

The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front

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Abstract: Here we argue that life emerged on Earth from a redox and pH front at *c.* 4.2 Ga. This front occurred where hot (*c.* 150°C), extremely reduced, alkaline, bisulphide-bearing, submarine seepage waters interfaced with the acid, warm (*c.* 90°C), iron-bearing Hadean ocean. The low pH of the ocean was imparted by the ten bars of CO₂ considered to dominate the Hadean atmosphere/hydrosphere. Disequilibrium between the two solutions was maintained by the spontaneous precipitation of a colloidal FeS membrane. Iron monosulphide bubbles comprising this membrane were inflated by the hydrothermal solution upon sulphide mounds at the seepage sites. Our hypothesis is that the FeS membrane, laced with nickel, acted as a semipermeable catalytic boundary between the two fluids, encouraging synthesis of organic anions by hydrogenation and carboxylation of hydrothermal organic primers. The ocean provided carbonate, phosphate, iron, nickel and protons; the hydrothermal solution was the source of ammonia, acetate, HS⁻, H₂ and tungsten, as well as minor concentrations of organic sulphides and perhaps cyanide and acetaldehyde. The mean redox potential (ΔE_h) across the membrane, with the energy to drive synthesis, would have approximated to 300 millivolts. The generation of organic anions would have led to an increase in osmotic pressure within the FeS bubbles. Thus osmotic pressure could take over from hydraulic pressure as the driving force for distension, budding and reproduction of the bubbles.

Condensation of the organic molecules to polymers, particularly organic sulphides, was driven by pyrophosphate hydrolysis. Regeneration of pyrophosphate from the monophosphate in the membrane was facilitated by protons contributed from the Hadean ocean. This was the first use by a metabolizing system of protonmotive force (driven by natural ΔpH) which also would have amounted to *c.* 300 millivolts. Protonmotive force is the universal energy transduction mechanism of life. Taken together with the redox potential across the membrane, the total electrochemical and chemical energy available for protometabolism amounted to a continuous supply at more than half a volt.

The role of the iron sulphide membrane in keeping the two solutions separated was appropriated by the newly synthesized organic sulphide polymers. This organic take-over of the membrane material led to the miniaturization of the metabolizing system. Information systems to govern replication could have developed penecontemporaneously in this same milieu. But iron, sulphur and phosphate, inorganic components of earliest life, continued to be involved in metabolism.

Keywords: greigite, hydrothermal conditions, iron sulphides, mackinawite, life origin, E_h .

'Life, geologically speaking, consists of the interference with secondary lithosphere-atmosphere reactions so as to produce a small but ever-renewed stock of organic molecules' (J. D. Bernal 1960, p. 34).

Darwin brought us down to Earth with the idea that 'all the conditions for the first production of a living organism' could be met 'in some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity, &c., present' (Darwin 1888, p. 18). This pond transmogrified to the 'hot dilute soup' of Haldane (1929) and Oparin (1938). Oparin (1938, 1957) suggested that life originated in 'coacervates', immiscible semi-permeable spherical droplets or micelles comprising large organic molecules that rained into the sea from a reduced atmosphere. In his later work, Oparin demonstrated the generation of complex organic molecules such as starch within these vesicles using biological catalysts (enzymes) (Oparin *et al.* 1962, quoted in Walde *et al.* 1994, p. 7541).

Oparin's ideas, relying as they did on the assumption of a reduced atmosphere, were seemingly endorsed by the Miller-Urey experiment (Miller 1953). This experiment demonstrated the feasibility of generating organic molecules from an atmosphere comprising methane, ammonia, steam and hydrogen; particularly the common amino acids, through electrical

discharges. Developments and variants of Oparin's ideas have been presented by Fox (1960) and Deamer (1985, 1986). Deamer assumed that the first cells had their organic polymers provided by molecules derived from the carbonaceous chondrites (but see Cronin *et al.* 1988; Shock 1992). This general line of reasoning led to the spectacular experiments of Luisi's group, who, apart from replicating organic droplets in water (Bachmann *et al.* 1991; Walde *et al.* 1994), also managed to bring about the synthesis, by replication, of DNA in vesicles enveloped by an organic membrane ('liposomes'; Oberholzer *et al.* 1995), using the polymerase chain reaction of Mullis & Faloona (1987). This experiment introduced information-bearing molecules into what was primarily a protometabolic system; it shows the way that replicating peptides, or PNA (peptide nucleic acids), RNA and eventually DNA, once generated, could have been incorporated and replicated in the earliest cells (Lee *et al.* 1996; Nielsen 1993; Gilbert 1986; Orgel & Crick 1993); a 'second origin of life' (Dyson 1985).

All these ideas require organic molecules on the primitive Earth and their subsequent strong concentration, admitted by most to be a problem (but see Matthews 1992 for an alternative view). As one possible solution, Bernal (1951) suggested that, following the generation of organic molecules by the action of ultraviolet radiation on ammonium carbonate and

sulphide, the concentration of colloidal organic molecules was brought about on hydrated clays. But this is like cooking the soup on a griddle, and an aluminium griddle at that. If this were so, and assuming congruence between the chemical pathways in the earliest stages of life and enzyme-catalyzed metabolism (de Duve 1997), we would expect aluminium and silicon to be implicated still in biochemical processes (Pringle 1953). They are not.

