

A Hydrothermally Precipitated Catalytic Iron Sulphide Membrane as a First Step Toward Life

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Abstract. We propose that life emerged from growing aggregates of iron sulphide bubbles containing alkaline and highly reduced hydrothermal solution. These bubbles were inflated hydrostatically at sulphidic submarine hot springs sited some distance from oceanic spreading centers four billion years ago. The membrane enclosing the bubbles was precipitated in response to contact between the spring waters and the mildly oxidized, acidic and iron-bearing Hadean ocean water. As the gelatinous sulphide bubbles aged and were inflated beyond their strength they budded, producing contiguous daughter bubbles by the precipitation of new membrane. $[\text{Fe}_2\text{S}_2]^{+0}$ or $[\text{Fe}_4\text{S}_4]^{2+/+}$ clusters, possibly bonded by hydrothermal thiolate ligands as proferredoxins, could have catalyzed oxidation of thiolates to disulphides, thereby modifying membrane properties.

We envisage the earliest iron sulphide bubbles (pro-

botryoids) first growing by hydrostatic inflation with hydrothermal fluid, but evolving to grow mainly by osmosis (the protocellular stage), driven by (1) catabolism of hydrothermal abiogenic organics trapped on the inner walls of the membrane, catalyzed by the iron sulphide clusters; and (2) cleavage of hydrophobic compounds dissolved in the membrane to hydrophilic moieties which were translocated, by the proton motive force inherent in the acidic Hadean ocean, to the alkaline interior of the protocell. The organics were generated first within the hydrothermal convective system feeding the hot springs operating in the oceanic crust and later in the pyritizing mound developing on the sea floor, as a consequence of the reduction of CO, CO₂, and formaldehyde by Fe²⁺- and S²⁻-bearing minerals.

We imagine the physicochemical interactions in and on the membrane to have been sufficiently complex to have engendered auto- and cross-catalytic replication. The membrane may have been constructed in such a way that a “successful” parent could have “informed” the daughters of membrane characteristics functional for the then-current level of evolution.

Key words: Botryoid — Chemiosmosis — Ferredoxin — Greigite — Hydrothermal — Iron sulphides — Membrane — Origin of life — Protocell — Proton motive force

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Glossary: Hollow pyrite botryoids: hollow hemispheres of cryptocrystalline pyrite (FeS₂) 0.1–1 mm across. Fischer-Tropsch syntheses: the highly exothermic catalytic hydrogenation of CO to hydrocarbons and aliphatic oxygenated compounds using finely divided iron. Greigite (Fe₃S₄): metastable iron sulphide precipitated from aqueous solution in a gel at 100°C and containing two-thirds of its iron as the high-spin ferric ion. Haber-Bosch process: the exothermic catalytic hydrogenation of nitrogen to yield ammonia. Probotryoid: a hydrostatically inflated colloidal iron monosulphide bubble; precursor to hollow botryoids and the progenitor to protocells. Proferredoxins: $[\text{Fe}_2\text{S}_2]$ and $[\text{Fe}_3\text{MS}_4]$ clusters (M = Fe, Mo, W, Ni, etc.) ligated by abiogenic thiols and thiolates. Protocell: a cell comprised mainly of abiogenic organics including thiols with subordinate iron sulphides, partly as proferredoxins; growth results from catabolism and osmotic pressure

Introduction

Cairns-Smith (1982) has emphasized the difficulty of imagining how organic compounds of any description

could have been generated and concentrated to compose the first forms of life. So instead of organics we suggest that iron sulphide membranes, precipitated on the deep sea floor at the interface between sulphidic, alkaline, and highly reduced hot spring waters and the acidic, mildly oxidized, iron-bearing Hadean ocean, formed the first reproducing “probotryoids” (iron sulphide bubbles). It was on these fresh membranes that such organics as there were became concentrated by adsorption, so protecting them from dissolution or oxidation. In this hypothesis metabolism was preceded in evolutionary terms by the hydrostatic inflation of the membrane comprising the “probotryoids” with the buoyant hot spring waters seeking to exhale into the Hadean sea (Russell et al. 1988, 1989, 1993).

Free energy in this far-from-equilibrium system is given by the contrast in the chemistries of the alkaline hydrothermal fluid and acid ocean water and their separation by the semipermeable composite membrane proposed here. Step-wise evolution in chemical complexity would have resulted from the growth and development of the successful membrane over millions of years. We can think of such a membrane as a flexible catalytic surface, partially hydrated when formed, but dehydrating and thickening with time. One advantage of this process of growth is that the hydrothermal solutes, trapped as they are within the iron sulphide bubbles, are always administered directly to where the inflating membrane weakens so that new probotryoids are generated over, and contiguously with, the parent probotryoid. Compare this contiguity with the limitations of crystal growth where, although information is transferred by the conformable copying of the stacking sequence from one structural layer to another, crystallization is terminated as (1) other similar crystals are intersected, (2) surface energy is lowered, or (3) crystal faces are “poisoned.”

The hypothesis presented here seeks to explain the emergence of life as an expression of the electron flow during gradual oxidation and loss in energy of our planet as hydrogen is freed from compounds and escapes to space. It is influenced and inspired by aspects of other hypotheses and knowledge: the suggestions of Leduc (1911) that inorganic osmotic membranes comprised the boundaries to the first living cavities and that sulphur may have been implicated in this encapsulation (the sulphobes of Herrera 1942); the affinities of organic surfactants for sulphides established in the minerals industry (Wark and Wark 1935; Leja 1982); the abiogenic generation of such organics in the Earth’s crust (e.g., Degens 1979); the significance in “prebiology” of the separateness of H^+ and OH^- (Williams 1961) and of the *dislocation* of activated reagents in space (Williams 1965); the antiquity and ubiquity of the iron-sulphur proteins recognized by Hall and his co-workers (1971, 1974) and the idea that earliest life was based on iron/sulphur chemistry (Hartman 1975); the anastro-

phic supramolecular organization that results in far-from-equilibrium conditions (Nicolis and Prigogine 1977; and see Kauffman 1993); the fact that submarine hydrothermal springs provide such conditions (Corliss et al. 1981); the significance of surfaces in catalysis including iron sulphides (Cairns-Smith 1982; Wächtershäuser 1988a, 1992); leading finally to the possibility of a simple evolutionary biogenesis (Morowitz et al. 1991; de Duve 1991).

