critical mass for productive biochemistry. Meanwhile, the partition itself becomes a non-aqeous matrix space for the accretion of nonpolar solutes which, as detailed in Morowitz [33], could potentiate the energy transductive functions requisite to endergonic synthetic functions in the inner vesicular compartment, and possibly facilitate dehydrative condensation reactions. See also [17]. There remains the problem, however, of such membranes as barriers to accretive diffusion (influx).

Closed or open systems? The conundrum

In the formation of liposomes from initially dispersed phospholipids, the vesicles (Figure 6) demonstrably incorporate constituents of the medium into the resultant inner compartment, including, when present, proteins, nucleic acids and other molecular species of biological significance. This has had widespread biotechnological application, where liposomes have been used as delivery systems in genetic engineering, pharmacotherapy, etc. To that point, the scenario envisioned by Darnell [8] (Figure 11) is credible. However, Morowitz [33] contests the sequence of complex molecules first, then cells, from the unlikelihood that molecules needed by the biosphere (e.g., proteins, nucleic acids) would be provided by the geosphere otherwise (lithosphere, hydrosphere, atmosphere), where matter exists at the thermodynamically lowest lying combinations. He notes that whatever munificence there might be

forthcoming from nature is chemically parsimonious. Morowitz [33] argues persuasively that (p. 156) "... in the long run any molecules needed by the biosphere must be synthesized within the biosphere from thermodynamically low-lying input molecules"; thus (p. 154) "macromolecules (become) a consequence of evolutionary processes rather than requiring that they be prerequisite to such processes! (The) necessity of macromolecules early in the process has been a weakness of the various soup models." Yockey [60] references critically the "*myth* of the prebiotic soup", noting, e.g., that while *biologically* generated keragens (so diagnosed from their $^{12}C/^{13}C$ ratios) have been detected in the most ancient sedimentary rocks, there is no geological evidence whatsoever for *non-biologically* derived kerogens which would have preceded the emergence of the protobiont on the early Earth. In any event, the encapsulating of evolving biochemistry by a lipid bilayer presents a major stumbling block in the evolution of cells scenario. The empirically demonstrable impermeability of lipid bilayers to the hydrophilic organic and inorganic precursors (carbohydrates, amino acids, phosphate, etc.) [1, 15, 27, 40, 41, 45, 49, 53] (Figure 12) of productive biochemistry (notably protein and nucleic acid synthesis) would result in closed systems (i.e., closed to "building block" molecules), and any system in isolation (enclosed interim polymers) will decay to its lowest free-energy state (monomers, initially, and then their dissolution into simpler constitutents). This is not just a theoretical prediction, but follows the readily observed effect of 55M water (i.e. wholly aqeuous conditions) on the integrity of peptide and phosphodiester bonds, respective proteins and nucleic acids in aqeuous solution/suspension. The availability, at this stage of evolution (protocellular), of anything like the kinds of permeability modifiers discussed below (**The biological solution**), would be an altogether unrealistic assumption, though some students of this problem [10], have entertained this notion. It has also been conjectured [33], in the face of the the more realistic alternative of a purely lipoidal membrane, that the nonpolar inorganics to which purely lipid bilayers *are* permeable could serve as building matter for the abiotic assembly of polar organics within the vesicle's interior compartment. Note, however, that while lipid bilayers are permeable to the ostensible inorganic precursors to amino acids etc. (carbon dioxide, nitrogen, inorganic CN derivatives, etc.), the processes envisioned for this sort of abiotic chemistry (requiring relatively high levels of purely physical energy sources [32, 48, and references therein]) would obliterate the protocell. Requiring, on the one hand, a barrier to diffusion from within to without, which is admirably satisfied by a lipid bilayer, the same structure, on the other hand, imposes an insurmountable barrier to biochemically requisite diffusion from without to within. One way around the latter debacle would be to impose a partition structured along the lines of that shown in Figure 8, i.e. a protein microsieve, in effect a dialysis membrane which would retain macromolecules, at least, within the inner vesicular compartment. However, by observation, protein-comprised membranes of this sort are, given the large pore size, remarkably leaky and unselectively so to smaller entities such as amino acids, phosphate, etc. This property defeats the requirement for selective concentration (inside *v.* outside) of reactants, especially at the molar concentrations imperative to non-enzymatic mass action reaction rate kinetics (see, eg., net yields from artificial "soup" chemistry [17, 32, 33, 36, 48, 60 and references therein]. By the passive diffusion process (the only feasible permeation principle that could be envisioned at this stage of biogenic evolution), what "leaks in", "leaks out" with equal facility; any net movement follows the concentration differential. This would apply to permeants of either of the aforementioned protocellular membrane models. Thus, following Fick's law, the rate of flow (J) is a function of the solute diffusion (mobility) coefficient (D), membrane surface area (A), the magnitude of the concentration gradient ΔC and the distance the solute travels across the interfacial partition (x):