Moreover, even if there was some way that organic protocells could have assembled, it was necessary in the experiments outlined above to seed the organic vesicles with DNA itself. We now know that the primitive atmosphere was not reducing (Gillett 1985), and that photons and electrical discharges will oxidize organic molecules and ammonia in a carbon dioxide/nitrogen atmosphere. Furthermore, large asteroids and comets impacting during the 'early bombardment' would have vaporized and obliterated not only 'Darwin's pond', but a good proportion of the Hadean ocean itself many times over (Maher & Stevenson 1988; Wardrup 1990; Chyba *et al.* 1995). Also, once the Hadean ocean finally condensed, there would have been no continental masses on which long-lasting standing bodies of water could have formed (de Wit *et al.* 1992; Rosing *et al.* 1996; Patchett 1996).

Experiments to date have at least encouraged researchers to believe that, in principle, an understanding of how life originated is possible. The difficulty is that these approaches are only successful within the limited boundary conditions assumed and imposed by the researcher at the laboratory bench, conditions which did not necessarily obtain in the hydrosphere or atmosphere at large. Little attention has been paid to the geochemical and geological conditions on the early Earth, why life originated at all, what geochemical role it played, the sources and forms of energy required for proto-metabolism, or the way in which an early organic soup might have been synthesized and contained.

In this paper we interweave aspects of some early notions with more recent ideas and experiments into a general hypothesis. These ideas derive from our research on iron sulphides in exhalative-sedimentary base metal deposits (Russell *et al.* 1981; Boyce *et al.* 1983; Russell 1988) and in unmineralized metasediments (Hall 1986), as well as from our studies of ultramafite-hosted magnesite deposits (Fallick *et al.* 1991). Shock & Schulte (1995) have pointed out that organic synthesis in certain submarine hydrothermal vents exhaling highly reduced fluids is 'thermodynamically similar to Miller-Urey synthesis driven by input of energy into an unstable mixture of gases (but does not require unestablished special circumstances that preserve an unstable system from some types of energy but not others)'. These same authors have also demonstrated, theoretically, that there is a strong thermodynamic tendency for carboxylic acids to be generated when extremely reduced hydrothermal solution reacts with seawater between about 50° and 200°C. As the temperature of the solutions drops, the larger fatty acids are favoured, particularly octanoic acid (C₇H₁₅COOH) and even longer chained acids (Shock 1996). Octanoate was the material used by Bachman *et al.* (1991) in their demonstration of self-replication in micelles at STP, but longer polymers would be required to resist dissolution in hot water. Thus it is now possible to imagine the development of a primitive living cell from a fatty acid coacervate or liposome containing complex nucleic and amino acids and their polymers; but how a protected environment could form for the production and self-organization of such an entity remains a difficulty.

Based on our knowledge of what life does rather than what life is, that is, its 'geochemical role', we have attempted to imagine life's very first stages. An off-ridge submarine hot spring interfacing with the Hadean ocean would have offered protection from bolide-induced vaporization, high energy photons, lightning, tidal oscillations and dissipation. Yet the environment would have also provided all the ingredients required by early life, as well as a steady supply of usable free energy, containment, catalytic surfaces, automatic waste disposal, and opportunities for development and evolution. It is possible that, just as a hydrothermal solution is effective in concentrating trace metal from the crust into orebodies, so a hydrothermal solution could glean traces of organic molecules residing in the crust, molecules ultimately derived from the carbonaceous chondrites, interplanetary dust particles and comets that were part of the later bombardment and coalescence of the planet (Oró 1961; Chyba & Sagan 1992; Owen *et al.* 1992; Matthews 1992). These molecules could have 'primed' the protocell for the production of further organic molecules. Alternatively, an alkaline, off-ridge hydrothermal system may have acted as a chemical reactor in which rudimentary organic sulphide components were generated and provided to the hot spring. In any event we argue that iron monosulphide bubbles distended over the hydrothermal spring were the hatcheries for the first organic protocells.

As geologists we often study the results of crystallization close to equilibrium, for example in petrographic and isotopic studies. But these studies focus on events recorded after much energy has been spent, though we are conscious of the energetic processes that preceded final equilibration. Our concern here is to understand the constant coursing of energy through a self-organizing dynamic system; a system which emerged and evolved from essentially inorganic beginnings towards life as we know it now. In the far-from-equilibrium system from which life emerged and evolved, energy and matter cascaded through generations of reproducing protocells and, subsequently, replicating cells. Both inorganic and organic molecules were involved in these processes, and had many overlapping roles; as energy providers, transducers, catalysts, and as the building blocks of cellular structure. The earliest truly replicating cells probably required only twenty or so elements (da Silva & Williams 1991), all of them available at submarine hot springs, and as many fundamental organic molecular components (Wald 1964; Eck & Dayhoff 1968).