Wächtershäuser (1988b, 1992) sees the exergonic formation of pyrite from hydrogen sulphide and ferrous sulphide as the first energy source for the fixing of carbon. In this vein Koch and Schmidt (1991) have argued that such energy, provided by the oxidation of hydrogen sulphide to provide protons, could be transduced (as electrons) across a primitive organic cell membrane to produce the first proton motive force. While we see the Wächtershäuser “pyrite-pulled” mechanism (albeit with both CO and CO₂ as carbon sources) possibly augmenting the abiogenic production of particular organic molecules in the consolidating hydrothermal sulphide mound (Fig. 1), our proposal contrasts with that of Koch and Schmidt (1991) as we argue that energy was provided by a natural proton motive force, already in place, a consequence of an acid ocean interfacing an alkaline hot fluid through a precipitating membrane. The “RNA world” (Crick 1968; Orgel 1968; Gilbert 1986; Joyce 1989; Orgel and Crick 1993) was, we believe, a later evolutionary development.

Below we outline a simple model for the emergence of life from probotryoids which evolve to aggregates of protocells up to 1 mm across that share a contiguous membrane with all their forebears.

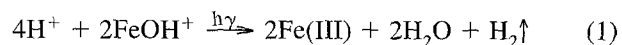
Initial Conditions

Although details of the environmental conditions that pertained during the Hadean are uncertain, some generalizations may be made. Assuming that the Earth’s upper mantle was somewhat more reducing than that presently maintained by the quartz-fayalite-magnetite (SiO₂-Fe₂SiO₄-Fe₃O₄) buffer (Arculus and Delano 1980; Kasting et al. 1993), the atmosphere at 4 Ga probably comprised ~10 bars of CO₂ and significant CO, as well as water vapor, N₂, some SO₂, and possibly traces of formaldehyde (H₂CO), CH₄, and NH₃ (Pinto et al. 1980; Walker 1985; Kasting et al. 1993). The iron-bearing minerals and complexes in the Earth’s lithosphere and hydrosphere would have been beginning to oxidize, mainly as a result of the thermal, electrical, and photolytic dissociation of water and hydroxyl, so the atmosphere would also have been host to transient hydrogen as it escaped the gravity field. There would have been a form of ocean floor spreading, but whether that floor was composed of basalt or the more mafic komatiite is not certain (Arndt 1983; Nisbet 1984, 1987;

Bickle 1986). Springs of superheated ($\sim 400^\circ\text{C}$) water broadly comparable to present-day black smokers (Edmond and von Damm 1983), i.e., powered by igneous intrusion, would have featured at oceanic spreading centers as well as at zones of ocean floor destruction. Land would have been ephemeral at these plate margins (Smith 1981; Moorbath 1985). Away from these margins, on the 4-km-deep ocean floor, sediments would have been thin and the high heat flow from the Earth's interior in those times would have driven about a million medium-temperature ($<200^\circ\text{C}$) springs at spacings of about 7 km (cf., Fehn and Cathles 1986).

Our favored site for membrane growth is at sulphide mounds developing at these hot ($150\text{--}200^\circ\text{C}$) springs on the deep Hadean ocean floor. The *initial conditions* were satisfied at some of these long-lived, stable hydrothermal springs; specifically at the interface between an iron-deficient, HS^- -bearing and thiolate (RS^-)-bearing, alkaline ($\text{pH} \sim 8$), reduced ($E_h \sim -0.5\text{ V}$) hot spring water at $150\text{--}200^\circ\text{C}$, and an iron-bearing, mildly oxidized ($E_h \sim -0.1\text{ V}$) and acid ($\text{pH} \sim 5$) ocean (Macleod et al. 1994; Grotzinger and Kasting 1993) (Fig. 1). Exhalation of such a hot alkaline solution into an acid ocean results in some significant effects. For example, as solubilities of many complexes (e.g., bi- and polysulphides) are much greater in alkaline solution, colloidal membranes are precipitated on acidification (Fig. 2). Perhaps traces of tungsten and molybdenum were introduced to the membrane in this way, i.e., as $[\text{WS}_4]^{2-}$ and $[\text{MoS}_4]^{2-}$.

For life's beginnings solar photons may have been an important, but indirect, energy source additional to thermal and chemical (free) energies. The oxidative photolysis of Fe^{2+} , derived from black smokers and dissolved in seawater, to FeOOH , could have provided the ultimate electron sink for prebiotic oxidation processes in the oceans as electrons were lost to space *via* hydrogen (Braterman and Cairns-Smith 1987):

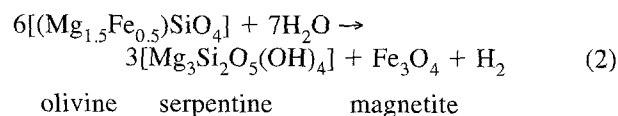


The Fe(III) produced will react with water to produce an iron oxyhydroxide sol. The ocean may have supplied ferric hydroxide for incorporation by sulphidation into the developing mixed valence iron sulphide membranes. At the same time an organic component in the ocean, scavenged by the ferric hydroxide, could have been brought to the membrane (Tatsumoto et al. 1961; Valentyne 1965; Collingwood et al. 1988). Another, or an alternative, source of Fe(III) would have been from the dissolution of magnetite in the high-temperature, acidic black smoker systems mentioned above (Seyfried et al. 1991).

Other possible electron sinks may have been sulphate or sulphite, and H^+ . Solar photons could also have generated prebiotic formaldehyde (H_2CO) from

CO_2 , CO , and water vapor, to be rained out into the oceans where it may have achieved concentrations of at least 100 ppm (Pinto et al. 1980). Formaldehyde is a compound of some potential in the building of more complex organic molecules (Reid and Orgel 1967; Cairns-Smith 1982; Cole et al. 1994).

We are confident of the alkaline nature of the off-spreading center hydrothermal solutions for there are modern examples of such springs (and groundwaters) in ultramafic rocks, rocks with compositions approximating those that could have comprised the Hadean ocean floor (Barnes and O'Neil 1969; Barnes et al. 1978; Neal and Stanger 1984). There is also experimental support for the concept (Janecky and Seyfried 1986). Moreover, theoretical rock:water thermodynamic modeling of such systems predicts aqueous fluids which, below 300°C , are invariably alkaline and reducing (Macleod et al. 1994). But the likely abiotic organic content of this fluid is unknown. On theoretical grounds Shock (1992), purposely conservative, suggests a tenor of around 1 ppm, although the overall concentration of abiotic organics was likely to have been higher, as we shall see. Reduction of CO and CO_2 to organics during geothermal convection of hydrothermal solution through the Earth's crust would have been concomitant with the oxidation of Fe^{2+} in silicates (olivine and pyroxene), sulphides (e.g., pyrrhotite— $\text{Fe}_{(1-x)}\text{S}$), wüstite (FeO), and, in places, residual native iron and iron-nickel alloys. (See Hall 1986). Hydrogenation and other reductions could have been catalyzed by both the sulphides and oxides, but most effective in this regard could have been filaments of awaruite (FeNi_3), which is common in such rocks (Ulrich 1890; Krishnarao 1964; Neal and Stanger 1984). So the organic compounds capable of acting as ligands for the iron sulphides may have been provided, at least in part, by these Fischer-Tropsch-like reactions within the free hydrothermal convective systems operating in the magnesium silicate and sulphide rich crust of the early Earth (Ferris 1992; Shock 1992) (Fig. 1). Certainly hydrogen would have been available for hydrogenation as a consequence of the well-established ease of oxidation of mafic rocks by water (Neal and Stanger 1984; Coveney et al. 1987; Peretti et al. 1992):