$$J = -DA \frac{\Delta C}{x} \tag{1}$$

the negative sign indicating that net solute movement is in the direction of the lesser concentration in the solvent space. As a kinetic energy expression, D = *w* RT (where *w* is solute motility); for electrically charged solutes, the above function (1) expands to the Nernst-Plank equation:

$$J = -w\Delta C \left(\frac{RT}{\Delta C} \frac{\Delta C}{x} + zF \frac{\Delta \Psi}{x}\right) \tag{2}$$

where z = the number of electrical charges (i.e., ion valence), F = Faraday's constant (96,000 coulombes/mol) and $\Delta \Psi / x$ = the charge gradient (voltage) across the membrane. The associated dissipation of energy, per the Gibbs' function, becomes

$$\Delta G = RT \ln \Delta C + z F \Delta \Psi \tag{3}$$

where ΔG is the free energy change involved in the move of 1 mol permeant across a non-restrictive interface.

If, in the protocell, the internal pool concentration of amino acids, etc., were (somehow) initially higher than the external concentration, the diffusion gradient would drive *efflux* . Note, it has been estimated that standing concentrations of putative organics in the "primordial soup" could have been no higher than micromolar [32, 33, 48 and references therein]. No less an authority on this subject than Stanley Miller himself has noted [32, p. 16] that "... an amino acid concentration of ... 10^{-4}M and an adenine concentration of about ... 10^{-5}M ... can be considered as a relatively concentrated prebiotic soup."

A variety of mechanisms to thwart the permeability barrier imposed by a lipid bilayer protocell plasmalemma have been proposed. These include the intervention of aldehydes to facilitate the excursion and subsequent internal concentration of amino acids by Schiff base formation [specific references in 33]. Reasonably low environmental pH (4-5) could repress the ionization of carboxylic acid groups rendering them more compatible with a non-polar partition [33]; likewise, at extraordinarily low pH (0-1), protons could partially neutralize even inorganic phosphate [17]. But how much more permeable pH *per se* would render such solutes, given the lipophobic properties of many amino acids, etc., otherwise, is debatable. There is to be considered as well the thermodynamic barrier to the diffusion of hydrophilics across hydrophobic domains that goes beyond ionization constants, *viz.* dehydration energies [14], which applies to amino acids and other solutes. The major potential energy barrier for a non-electrolyte (non-electrolytically active) molecule moving passively according to a chemical gradient includes the necessity for the permeant to lose its water of hydration as a condition of passing the polar barrier of the lipid groups [3, 14] as well as achieving solubility in, or compatibility with, the hydrocarbon (hydrophobic) constituents of the membrane.

However passaged, solutes would have to be retained. Internal trapping of amino acids (and other organics) by non-enzymatic inorganic phosphorylation has been suggested [33] (reaction (a) below), which, in the case of amino acids would concommintantly facilitate peptide synthesis [17, 33] (reaction (b) below). However, this would require, in our estimation, unattainably high reactant concentrations (in an aqueous milieu) of pyrophosphate, where, e.g., [17]:

Amino acid + Pyrophosphate ---> Aminoacyl phosphate + Phosphate (a)

Aminoacyl phosphate + Amino acid ---> Dipeptide + Phosphate (b)

Note that a system freely permeable to inorganic phosphate(s) presumably would be likewise permeable to Ca^{++}, resulting in the formation of frankly insoluble precipitates of calcium phosphate/pyrophosphate. Under such conditions, only trace quantities, at best, of pyrophosphate would be available in an aqueous milieu for the above reactions. While extant prokaryotes are known to trap permeant organics by post-transport phosphorylation [25], the complex biochemical mechanism (involving micromolar concentrations of reactant : product ratios, spacially organized enzymatically catalyzed and highly specified reaction sequences) is beyond credible expectation for an evolving protocell.

Getting around the problem of vesicle impermeability at the outset, Blobel [5] envisions the evolution of protocells from initially *inside-out* liposomal vesicles (Figure 13), a process however which fails to accommodate Morowitz's trenchant observation concerning the limited munificence of the abiotic geosphere [33]. In particular, it does not solve the problem of diffusional loss of products (or achieving sufficient reactant concentrations) in structurally "open" systems, and only begs the lipid membrane permeability problem, since this would apply once the vesicles turned right-side in. Meanwhile, the mechanism for the remarkable turn of sidedness illustrated in Blobel's model is not intuitively obvious. Its major virtue, if that, is its conjecture as to how the brute structure of gram negative bacteria (with their two membranes) (Figure 14) - putatively the evolutionarily first kinds of prokaryotes - might have developed from a "cell" initially structured of a singular membrane. A critique of that hypothesis is beyond the scope of the present paper.

The biological solution

Per deDuve [10, p. 203] "*Unless* we subscribe to special creation, we *must assume* that, where life started, local conditions were adequate to overcome the concentration problem" (emphasis added). deDuve of course is not advocating creationism, but, at this point, the reader may wonder if special creation is not the better scientific explanation afterall. An assumption forced by the thesis then used as the best *evidence* for the thesis is not inherently scientific protocol. What perforce gainsays the alternative assumption? Only one's world view? But that's not science, or so we are told, irrespective of its alleged

basis. Should *ad hoc* presumptions force a *scientist* to an inescapable inference of evolutionary processes as fact?