Initial conditions

The emergence of life is a geological issue. Our aim here is to enquire how geochemical disequilibrium led to the first metastable metabolizing system. We surmise that such a system was born of the attempts of a hydrothermal fluid to reach equilibrium with the most ancient (Hadean) ocean, attempts that were continually frustrated by the spontaneous precipitation of an intervening iron monosulphide membrane (Russell *et al.* 1994). Prebiotic conditions in which life could have emerged have been outlined by Osborn (1917), Rubey (1951), Bernal (1960), Sillen (1965), Walker (1985), Corliss (1986a), Kasting & Ackerman (1986), Russell *et al.* (1989, 1994), Shock (1992), Kasting *et al.* (1993), Grotzinger & Kasting (1993), Macleod *et al.* (1994), Shock *et al.* (1995), and Shock & Schulte (1995). The Hadean atmosphere was not reduced and the Hadean ocean was not an organic soup (Goldschmidt 1952; Gillett 1985; Krupp *et al.* 1994). Indeed, it is possible that some enhancement of UV radiation from the relatively young Sun

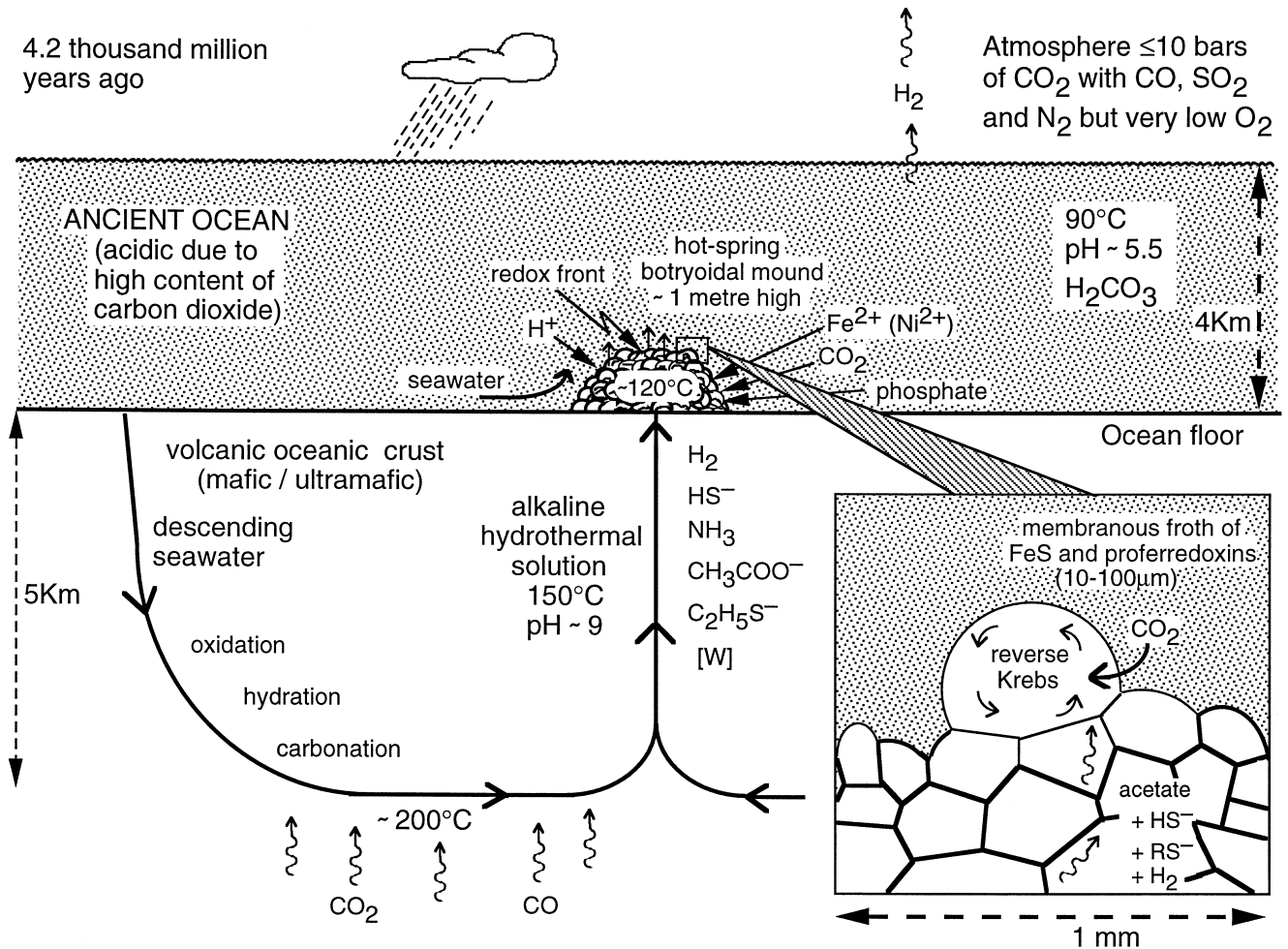


Fig. 1. Model environment for the emergence of life on the ocean floor at a submarine alkaline hot spring, 4.2 billion years ago. The Krebs cycle is also known as the citric acid cycle or tricarboxylic acid cycle.