Also, given the increase in solubility of nitrogen with pressure, Haber-Bosch-like reactions for generating ammonia and hence amines are possible (and see Cole et al. 1994). Moreover, the experiments of Hennet and his co-workers (1992) indicate that certain amino acids could have been produced as metastable phases in

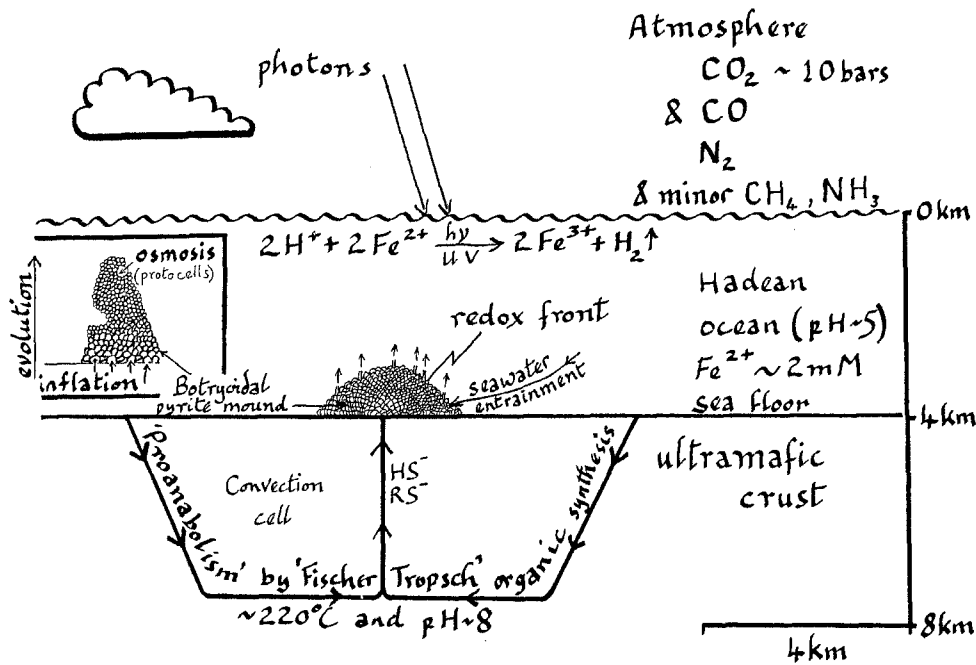


Fig. 1. Model environment for the emergence of life as contiguous iron monosulphide probotryoids on the 4-km-deep ocean floor four billion years ago above a convection cell involving modified Hadean seawater. Note that the mound and probotryoids are not to scale. *Inset* shows the evolution of probotryoidal FeS aggregates with only a minor organic content where growth is primarily driven hydrostatically,

through to protocells where the drive is osmotic and the membrane is mostly organic with the FeS sequestered. The hydrothermal spring operates for 1–10 million years. (See Russell and Skauli 1991.) If the system is considered overall then life began autotrophically; if only the probotryoids are considered then life emerged heterotrophically.

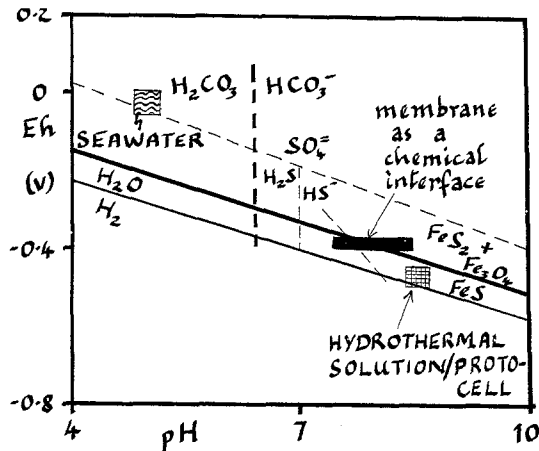


Fig. 2. An Eh-pH plot of the putative Hadean (4-billion-year-old) seawater and hydrothermal solution demonstrating the iron monosulphide membrane as a chemical interface (redrawn from Macleod et al. 1994). The oblique dashed line through the membrane represents the boundary between soluble Fe^{2+} at lower pH and Eh, and insoluble Fe^{3+} at higher pH and Eh. The Eh contrast between the two solutions provides a potential across the membrane of at least 0.4 V to initiate and drive catabolic processes.

hydrothermal convection cells operating in the crust at $\sim 150^\circ\text{C}$. In their experiment the Earth's crust was represented by pyrite, pyrrhotite, magnetite, and titanium oxide; platinum was used as a sink for any free oxygen. Their model hydrothermal solution contained KCN (0.19 M), NH_4Cl (0.23 M), H_2CO (0.18 M), and NaHS

(0.05 M) in the presence of a 10-atmosphere mixture of CO_2 and H_2 (3:1). The main amino acids synthesized in this way were glycine ($\sim 10 \text{ mM}$), aspartic acid ($\sim 0.3 \text{ mM}$), and alanine ($\sim 0.1 \text{ mM}$).

Although the actual tenors of organics in Hadean hydrothermal solutions of the type considered here are an unknown quantity, their occurrence in association with hydrothermal minerals in certain meteorites, the carbonaceous chondrites (Cronin et al. 1988; Anders 1989; Tomeoka 1990), could, by analogy with parent asteroids, indicate an environment on the early Earth richer than some theoretical calculations imply. They could have been introduced late during cold accretion and then differentiated into the Earth's crust, to be augmented by cometary dust (Anders and Owen 1977; Chyba and Sagan 1992), finding their way into the oceans (to join formaldehyde) and thereby into hydrothermal solutions. Be that as it may, there would certainly have been another potentially polymerizing nonmetal available in the hydrothermal solution—sulphur. Macleod et al. (1994) estimate its concentration (as HS^-) in the hydrothermal solutions as approaching 1,000 ppm ($\sim 0.03 \text{ M}$). We have found that a variety of organosulphur compounds are produced in experiments simulating hydrothermal conditions. The main products are thiols, dithiols, disulphides (Kaschke et al. 1994), carbon/sulphur ring compounds, and secondary amines (Cole et al. 1994). Organosulphur compounds would have contributed to the membranes simply by adsorption on to

the fresh iron sulphide surfaces (Leja 1982). Some of the organics in the hydrothermal solution could also have escaped directly through fine chimneys into the seawater to bathe the exterior of the sulphide mound (Fig. 1).