The plasmalemma of most extant cells is of course both selectively permeable and concentrative respective the cell's biochemical and physiological requirements. Exceptions include the remarkably leaky membranes of some of the intracellular parasites (e.g., mycoplasmas, rickettsiae), on the one hand, and the insulative (electrolyte impermeable) membrane which comprises the myelin sheath of neuron axons, on the other. In addition to phospholipids, naturally occurring plasma membranes are invested to one degree or another with a variety of highly specified proteins which comprise the structure of highly selective permeant channels. (Germane to the present observations, we note that the notorious impermeability of myelin - the plasma membrane of periaxonal Schwann cells - coincides with its remarkably high lipid/low protein ratio of ca. 4-6 : 1 [52]; for plasma membranes otherwise, the lipid : protein ratio is ca. 1 : 1). These proteins take the form of more or less static pores (those, e.g., at "gap" or nexus junctions and the "porin" complexes of bacterial cell envelopes) (Figures 15 and 16) and conformationally variable channels and kinetic "carriers" (Figures 17-19). The latter, apropos of the term "permeases", are both catalytic, respective the rate kinetics of transport, and highly specific respective their solute interactions; some are vectorally specific independently of the solute concentration gradient (the phenomenon of "active transport"). Their molecular structures, ergo functional properties, are rigorously specified by genetic information *per se* and its transcription/translation *viz.* nacent protein synthesis and a variety of post-translational regulatory factors (see, e.g., the role of chaperones [13]).

To surmise that equivalent entities could have formed abiotically, *sans* informational determinants, concommitant with or prior to the first lipid bilayers in the course of protocellular evolution begs for nothing short of a miracle. de Duve [10, pp. 100-101] nonetheless offers that "The most likely (ancestral membrane) ... (would have) consisted (simultaneously) ... of lipids *and* proteins ... (the latter) ***must*** have included a minimum set of transport systems to maintain an adequate intracellular milieu and to mediate the necessary exchanges of matter between the cell and its environment" (emphasis added). While descriptive of the *need*, such statements do not address the *plausibility* of spontaneous occurrence or provide any *evidence* for it (*cf.* the "inescapable inference"). The bane of any theory is the *ad hoc* hypothesis - in this instance, the inexplicable simultaneous co-development of lipid membranes and selective protein permeases. When a hypothetical phenomenon is both implausible and undemonstrable, can it remain a scientific principle? Or does it become a matter of faith? The always germane theroretical constraints [57, 60] momentarily aside, there are formidible *physical* limitations that impact de Duve's account. Proteins serving as permeability modifiers must first of all be integrated into the matrix of the bilayer (Figure 20); i.e., they are integral (intrinsic) membrane proteins *cf.* extrinsic (peripheral) membrane proteins [44, 52]. Accordingly, their lipid-interfacing external surfaces must be at the outset to the largest extent hydrophobic [14]. And, empirically, so they are [20]. By observation, such water immiscible proteins tend to precipitate and denature in an aqeous environment; hence the laboratory requirement for detergents in their extraction, stabilization, etc. How, then, could such proteins originate spontaneously in an aqueous "soup"? Note, in extant cells, this problem confronting the abiogenicists is solved biologically by the mechanisms illustrated in Figures 21-23 [13, 21, 54]. While perhaps accommodated by Blobel's model (Figure 7), the flaws in reasoning for that model, discussed above, render it untenable in the first place. Otherwise, the necessary transitional conformations are not accomodated by sheerly stochastic processes [20].

Extant proteinaceous pores and permeases tend to be multi-subunit arrays with molecular weights for the polypeptides in the 10's to 100's of kilodaltons and higher [23, 40, 46], and have structurally complex colateral associations with other membrane constituents. From the engineering point of view, they are highly sophisticated machines. Conjuring the existence of simpler, i.e., smaller and less selective permeability-modifying entities analogous to present day antibiotic peptide ionophores or functionally equivalent other kinds of molecules (e.g., macrotetrolides, cyclic polyethers, trialkyltins, etc.) [14, 23, 40] is fallacious. As such initially exogenous modifiers are known, they are functionally limited to affecting the diffusional excursion across lipid bilayers of small ions (e.g., Na+, K+, H+, Cl^-, OH^-), ordinarily not larger ionorganics (e.g., phosphate, pyrophosphate) or organic molecules. A possible exception to this principle is the relatively small (albeit 8 kd) polypeptide component of the otherwise complex phosphate transport system in mitochondria, and perhaps ionophores like A23187 and beauverein which have a high affinity for divalent cations. Since these ionophores nonetheless operate according to the diffusional kinetcs shown in equations (1) and (2) above, the requisite co-parameter of concentrative absorption (or exclusion) is precluded (hence their physiological effect - of collapsing pre-existing gradients - as antibiotics). The net result of these kinds of modifiers, absent other principles, is equilibrium (a chemical definition of death, not life!).