would have not only destroyed organic molecules and ammonia but also generated very low concentrations of oxygen and ozone from water and carbon dioxide in the prebiotic atmosphere (Canuto *et al.* 1982; Des Marais 1994; and see Arrhenius 1987). But photolytic oxidation of Fe^{2+} to Fe(III) did provide the ocean with its main oxidized species, constituting a major potential electron acceptor (Braterman *et al.* 1984).

The initial conditions were all-important because 'protolife' could not have been inventive. Given the aqueous nature of the living cell, it follows that life itself must have begun in water, presumably in the Hadean ocean. This ocean condensed from the early atmosphere as accretionary heating diminished (Pollack 1990). The original supercritical H_2O was probably partly derived from icy asteroids (Chyba 1990; Hunten 1993) and partly from a degassing Earth (Pollack 1990), as was atmospheric CO_2 and subsidiary CO (Oró 1961; Owen *et al.* 1992; Kasting *et al.* 1993). As a result of the high partial pressure of CO_2 , the Hadean ocean was likely to have been acidic (Grotzinger & Kasting 1993), although Kempe & Kazmierczak (1994) have argued that it quickly became highly alkaline in the Archaean. Such a high partial pressure of CO_2 would also have induced a strong greenhouse effect and high ocean temperatures, between 85° and 110°C according to Kasting & Ackerman (1986). Here we assume a temperature for the ocean at *c.* 4.2 Ga of about 90°C . Hadean ocean water

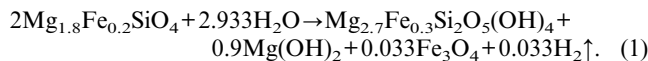
was continually recycled, in part by black-smoker-like acidic hot ($\leq 400^\circ\text{C}$) springs (Corliss 1986a), in part by 'acid rain' (Pinto *et al.* 1980), but probably also by myriad hot ($\leq 200^\circ\text{C}$) alkaline seepages on the deep ocean floor (Fig. 1) (Anderson *et al.* 1977; Fehn & Cathles 1986; Mottl 1992; Macleod *et al.* 1994). In the absence of continental masses, surface weathering would have been restricted to ephemeral volcanic islands and collision zones (Abbott & Hoffman 1984; Macleod *et al.* 1994).

The Earth itself was, at that time, even more strongly reduced than now (Arculus & Delano 1980). Because of continual additions from meteorites, the crust would have been heterogeneous, with nickel-iron asteroids rendering portions of the crust very reduced indeed (Melosh 1989). But the recycling of seawater through the upper oceanic crust began a process of oxidation of iron in iron-bearing silicates, in reactions that liberated hydrogen which eventually escaped the Earth's gravitational field as a gas (Neal & Stanger 1984; Coveney *et al.* 1987; Corliss 1986a) (Fig. 1). This has been the pattern ever since, with redox fronts or waves, expressed as contrasts in mineralogical redox assemblages within exposed sediments and rocks, revealing the 'frozen' pathway of migration of surface derived waters (e.g. Spooner *et al.* 1977; Mohamad *et al.* 1992).

It has occurred to several researchers that life may have originated in a hot spring (Copeland 1936 in Fox 1995; Fox

1959, 1960; Degens 1979), though Corliss *et al.* (1981) were first to propose a fully coherent theory based on the then recent discovery of hot springs at the ocean ridges (see also Baross & Hoffman 1985; Corliss 1986b). Pringle (1953) had foreseen the advantages for emerging life of being shielded from the Sun in the ocean deeps. To this he added the idea of a redox contrast in the ocean driving dynamic chemical activity (though implicating oxygen) and, citing Bresler *et al.* (1949), pointed to the 'greater ease with which condensation reactions involving the removal of water take place at high pressures' (Pringle 1953). Since then Neal & Stanger (1984) have suggested that, in prebiotic times, hydrogen in hot springs could have played a part in the synthesis of the first organic molecules. More specifically we have argued that life may have originated at highly reduced alkaline submarine springs operating at a distance from oceanic spreading centres (Russell *et al.* 1988, 1989, 1993, 1994).