The chemistry of the Hadean ocean would have been governed by interactions with the atmosphere and the oceanic crust, given that the land area at a time when there were no continents (Moorbath 1985) would have been relatively insignificant. A 10-bar CO_2 atmosphere would have acted as a very effective "greenhouse" (Walker 1985; Kasting and Ackerman 1986) although the actual temperature of the ocean then is disputed. Henderson-Sellers and Henderson-Sellers (1988) calculate a temperature of only up to 16°C , whereas on isotopic evidence Ohmoto and Felder (1987) suggest $30\text{--}50^\circ\text{C}$ and Karhu and Epstein (1986) favor a hot, 80°C early Archaean ocean. Costa and his co-workers (1980) consider an even hotter ocean more likely, a condensate from the secondary atmosphere at $100\text{--}120^\circ\text{C}$, maintained for a time by the "greenhouse," notwithstanding the relatively low solar flux (Newman and Rood 1977). High CO_2 in the atmosphere would also have imposed a low pH on the Hadean ocean, estimated to be around 5 once saturated in dolomite (Fig. 3). About 100 ppm or $\sim 2\text{ mM}$ of dissolved iron would have been supplied to the Hadean ocean by black-smoker-like hot submarine springs (von Damm et al. 1985). Assuming a 10-bar CO_2 atmosphere, the Fe^{2+} carrying capacity of ocean water saturated in siderite would have been dependent on temperature, and calculations using Geochemist's Workbench (Bethke 1992a,b) give 225 ppm (4 mM) at 20°C and 63 ppm ($\sim 1\text{ mM}$) at 80°C (and see Edmond and von Damm 1983). Nickel may also have been present at the ppm level in the ocean, derived from the acid weathering of the ephemeral land surfaces. (See Hirst 1971.) Minor concentrations of formic and phosphoric acids in the ocean are also a likely consequence of volcanic exhalations (Kasting et al. 1993; Yamagata et al. 1991).

A continual supply of protons to the exterior of the sulphide mound would be guaranteed by the secondary entrainment and convection of these acid Hadean ocean bottom waters (Fig. 1).

Membranes in Laboratory Experiments

Chemical (silica) gardens illustrate how inorganic membranes can form. These grow osmotically by means of precipitation of a semipermeable membrane at the interface of acid and basic solutions (cf. Cairns-Smith 1982). Seeds for the resulting garden spires come in the form of hydrated salts of a weak base and a strong acid (e.g., $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) cultivated in a solution of sodium silicate, a salt of a strong base and weak acid (Coatman et al. 1980; Garcia-Ruiz 1985; and see Leduc 1911). Comparable structures can be produced by the intro-

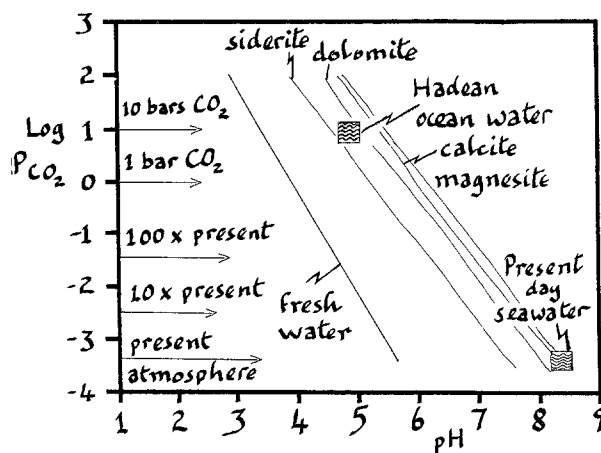


Fig. 3. Calculation of the pH of fresh water, as well as of water saturated in carbonates, in equilibrium with carbon dioxide at varying pressures (after Macleod et al. 1994). This leads to an estimated maximum pH of ~ 5 for Hadean ocean water assuming (1) that it was saturated in carbonate and (2) a 10-bar CO_2 atmosphere (Grotzinger and Kasting 1993). Present-day near-surface seawater is buffered by calcite.

duction of an alkaline solution of sodium silicate through a fine aperture into a mildly acidic solution of a metallic salt, and an iron sulphide version may be produced by introducing a solution of sodium sulphide into iron chloride solution (Russell 1988), an experiment originally designed to mimic the formation of fine hydrothermal chimneys at sedimentary exhalative lead-zinc deposits in Ireland such as Silvermines and Tynagh (Larter et al. 1981; Banks 1985). Apart from fine hollow spires of iron sulphide, bubbles may also form, measuring 0.1–1 mm in diameter (Fig. 4), comparable to sulphide structures fossilized as hollow pyrite botryoids in those same ore deposits (Fig. 5, 6) (Russell et al. 1989). The semipermeable nature of these iron monosulphide membranes is demonstrable in imitations of the porous pot experiments of Pfeffer (1877) as well as in our chemical garden experiments.

The iron-sulphur membranes, formed by the injection of a 0.1 M solution of sodium sulphide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) into 0.1 M solution of ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), have about 20–40 times the durability when a 100-ppm mixture of (potentially) abiogenic organics is added to a liter of each of these fluids at standard temperature and pressure (STP). We chose the following on the basis of previously published work (e.g., Degens 1979; Pinto et al. 1980; Hennet et al. 1992): formaldehyde (15 ppm), formic acid (15 ppm), methanol (30 ppm) glycine (15 ppm), valine (15 ppm), alanine (5 ppm), and aspartic acid (5 ppm). Increasing these concentrations proportionately to total 1,000 ppm also led to a 20-fold increase in the lifetime of the iron sulphide bubbles, but only minor effects were observed on addition of a 10-ppm mixture (Carr and Russell, unpublished research).