When viewed independently of one another, plasmalemmal proteins integrate the cell with its environment, while the lipids, re: permeability, passively isolate. These principles are not working at cross purposes, however. Plasmalemmal lipids ensure that the selective permeability afforded by protein-specified conduits is not overridden by non-selective permeability elsewhere in the interfacial structure between interior and external compartments. This is a function that notably fails the protein-first models (i.e., passive sieves) for protocellular evolution addressed above.

Equally important to the biologically productive properties of cell surfaces are their para-investments, *viz.* the ubiquitous glycocalyx (Figure 3). Noting that phospholipid bilayers, amphiphilicity nothwithstanding, are sparingly hydrophilic at best [52], this dominantly carbohydrate-comprised (hence highly hydrophilic) structure [38, 43, 58] endows cells with their especially important characteristic of surface "wetability". For discussion, see [26, 28, 29, 58]. The glycocalyx, as a composite of highly specified peptidoglycans and glycolipids, adds yet another dimension to the functional and structural complexities challenging the evolutionary paradigms.

CONCLUSION

In sum, the inordinate complexity, demonstrable specificity, and low probability of naturally occurring plasma membranes are features daunting to current evolutionary explanations of their origin by stochastic processes. The adequacy of the more simplistic, ergo physicochemically feasible, versions contemplated for the protocell falls well short of their patent requirements. The speculation of more elaborate models at the outset defies the principle of Ockham's razor, not a trivial failing of reason, and imposes on these models an inexplicable release from a number of real physicochemical constraints. Meanwhile, the shortcomings of the most plausible evolutionary scenarios are heuristic to the conclusion drawn here from the existing data. Indeed, the most reasonable explanation for the existence of the plasma membrane, ergo cells, ergo life, is the evolutionist's anathema - deterministic, intelligent design. High complexity, high specificity, and low probability of spontaneous occurrence are the hallmarks of engineered systems, not happenstance phenomena. While he is certainly ill-disposed to the creationist view *per se*, Hubert Yockey, who is among the leading contributors to information theory in molecular biology for nearly three decades, has concluded, and we concur, that "The currently accepted ... (evolutionist origin of life) ... scenarios are untenable and the solution to the problem will not be found by continuing to flagellate these conclusions" [60, p. 289]. Conjecture, the power of imagination and rank suggestion notwithstanding, the Emperor has no clothes.

ACKNOWLEDGEMENTS

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FIGURES

Abbreviations: AMb, ammonium molybdate; Os, osmium tetroxide; PTA, phosphotungistic acid; SEM, scanning electron micrograph; TEM, transmission electron micrograph; UAc, uranyl acetate

Figure 1. Biogenesis as an evolutionary process. Courtesy of Dr. Harold Morowitz [33].

Figure 2. Complexification of systems as a function of energy flow. Courtesy of Dr. Harold Morowitz [33].

Figure 3. TEM of a plastic embedded thin section of a plasma membrane (tegument plasmalemma of *Moniliformis dubious* [27]), resolving the bilayer (here ca. 12 nm thick) and filamentous elements of the glycocalyx (arrows).

Figure 4. TEM of a plastic embedded thin section of an artificial lipid bilayer (formed from phosphatidyl ethanolamine in n-decane per the method of [49]), fixed in lanthanum nitrate and potassium permanganate; electron opaque "lines" reflect position of polar head groups and olefin (C=C) groups which have bound the metal fixatives; the intervening hydrocarbon chains do not react with these fixatives and remain electron transparent. The thickness of this membrane is ca. 7 nm.

Figure 5. Schematic interpretation of Figure 4.

Figure 6. TEM of liposome vesicles formed from phosphatidylcholine per the method of [2], negatively stained with PTA.

Figure 7. Os-fixed proteinoid microspheres, TEM of plastic embedded thin section, courtesy Dr. Sydney Fox [18]. Where the plane of section becomes tangential to the surface, there is a suggestion of pores (arrows); the original interpretation [18] is otherwise that of a bilayer.

Figure 8. TEM, *en face* view of a proteinaceous (albuminoid) "membrane", prepared according to [50] and fixed in Os vapor, showing sieve-like microstructure with a pore size ranging between ca. 4 - 10 nanometers.

Figure 9. SEM of a polymeric "megamolecular" gel; the larger pores have a diameter of ca. 1 micrometer.

Figure 10. TEM of crystaline protein (catalase), AMb negative stain; electron opaque spaces between structural units (transparent) are ca. 7 - 10 nm wide.

Figure 11. Pre-biotic formation of the primordial cell according to Darnell [8]; **a**, the "soup" of abiogenically derived amino acids, nucleotides, oligo/polynucleotides and peptides; at center, amphiphilic lipids are assuming a bilayer configuration around constitutents of the soup, which includes a potential RNA-type genome; **b**, the liposomal "cell" so formed.