Such low to medium temperature, freely convecting hydrothermal systems (rather than magma-driven hydrothermal convection) operating in the deep ocean floor (cf. Russell *et al.* 1981; Fehn & Cathles 1986) would have been primarily responsible for oxidizing the crust, while the reduced and alkaline fluid that resulted from serpentinization of the ultramafic rock (Mottl 1992) issued as submarine seepages. This can be illustrated using an olivine/water reaction (Janecky & Seyfried 1986):



Much of the carbonic acid in the hydrothermal solution would have been lost to carbonation of the mafic crust in the down-draw limbs of the convection cells (Griffith & Shock 1995). Nevertheless abiotic organic molecules could have been generated by hydrogenation of the carbon monoxide and dioxide (Shock 1992) degassing from the mantle (Neal & Stanger 1984; Kelley 1996), perhaps catalyzed by awaruite (Ni_3Fe) filaments (Krishnarao 1964), or even larger bodies of nickel-iron derived from meteorites of the 'late bombardment' (Melosh 1989). But we cannot be certain if, or what hydrothermal prebiotic organic molecules were synthesized, let alone in what concentration, because present-day exhalations are hopelessly compromised by life.

Iron, though absent from the alkaline hydrothermal solutions (Macleod *et al.* 1994), would have been present in the Hadean ocean, being derived from the acid black smokers where concentrations would have approached 0.02 mol kg^{-1} , to judge from the southern Juan de Fuca data (Von Damm 1990). Due to the high oceanic temperatures and acidity, the cloudiness of the atmosphere, the high partial pressure of CO_2 , and the dearth of free oxygen, much of the iron may have stayed in solution (Fig. 2), as it does in Lake Nyos and Lake Monoun in Cameroon (Kling *et al.* 1989; Kusakabe *et al.* 1989; Bernard & Symonds 1989; Evans *et al.* 1994), only to have been precipitated on mixing with the bisulphide-bearing (HS^- also *c.* 0.02 mol kg^{-1}), alkaline spring waters on the ocean floor (Macleod *et al.* 1994) (Figs 3, 4). From our experiments (Russell *et al.* 1994), we infer that iron monosulphide would have precipitated in the form of a membrane, thus stemming the flow of the other 'electron-rich' molecules, generating and constraining a 'hot organic soup' in tiny botryoidal 'tureens' (Russell *et al.* 1988) (Figs 1, 5a, b). Evidence from the Tynagh orebody in Ireland indicates that the outer surface of the mound might have comprised bubbles of (colloidal) iron

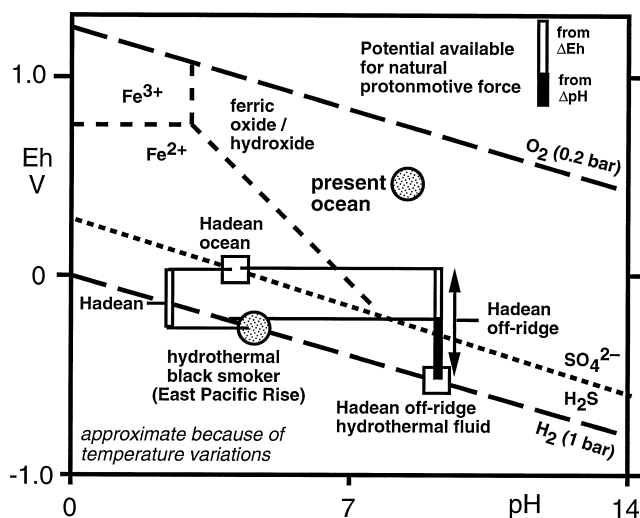


Fig. 2. Schematic Eh-pH diagram showing the average positions of present day and Hadean hydrothermal fluids and ocean water. Eh values of redox couples may differ greatly from the estimated average position. Note that fluid temperatures differ for the fluids represented but this produces no significant changes in the schematic relationships illustrated. Construction lines show how total average potential energy for protonmotive force is derived from pH and Eh components in the case of Hadean off-ridge hydrothermal systems. Because of inverse pH relationships, there is no pH component to add to the redox potential energy available for metabolism in both present day and Hadean black smoker hydrothermal systems.