Gelatinous iron monosulphide ($\text{Fe}_{(1-x)}\text{S}$) precipitates

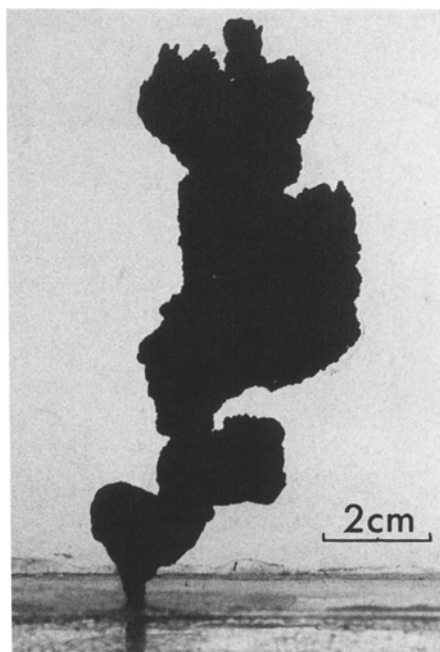


Fig. 4. Probotryoid bubbles comprising an iron monosulphide gel produced at the interface between model alkaline “spring” water bearing HS^- and the ambient seawater analogue containing FeCl_2 .

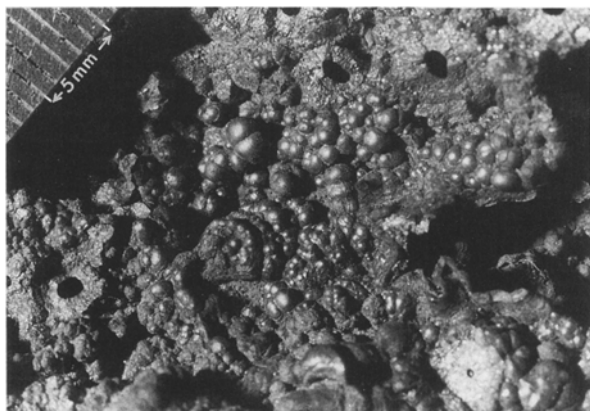


Fig. 5. Pyrite botryoids from Tynagh Mine, Ireland. Scale in mm.

are difficult to characterize (Rickard 1975; Stanton and Goldhaber 1991) but in certain conditions may pass, by dehydration of hydrated iron bisulphides (Rickard 1989), through mackinawite ($\text{Fe}_{(1+x)}\text{S}$), and are well known to oxidize, even at relatively low oxygen fugacities (low redox potential, see Fig. 2), to form the metastable ferrous/ferric sulphide, greigite (Fe_3S_4) (Skinner et al. 1964; Berner 1967; and see Russell 1988). Vaughan and his co-workers (1991) have demonstrated the presence of the greigite structure in fresh “amorphous iron sulphide” precipitated from aqueous solution at 100°C . Greigite (Fig. 7) is an inverse spinel (Vaughan and Tossell 1981) for which the formula accounting for charge and coordination may be written:

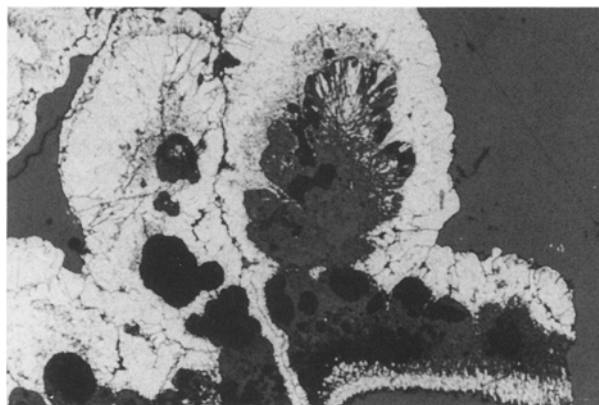
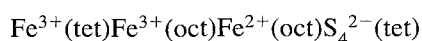
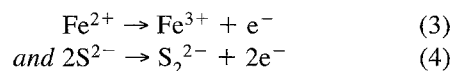


Fig. 6. Section through hollow pyrite botryoids from Tynagh. Field of view 2 mm across.

As can be seen from the half cell depicted in Fig. 7, it can be considered to consist, in part, of a *primitive* $[\text{Fe}_4\text{S}_4]$ cube with each Fe linked “externally” to three S atoms and each S linked “externally” to one iron atom. Ultimately, although nucleation is difficult (Schoonen and Barnes 1991), highly stable ferrous disulphide (pyrite), $\text{Fe}^{2+}(\text{S}_2^{2-})$, is produced on progressive oxidation of iron sulphide precipitates. But the $[\text{Fe}_4\text{S}_4]^{2+}$ cluster itself, if protected from complete oxidation (or reduction) by organic ligands, has the quality of an electron transfer agent.

Greigite itself is a metastable, somewhat “ionic” mineral, which is rare in the geological record because of the instability imparted by the large number of electrons in antibonding orbitals (Vaughan and Craig 1985; Vaughan and Lennie 1991). It is only found in relatively recent sediments, and then only where it has been well protected from oxidation to pyrite or reduction to pyrrhotite ($\sim\text{Fe}_7\text{S}_8$) by surrounding impermeable clays, carbonates, or borax (Skinner et al. 1964). Otherwise the final products depend mainly on the degree of oxidation *via* both:



But greigite also occurs in a magnetotactic bacterium (Mann et al. 1990) where, apart from its navigational use, it probably serves as an iron store (Williams 1990), and could even be acting as a redox buffer. So in this “primitive” sulphur bacterium the greigite is protected from oxidation by organic ligands in the same way that $[\text{Fe}_4\text{S}_4]$ clusters are protected by organic ligands in ferredoxins. And bacteria may be the source of greigite at least in lacustrine sediments (Mann et al. 1990).

We conclude that a membrane of some complexity comprising a dehydrating iron and organic sulphide gel is a likely consequence of fluid mixing on the Hadean ocean floor, and that it had the capacity to stem much of the energy flow from one solution to the other. In

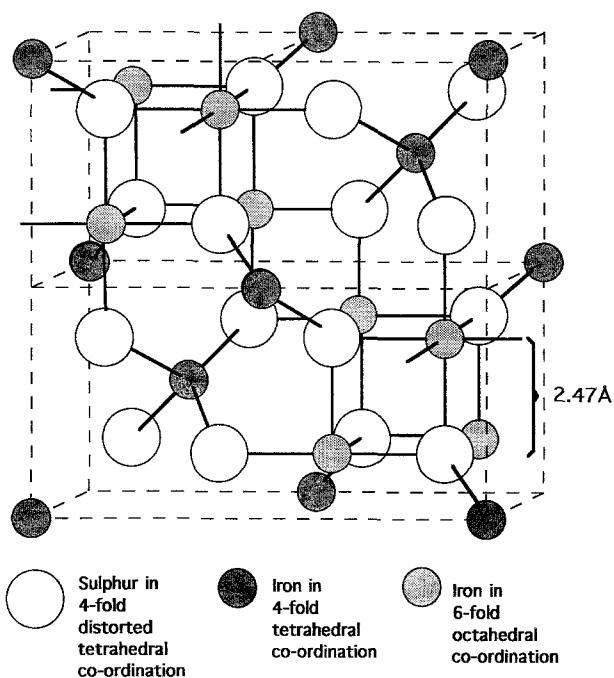


Fig. 7. The structure of greigite (Fe_3S_4) drawn to emphasize the different iron sites in contrast to a similar network of sulphur atoms. Note the *primitive* cube to the bottom right of this half cell in which the four iron ions may be considered as being in the $\text{Fe}^{2.5+}$ state.