Figure 12. Relative permeability of a phospholipid bilayer to water and various organic and inorganic solutes (see text for references); A, H_20; B, glycerol; C, amino acids; D, monosaccharides; E, purines and pyrimidines; F, Cl^-; G, K^+; H, Na^+; I, phosphate, Ca^{++}, and nucleotides. Note, the scale for the comparisons of permeability coefficients (ordinal values) is in *negative* orders of magnitude. The actual rate of flow across the membrane (mol/sec/cm^2) would be a function of the concentration difference (dmol/cm^3) on the two sides of the membrane multiplied by the permeability coefficient (cm/sec). For the amino acid tryptophan, e.g., at a concentration difference of 0.1 mM, its transmembrane diffusion rate would be 10^{-11} mol/sec per cm^2 of membrane surface area.

Figure 13. Pre-biotic formation of the primordial cell according to Blobel [5]. From left to right, a liposome has adsorbed already evolved ribosomes, other macromolecules (proteins and nucleic acids developed in the "soup") to its external surface; surface bound material undergoes nonrandom arranging, with the generation of a concave plane; continued involution to a "double membrane" bound semi-closed vesicle; fusion at the "orifice" produces a structure analogous to a gram-negative bacterium.

Figure 14. TEM of a thin-sectioned, gram-negative bacterium (*Escherishia coli*), showing its two membranes; courtesy Dr. Wouter van Iterson and Bacteriological Reviews.

Figure 15. TEM of protein particles with the ultrastructure of a pore isolated (by detergent extraction) from membranes of the electric organ of *Torpedo californica*, negatively stained with phosphotungstate; magnification (inset) ca. 1 million X (micrograph by J. Telford, courtesy Dr. A. Schamoo and the NYAS); these pores function as Na+ permeation channels.

Figure 16. The diagrammatic interpretation of the kind of pore shown in Figure 15; water-filled channels for the passage of ions and hydrophilic organics are formed by groupings of protein subunits.

Figure 17. TEM (courtesy Dr. John Oaks) of a cryofixed, freeze-fractured and etched microvillous "brush border" membrane preparation of the *Hymenolepis diminuta* tegument [28, 29]. This membrane is dedicated physiologically to nutrient absorption functions and accordingly contains a wide variety of transport sites for amino acids, nucleosides, sugars, etc.; note numerous particles embedded in the continous lipid matrix; original magnification is ca. 530,000X.

Figure 18. TEM of a thin section of the *H. diminuta* tegument membrane (Figure 16), cryofixed, freeze-dried, negatively stained with Os, and embedded in plastic [29]. A putative transport carrier complex in longitudinal section is demarcated at the arrows, where two subunits and the intervening aperture are resolved; a cross-sectioned carrier is seen at X; original magnification is ca. 4 million X.

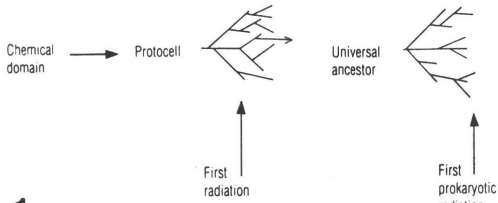
Figure 19. The diagrammatic interpretation of the structure shown in Figure 18; **a**, binding of the permeant solute to an "active site" triggers a kinetic conformational change in the structure of the protein subunits opening a permeation channel to the opposite side of the membrane (**b**). See [31, 44].

Figure 20. Integral (intrinsic) protein particle within the lipid bilayer of a membrane.

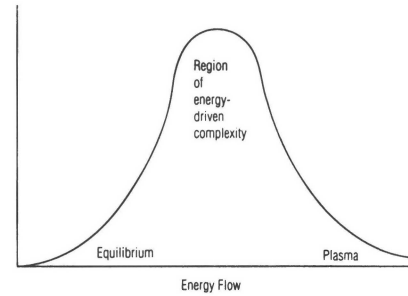
Figure 21. TEM of an isolated L cell plasma membrane with adherent ribosomes [21] (courtesy Dr. Marion Glick and Raven Press); such *in vitro* preparations incorporate ^{14}C-amino acids into membrane proteins.

Figure 22. The interpretation of Figure 21.

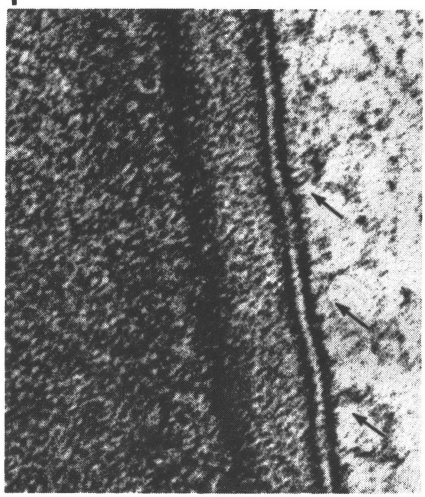
Figure 23. An alternative mechanism, where membrane protein synthesis occurs on cytosolic ribosomes; the peptide chains are complexed with "chaperones" which provide them a configuration and surface presentation compatible with the initially aqueous mileu and facilitate their incorporation into the membrane proper. See text for references.