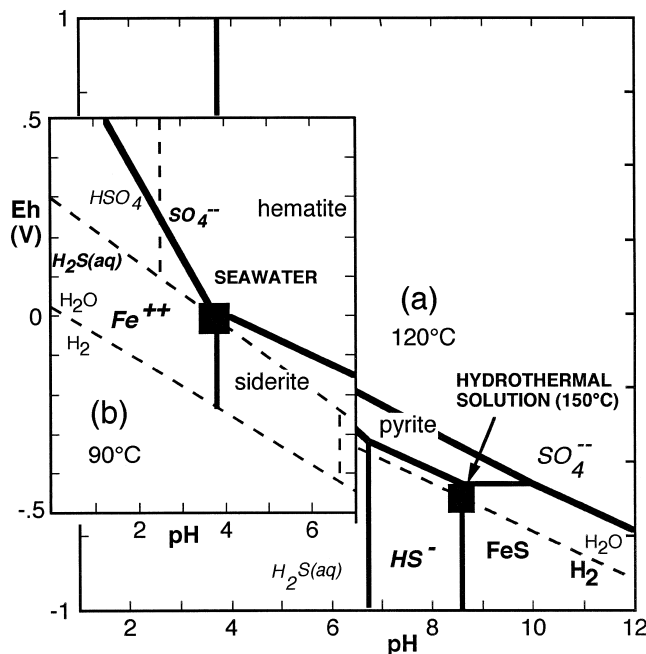


Fig. 3. Eh-pH diagrams calculated using Geochemist's Workbench (Bethke 1992a, b; 1996) illustrating estimated Eh, pH and temperature contrast in the submarine hot-spring mixing environment between: (a) off-ridge hydrothermal solution (120°C , activity $\text{H}_2\text{S}(\text{aq}) = 10^{-3}$, a $\text{Fe}^{2+} = 10^{-10}$) cooling from 150°C ; and (b) estimated Hadean (*c.* 4.2 Ga) seawater (90°C , saturated in siderite, a $\text{H}_2\text{S}(\text{aq}) = 10^{-10}$, fugacity $\text{CO}_2(\text{g}) = 10$ bars). Eh and pH are assumed to be controlled by dominant equilibrium couples.

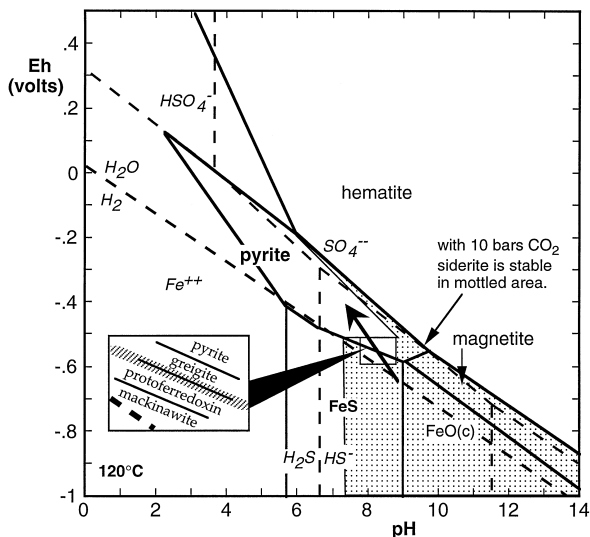


Fig. 4. Eh–pH diagram produced using Geochemist’s Workbench (Bethke 1992a, b; 1996) to illustrate the very low oxidation state required for the stability of iron monosulphide, FeS and the general redox relationships of FeS and pyrite, FeS₂. The inset shows notional phase relations emphasizing the intermediate oxidation state of the FeS component of membrane protoferredoxins. The inset is positioned to indicate the Eh–pH conditions pertaining to Hadean hydrothermal fluid as it enters Hadean seawater. The arrow indicates the trajectory of the Eh–pH change on mixing. Also illustrated is the influence of high CO₂ pressure on the phase relations. The high partial pressure of carbon dioxide leads to decrease but not elimination of the stability field of FeS in favour of siderite. However, FeS precipitates forming at early submarine hot-spring sites will be prone to carbonation externally to siderite, as well as oxidation to pyrite, unless protected by, for example, adsorbed and bonded abiogenic organic molecules.

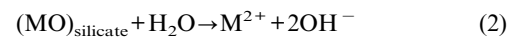
monosulphide, each about 100 μm across (Figs 5c, d) (Russell 1996a). Our experimental simulations on the other hand have produced contiguous bubbles, forming compartments about 10 to 20 μm across (Fig. 5b), and contrasting with typical bacterial cells which measure about 1 to 5 μm. In order to achieve comparable morphologies, however, these compartments were generated at 90°C with Fe²⁺ and HS[−] solutions enriched five to twenty-five-fold over their concentrations calculated for a Hadean spring site. At calculated concentrations of ferrous iron and sulphide (i.e. 20 mmoles of each), only a simple membrane formed. Experiments at higher temperatures (and pressures) are not feasible at present.

Hadean ocean water and the two hydrothermal systems

Degassing of high-temperature mafic and ultra-mafic magmas (between 1400° and 1000°C) produces volatiles that are relatively oxidized, e.g., carbon monoxide, carbon dioxide, sulphur dioxide, polyphosphates, steam and nitrogen, but not oxygen (Kelley 1996; Pineau *et al.* 1976; Chivas *et al.* 1987; Grotzinger & Kasting 1993; Yamagata *et al.* 1991). The soluble volatiles contributed to the relatively oxidized state of the hydrosphere. Water–rock reactions associated with convective hydrothermal

systems operating in the oceanic crust at moderate temperatures (up to about 400°C), result in reduction of water by the ferrous silicate component of mafic and ultramafic rocks. The concomitant oxidation of these rocks results in the generation of a ferric component to the silicates and oxides (Spooner *et al.* 1977; Kasting *et al.* 1993). In the higher part of this hydrothermal temperature range, at oceanic spreading centres, a portion of the water is dissociated so that hydroxides are precipitated and hydrogen ions (protons) are fed to the oceans *via* the acidic black smokers (Seyfried *et al.* 1991). Soluble ferrous iron is carried in solutions from the acid black smokers at a concentration of up to 0.1% (Von Damm 1990). It would have remained dissolved in the acidic Hadean ocean. Nickel (and cobalt) may have been introduced into the ocean through sulphur-deficient high temperature acidic springs.