general gels are neither liquid nor solid but exist at the boundary between these states and consist of a distribution of self-similar clusters over a range of sizes, fractal on all length scales (Kopelman 1989). When gel membranes thicken, diffusion through them becomes exponentially slower, which is significant since many chemical reactions are diffusion controlled.

Membrane Catalysis

A colloidal gelatinous ferrous/ferric sulphide membrane would, with the help of organic ligands, develop the potential to act as a catalyst by offering the possibility of electron transfer, owing to $\text{Fe}^{2+}/\text{Fe}^{3+}$ transitions in the membrane, before the evolution of ferredoxins (cf. Williams 1965). Iron-sulphur proteins are ubiquitous in current forms of life. Moreover, in "primitive" forms they play the role of NADP(H) (Daniel and Danson in review), and Hall and his co-workers (1971) have suggested that they are primordial catalysts, a view supported by the work of George et al. (1985) and by the theoretical proposals of Wächtershäuser (1992). They are effective and versatile redox catalysts and, especially if incorporating nickel, can carry out hydrogenase reactions of the type shown in Fig. 8. There is good reason to believe that simpler versions with short polymers of prebiotic organic compounds would also be extremely active (Williams 1965; Müller and Schladerbeck 1985; da Silva and Williams 1991) and, because of their thermodynamic stability, spontaneously self-assembling (Hagen et al. 1981). Given the propensity of ferrous/fer-

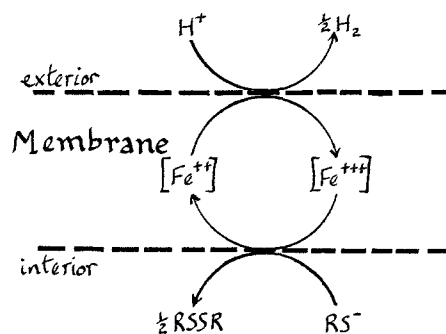


Fig. 8. Natural hydrogenase reaction involving acid seawater activating an iron center within the membrane and, in turn, precipitating an organic disulphide.

ric sulphide to conform in part to a *primitive* cube in metastable greigite, we can imagine this molecule either developing into that iron sulphide mineral, or, as the "cubane" (Holm 1992), developing into a proferredoxin in the membrane, depending on the local availabilities and activities of iron and prebiotic organics.

Notwithstanding the findings of Hennes et al. (1992) it is by no means certain that primitive peptides were the only ligating agents for the iron-sulphur centers, because it appears that apart from glycine, amino acids would not have been developed in quantity abiogenically in the hot-spring environment. For example, in an attempt to reproduce the findings of an earlier aqueous synthesis of amino acids (Kimoto and Fujinaga 1988, 1990) we found only methylaminomethanethiol, carbon/sulphur ring compounds, and insoluble polymers which, combined, had the elemental analyses corresponding to the formula $\text{C}_8\text{H}_{16}\text{S}_5\text{N}_2$ (Cole et al. 1994). Even in the cases where amino acids have been generated in the laboratory they are outweighed by other organic molecules, and anyway, peptidlike polymers are not produced (Miller 1955; Hennes et al. 1992). So perhaps the ligands were more like those used by Bonomi et al. (1985) in the generation of ferredoxin model compounds in aqueous solution. These workers have shown that it is possible for $[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]^{2-}$ to assemble in water from FeCl_2 , sulphur, and thiophenol ($\text{C}_6\text{H}_5\text{SH}$) at STP; 2-mercaptoethanol ($\text{HOCH}_2\text{CH}_2\text{SH}$) has a similar ligating ability, producing $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{OH})_4]^{2-}$. In modern iron-sulphide proteins the wide redox potential range (from +0.2 to -0.4 V at pH 7, 25°C) is obtained largely through the effect the particular protein has on the iron sulphide center, but the redox potential in the probotryoidal membrane we propose would probably have been more restricted (approx. -0.3 V at pH 7, see Fig. 2). Proferredoxins may have been important at this earliest stage of evolution as the FeS clusters could have been protected from oxidation to inert but conductive crystalline pyrite by ligands which prevent the reaction $2\text{S}^{2-} \rightarrow \text{S}_2^{2-}$. On the other hand, $\text{Fe}^{2+}/\text{Fe}^{3+}$ switching is permitted in clusters (Fig. 8), allowing the complex to behave as an electron transfer agent (see equations 3 and 4) with distortion but without a change

in conformation of the active center (Noodleman and Case 1992). In this way these agents may have helped the membrane to resist the aging process. We speculate that traces of $[\text{MoS}_4]^{2-}$ and $[\text{WS}_4]^{2-}$, complexes soluble in alkaline solution, were carried in hydrothermal solution to the hot spring mounds where they reacted with iron in ocean water to form $[\text{MFe}_3\text{S}_4]$ clusters of the type to become significant in some hyperthermophilic archaeobacteria (e.g., Makund and Adams 1991; and see Holm 1992).

Our proposed role for iron sulphides contrasts with that of Wächtershäuser (1988a, 1990), who envisaged the growth of insoluble pyrite providing both a supply of electrons (energy) as well as a substrate for the first chemoautotrophic (but noncellular, two-dimensional) metabolism. As touched on above, we consider that pyrite crystals would not only destroy the flexibility and insulating properties of the membrane, but that they would also severely limit its chemical reactivity: once formed, pyrite is extremely difficult to reduce. Iron is usually bonded in fourfold coordination to sulphur in biological systems, and if ligated, it can tolerate oxidation/reduction, as we have seen, but in pyrite it is bound as Fe^{2+} in sixfold, octahedral coordination to $(\text{S}_2)^{2-}$ (Finklea et al. 1976; and see Cairns-Smith et al. 1992).