Chemical domain
Protocell
Universal ancestor
First radiation
First prokaryotic radiation

1


Complexity measure
Region of energy-driven complexity
Equilibrium
Plasma
Energy Flow

2



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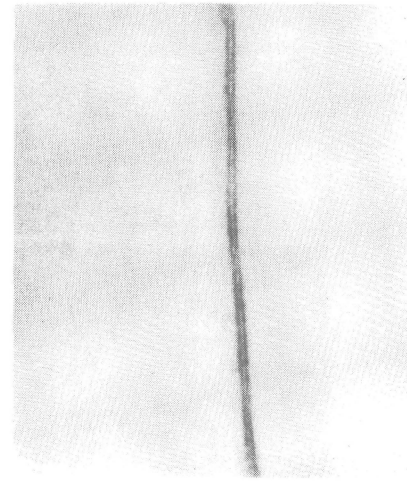

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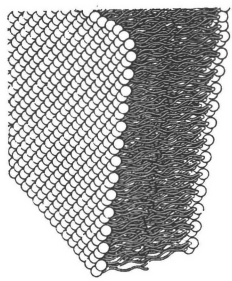

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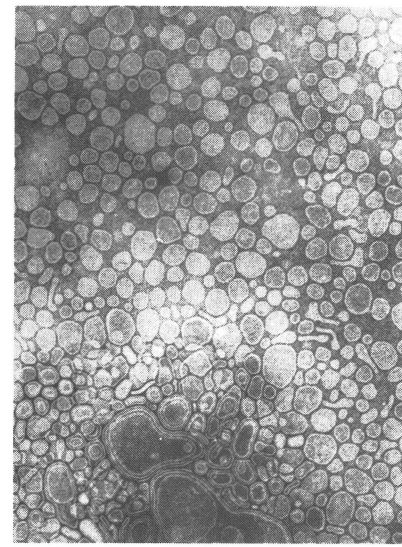

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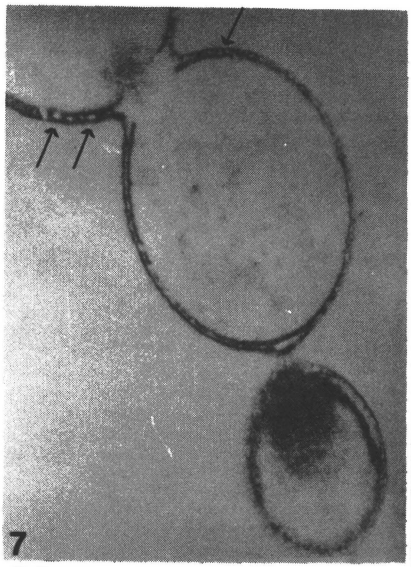

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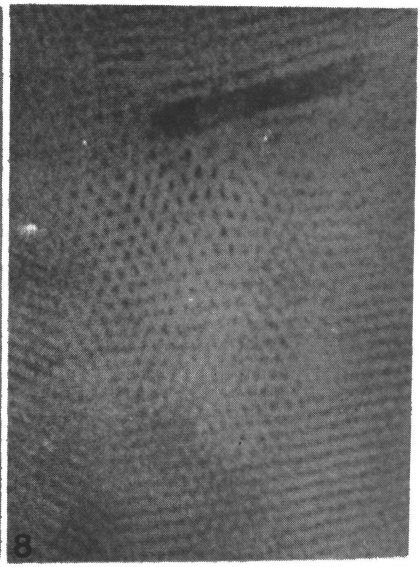

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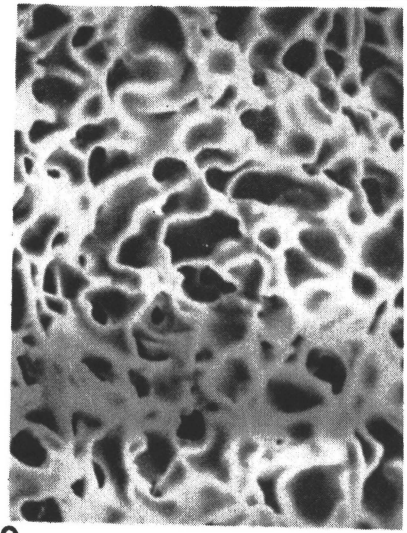

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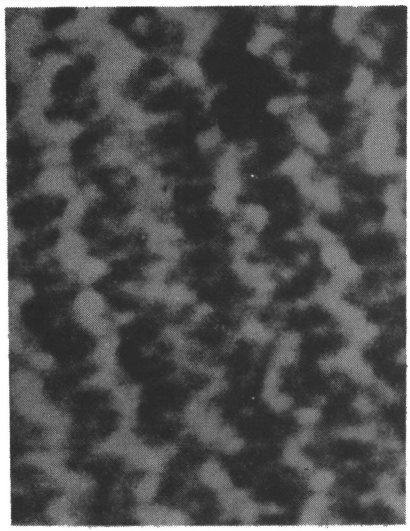