Off-ridge, deep ocean hydrothermal springs and seepages are the predicted result of lower temperature (100–200°C) rock–water interactions. Here, a portion of the HOH splits to hydrogen gas (eqn 1, and see Janecky & Seyfried 1986) and some soluble hydroxide:



where M=Mg and Ca. Therefore this alkaline hydrothermal fluid, generated away from spreading centres, would have been extremely reduced (Macleod *et al.* 1994). Yet the chemistries of the million or so low to medium enthalpy springs we estimate to have been operating at any one time in the Hadean ocean would be quite variable, depending on the temperature of the solutions, the age of the system, and the rock composition hosting the hydrothermal system. Numerical modelling and laboratory experiments with sulphide-bearing ultramafic rocks (Macleod *et al.* 1994) indicate that a reduced alkaline solution will dissolve up to c. 750 ppm of sulphide as HS[−] at 250°C. Organic thiolates (RS[−]) may also be generated (Kaschke *et al.* 1994; Heinen & Lauwers 1996). If so, other possible sulphide species in these extremely reduced alkaline fluids are WS₄^{2−} or WS₃^{2−}, i.e., in valence states of (VI) and (IV) respectively (Nekrasov & Konyushok 1982). Tungsten might also have been present as the tungstate (WO₄^{2−}) in these solutions. Circumstantial evidence in support of the presence of this metal in these solutions comes from the fact that tungsten is a pathfinder element for gold mineralization in mafic terrains (e.g., Phillips *et al.* 1984; Mueller 1991), and it is well known that gold is transported as a sulphide complex (Au₂(HS)₂S^{2−}) in alkaline hydrothermal fluids (Seward 1973; Boyle *et al.* 1975; Phillips & Groves 1983). Moreover, the rare sulphide tungstenite (WS₂) has been found associated with graphite in listwaenite in an ultramafic massif in Eastern Siberia (Voevodin *et al.* 1979; Nekrasov & Konyushok 1982). But generally the metal only occurs in the form of tungstates in natural conditions (Evans 1974). In contrast to the metals mentioned above, iron is virtually insoluble in alkaline solutions (Macleod *et al.* 1994).

It is the interfacing of the alkaline hot springs with the acid ocean that brings about the precipitation of an iron monosulphide (mackinawite) membrane (Figs 4, 5a, b). Electrons could have been transferred across such a membrane. Also the hydrogenating potential of the mackinawite could have been enhanced by the presence of nickel (Kouvo *et al.* 1963; Vaughan 1969; Morse & Arakaki 1993). We assume the membranes to be semi-permeable by analogy with chemical garden growth (Coatman *et al.* 1980; Russell 1988) but just how protons and other entities could have been translocated