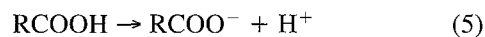
But Wächtershäuser's mechanism may have provided a process in the development of the ever-enlarging hydrothermal hot-spring mound which would have increased autonomy of cellular colonies, perhaps a step toward autotrophy. The original hydrothermal circulation system supplying the organic compounds, in part by "proanabolism" (Fig. 1), could have been supplemented by organic synthesis of a restricted number of organics generated on the growing pyrite crystals replacing and nucleating upon the old iron monosulphide membranes within the mound itself. That is, once the sulphide mound had started to form, seawater-bearing formaldehyde and carbonate could have been entrained through the base (Fig. 1) and monosulphide oxidized to pyrite, perhaps accompanied by the concomitant fixation of carbon as in the Wächtershäuser model—a final contribution to earliest "life" as probotryoids (cf. Fig. 4) converted to true pyrite botryoids (Figs. 5, 6). So the mound could be considered as a chemical reactor (cf. Corliss 1990).

Membrane Growth

We propose that an iron-sulphide membrane would have grown in the first instance by inflation with the buoyant hydrothermal solution at the submarine hot spring (probotryoidal stage), and only later by osmosis (protocellular stage). Membrane area expansion could occur by autoinsertion of hydrophobic moieties (e.g., fatty acids + SH^- = protolipids) as the membrane expanded. There are precedents for such insertion in cur-

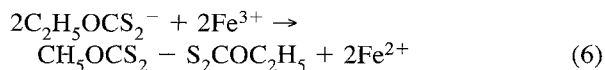
rent biological membranes—for example, the spontaneous monomeric diffusion of cholesterol into them (Phillips et al. 1987). The organic contribution to the membrane may have included those organic sulphides which we presume to have been produced within the hydrothermal system. (See previous section).

There are three obvious possibilities that could act as the driving force behind osmosis as the probotryoids evolve to protocells. *First*, the catalytic (catabolic) splitting of large, chemically fragile molecules on the membrane's inner wall (cf. Hall et al. 1971; and see Weisser and Landa 1973). Fermentation is one process in which oxidation of organics could be encouraged by hydrogenase activity of the iron-nickel sulphide clusters driven by the protons in the acidic ocean water at the membrane exterior ($2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$) (Fig. 8). *Second*, a portion of the hydrophobic compounds (e.g., neutral fatty acids) capable of dissolving in the membrane could be converted in the membrane, or at its inner face, to a more hydrophilic form, possibly by redox reaction or ionization, thus becoming more soluble:



Those hydrophilic forms dissolving in the interior of the protocell will add to the internal osmolality. This second possibility has an analogy in current mitochondrial metabolism. The retention of tricarboxylic acid cycle intermediates in mitochondria occurs because the di- and tricarboxylic acids which make up the intermediates have larger dissociation constants (i.e., a higher proportion is in the charged state) than is the case for the monocarboxylic acids which are the feeders for the cycle. Uncharged molecules can dissolve in, and diffuse through, biological membranes, but charged molecules cannot. *Third*, the transport of positively charged ions in the external, acidic ocean (including organics ultimately derived from cometary dust and the primitive crust) into the membrane, driven by the natural chemiosmotic gradient (cf. Mitchell 1961, 1979; Williams 1961, 1965) arising as a consequence of the acid ocean and an alkaline hydrothermal fluid (Macleod et al. 1994).

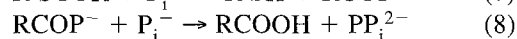
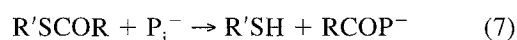
At this stage of evolution all these products would be maintained at a relatively constant level by volume increase as the protocell grows, buds, and produces contiguous offspring. Nevertheless, as we have seen, not all the catalytic conversions would have been destructive or catabolic. With the ligated iron-sulphide centers in the membrane acting as redox catalysts or proferredoxins (Fig. 8), oxidation and concomitant polymerization of prebiotic thiolates to insoluble disulphides would also have taken place—e.g.:



We assume aqueous voids in the membrane interior

were quickly filled by the proferredoxins and also abiogenic organic compounds, attracted to the membrane by surface effects, and possibly rendered less soluble by the catalytic oxidation outlined above (equation 6).

A major implication of our proposal is that the proton motive force, which forms a fundamental part of energy transduction and conservation across cell membranes (Jones 1988), including those of archaebacteria, has existed from the time of the first abiogenic steps toward life. An important role for thioester (RCOSR') bonds, which would be available in the primitive sulphide-rich organic membrane, is favored by de Duve (1991) for the initiation of phosphate-based metabolic cycles, as inorganic phosphates (and thiophosphates) can attack thioester bonds to produce acyl phosphates and pyrophosphates, possible precursors of ATP:



Three key components for the development of metabolism are therefore incorporated in the membrane we envisage: a proton gradient (ultimately providing the proton motive force mechanism of energy conservation) (Fig. 8); electron transfer agents (ultimately participating in the proton motive force through respiration and photosynthesis); and thioester bonds (important in the production of polyphosphates with "high-energy" bonds for diverse processes of biosynthesis).

In this model the Hadean ocean not only stores ferrous iron and protons but also ferric iron complexes, ions of phosphoric acid, and perhaps residual prebiotic organic acids. The hot spring supplies reduced sulphur as HS⁻, RS⁻, and possibly glycine (Hennet et al. 1992).

Membrane Reproduction

As the young iron-monosulphide-dominated membranes are semipermeable, indicating some hydrophobic character, they are self-sealing. Sealing of ruptures would also be facilitated by spontaneous precipitation of new membrane, the composition of which, nucleating and propagating from the old, would tend to have a similar chemistry and structure: i.e., reproduction would be self-seeding even at an early stage. A sense of how this might work for the simplest of systems prior to the emergence of life may be gleaned from a chemical garden experiment. If a crystal of hydrated ferrous chloride (FeCl₂ · 4H₂O) is dropped into a petri dish containing a saturated, filtered solution of sodium sulphide (pH 13.6), submillimetric vesicles are observed to "germinate," one or two coming from another. These vesicles are morphologically comparable to, though smaller than, the pyrite botryoids found at Tynagh (cf. Figs. 5, 6).

As with the chemical garden experiment, the membrane at the submarine mound would have formed at the

interface between an HS⁻ (and probably RS⁻)-bearing alkaline hydrothermal solution and the iron-bearing, acid ocean. Positively charged colloidal ferric oxyhydroxide would have been attracted, along with the Fe(II) complexes, to the negatively charged membrane. Here the iron species would have been precipitated to produce a mixed valence iron sulphide, a portion of which may have been ligated by organosulphur compounds. As Williams pointed out in 1965, "RS⁻ (or RS-SR or RS₂⁻) could have the function of making the iron ions (II and III) in their complexes *extremely active* both as electrophilic (III) and as nucleophilic (II) centers, showing much of the capacity of free radicals in these respects." The catalytic properties of these proferredoxins would encourage chemical ordering; the proferredoxins would have participated in chemical cooperation.