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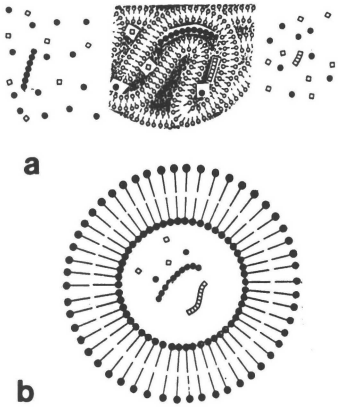
a
b

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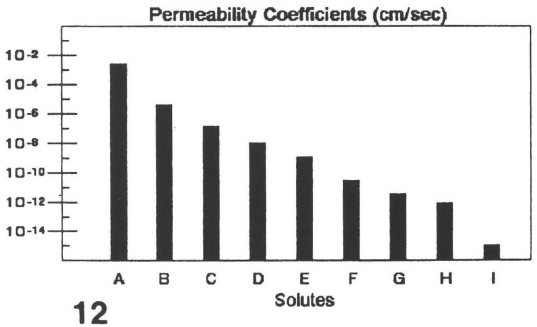
Permeability Coefficients (cm/sec)
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10-6
10-8
10-10
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A
B
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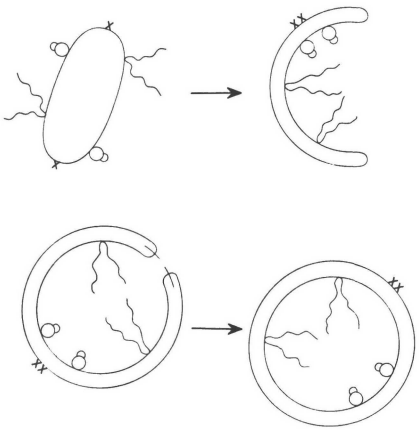
G
H
I
Solutes

12



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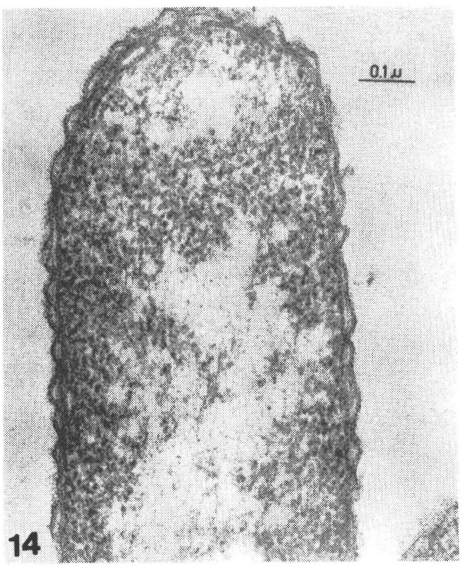
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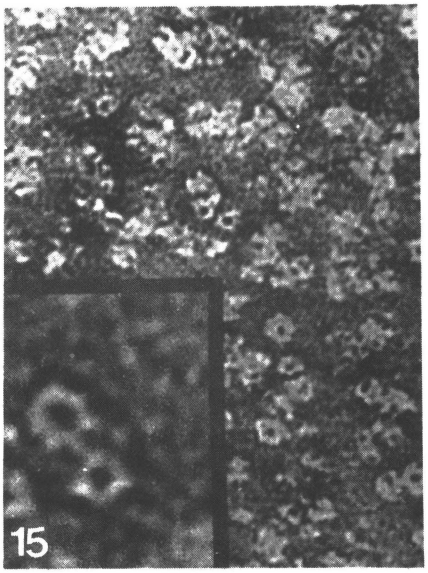

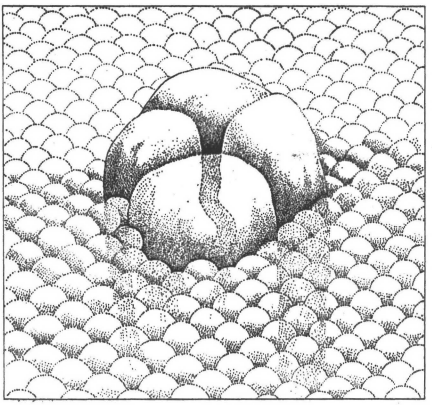

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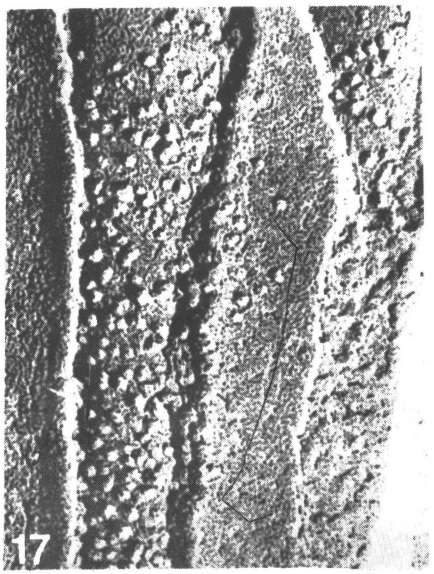
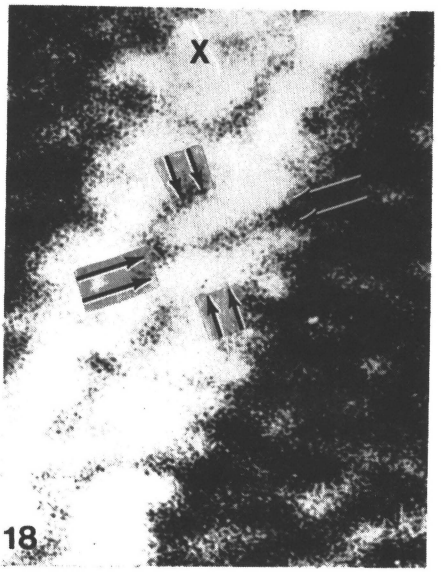
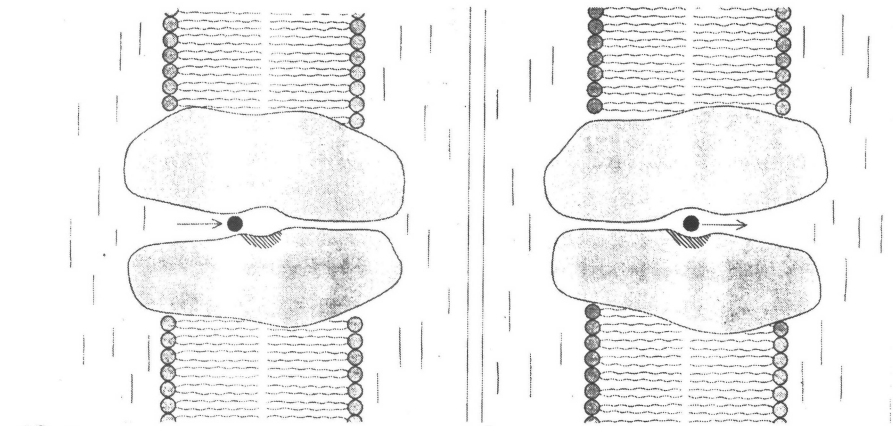
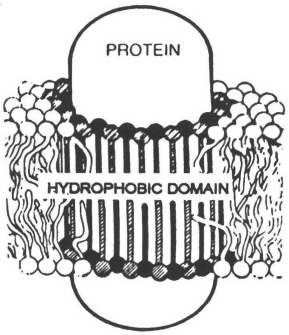


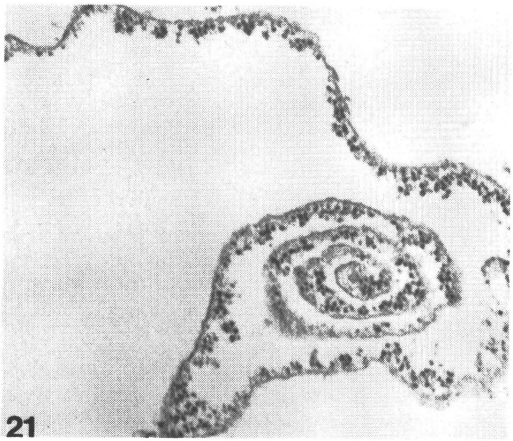




19 a b


PROTEIN
HYDROPHOBIC DOMAIN

20



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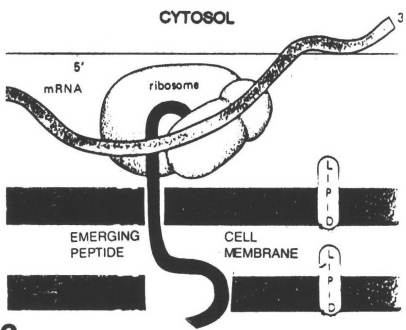
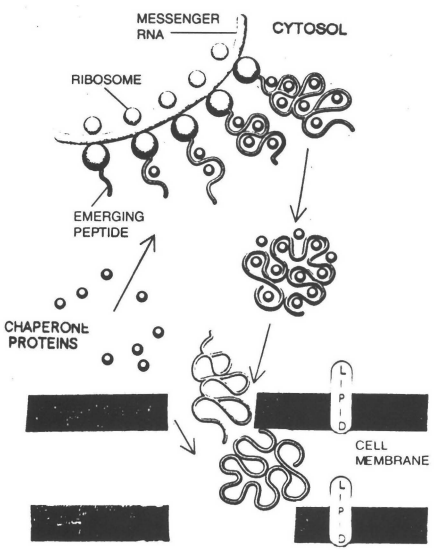

CYTOSOL
3
5'
mRNA
ribosome
LIPID
EMERGING PEPTIDE
CELL MEMBRANE
LIPID

22


MESSENGER RNA
CYTOSOL
RIBOSOME
EMERGING PEPTIDE
CHAPERONE PROTEINS
LIPID
CELL MEMBRANE
LIPID

23