The leading surface of the membranous sulphide mound is always young and ever growing (Fig. 1). Probityoids of iron sulphide up to 1 mm across could have a lifetime of hours or even days because those iron-sulphide clusters which have been ligated will not participate in the normal pyritization process, and will, along with other adsorbed hydrophobic organics, block, or at least inhibit, the crystallization of those unligated clusters beyond greigite.

But after aging the membrane would not have been good at self-maintenance. As we have seen, thickening is likely to have led to overall loss of expansivity and therefore to budding as the hydrothermal system pumped more fluid into the sulphide mound. Organic disulphides resulting from oxidation of thiolates (equation 6) within the membrane could have adhered to it. We have produced amines and carbon/sulphur ring systems in experiments partly designed to mimic such a system (Cole et al. 1994). Such compounds could have conferred chemical stability and physical flexibility to the membrane. So in our view growth involves the oxidation of RS⁻ to give a disulphide linkage (Fig. 8) and the bonding of metal ions with anionic functional groups of organosulphur compounds (Kawka and Simoneit 1987; Cole et al. 1994; and cf. Wu and Orgel 1991).

We propose an evolution toward an initially pliable but gradually aging membrane, a "smart structure" able to withstand the vagaries of the hydrothermal supply. We go on to speculate that in this primitive system the composition of the membrane contains information that determines the environment for further growth. The longevity of the system will influence how effective the membrane is in providing an environment in which more complex prometabolic and self-replicating systems can develop over time. But crucially, the overall composition of the membrane will determine future composition. Hydrophobic, or better still, amphipathic, molecules can be expected to self-insert into such a hydrophobic membrane, and the compounds inserted will depend partly on the membrane's composition (e.g.,

hydrophobicity) and partly on the concentration of the various organics in the hydrothermal solution and the surrounding ocean waters. The more successful the discriminating system is in retaining osmotically active, and in particular organic, species, the more likely it is to evolve from reliance on hydrothermal inflation toward independent, osmotically driven growth and division, so increasing the number and survival of “daughter” protocells. The more successful the exterior is at controlling the insertion of organics, the more likely any daughter protocell is to resemble the parent. These steps have to precede the complete separation of daughter cells.

Conclusions

The minimum requirements for life are (1) maintenance of a volume (procytoplasm) separated from the bulk medium, (2) ability to carry out some form of catalytic conversion, (3) energy, and (4) reproduction, including the passage of enough information to ensure that offspring is relatively similar to the parent. Although an isolated reproducing cell is usually seen as a minimal life form, it is evident that a hydrated iron-sulphide-containing membrane has the advantage of potentially embodying all these requirements within a single entity. A membrane, separating a volume from the external medium, could, as a probotryoid, grow by hydrothermal inflation, and subsequently evolve, in stages, by organic takeover of the greigite component, to encapsulate protocells which would grow by osmosis. It could have the potential to carry out catalytic conversions as a contribution to chemical cooperation, and perhaps to act as “genetic” information in the sense that the membrane’s composition could exert some control on the nature of components inserted into, or permeating through, it and its progeny, as it increases in area in an environment of persistent chemical and thermal disequilibrium—i.e., where those gradients are high and are maintained by the membrane.

As hydrothermal (hydrostatic) inflation precedes catabolically driven osmosis as the means of growth and reproduction, the old argument over the prior appearance of metabolism or genetic information is avoided. They may have developed interactively: the structure of the membrane holding information, and, at the same time controlling, its semipermeable nature. Also the dichotomy between the heterotrophic and autotrophic theories of the origin of life is dissolved. It is a matter of scale. If the overall hydrothermal system is conceived of as the first approach to life then it is autotrophic, but if the focus is restricted to the probotryoidal bubbles it may be considered heterotrophic.

So here we have attempted to show that life emerged as a natural consequence of the drive for equilibrium between the Earth’s crust and hydrosphere, itself in equi-

librium with the atmosphere. It was not an event that merely happened on Earth: it was a part of its functioning, one result of the loss of free energy.

The central features of our proposal are:

1. Semipermeable membranes comprising iron and organic sulphides formed probotryoids which were capable of growth and “reproduction” by hydrostatic inflation-driven swelling and budding while maintaining the strong Eh-pH (chemiosmotic) contrast between an internal alkaline, highly reduced hydrothermal fluid, and the external, mildly oxidized, and acid seawater.
2. Depending on the local availabilities and activities of iron and prebiotic organics the “cubic” ferrous/ferric sulphide clusters $[\text{Fe}_4\text{S}_4]$, newly precipitated and comprising the membrane, either developed into metastable greigite, or, as the “cubane” (Holm 1992), formed the centers to proferredoxins.
3. A large number of iron-sulphide mounds (natural chemical reactors) were formed as a consequence of a multiplicity of submarine hydrothermal convection cells away from oceanic spreading centers. The chemical composition of the hydrothermal fluid would not in all cases have been favorable to the development of the protocellular systems we propose, but the variation in the compositions increases the probability of success for one of them.
4. Initially, at the probotryoidal stage of development, catalytic conversions in and on the membrane would have been catabolic, apart from the oxidative dimerization of thiolates to organic disulphides. Early “anabolic” synthesis of abiogenic organics was provided *within* the submarine hydrothermal system using, in part, CO, CO₂ and/or H₂CO as carbon source(s), although carbonaceous deposits in the crust indicated by meteorites could also have been a source at least early on.
5. Later, at the protocellular stage, selection of protocells with particular membrane compositions, now comprising a greater proportion of organics, would have depended upon osmosis, as catalytic conversions of organic molecules (the fuel supplied in the hot springs and from the ocean water) by proferredoxins in or on the membrane resulted in an increase in intracellular osmotic pressure and maintenance of a relatively high pH.
6. The catalytic membrane provided an environment for the development of a self-replicating system and of metabolism prior to the physical separation of isolated daughters.

Our proposal does not purport to bridge completely the gap between abiogenic chemicals and cellular life forms but rather suggests some initial conditions, available materials, mechanisms, energy sources, and a plau-

sible environment in which the latter could develop from the former. We see the advantage of our hypothesis as requiring a minimum of special conditions and fitting well with what we know of the early Earth. Many of the elements of our proposal are either well known and have been, or can be, tested in the laboratory.

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