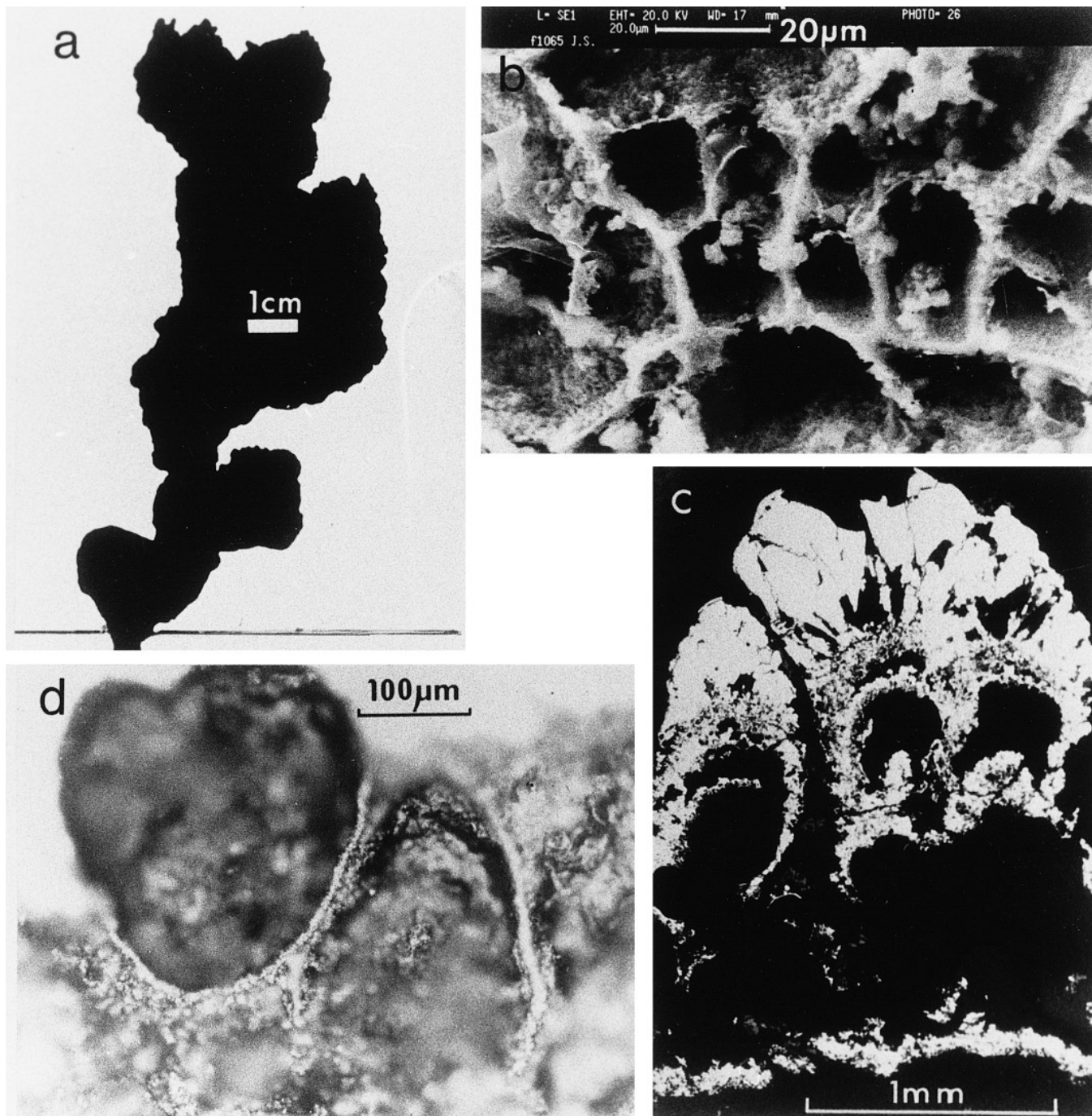


Fig. 5. Comparison of laboratory generated iron sulphide mound (Russell *et al.* 1989) comprising compartments, with iron sulphide structures from the Irish orebodies (Boyce *et al.* 1983; Banks 1985). (a) Iron monosulphide edifice generated as a 0.5 molar solution of sodium sulphide (representing alkaline seepage water) is injected into a 0.5 molar solution of ferrous chloride solution in a visijar (representing Hadean ocean; (b) freeze dried section of (a) prepared and photographed by John Sherringham; this section reveals a complex of iron monosulphide compartments offering a potentially extensive catalytic surface for *in vitro* organic synthesis (cf. Shock 1996); (c) cross-section through the top of a sulphide mound from the exhalative centre of the Tynagh orebody (Banks 1985); what appear to be bubbles of sulphide have been epitaxially overgrown with pyrite; (d) magnification of the lower central portion of (c).

across the membrane is unknown. One speculative possibility is that cages in microporous sulphides may have acted as such locales (cf., Bedard *et al.* 1989). Within zones of low permeability in the hydrothermal system (where the water/rock ratio was very low), it may be that carbon oxides, from the degassing mantle or ocean, could have been exothermically

hydrogenated to a series of kinetically stable organic molecules, particularly formate, acetate, acetaldehyde and ethanol (Shock 1992; and see Williams 1965). At the seepage site the membrane could have encapsulated culture chambers in which further organic syntheses took place, the prelude to life itself (Russell *et al.* 1989) (Figs 1, 5). In particular, methane thiol

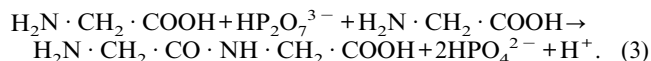
(CH₃SH) and ethane thiol (CH₃CH₂SH) may have formed where hydrogen or protons invaded the membrane (Heinen & Lauwers 1996).

Yet the black smoker as well as the cooler, off-ridge springs, may be considered to be far-from-equilibrium systems. Although there is more thermal contrast with the ocean at black smokers, it is the off-ridge spring that is further from electrochemical equilibrium (Fig. 2). As Prigogine & Stengers (1984, pp. 175–176) remarked ‘the biosphere . . . as well as its components . . . exist in far-from-equilibrium conditions. In this context life . . . appears as the supreme expression of . . . self-organising processes . . . Once the conditions for self-organisation are satisfied, life becomes as predictable as the Bénard instability . . .’ So we should not be surprised by the coupling of dissipative (i.e., kinetic) structures such as the steady state off-ridge convection cells and earliest life, life as a chemical exchanger. Shock (1996) and Shock & Schulte (1995) have shown theoretically that the conversion of the inorganic carbon dioxide to organic compounds on mixing of the two solutions (ocean water with highly reduced hydrothermal solution in equilibrium with quartz, magnetite and fayalite) lowers the overall Gibbs free energy. It follows that this environment is ripe for the emergence of the most complex of dissipative structures, life.

The largest initial overall pH and redox contrast between aqueous species occurred either side of the semi-permeable iron monosulphide membrane which acted as the spontaneously precipitated interface between hydrothermal fluids that emanated from the medium enthalpy springs and Hadean seawater (Russell *et al.* 1994; Macleod *et al.* 1994). In other words, this is where the total free energy of the system was focused (and see Shock *et al.* 1995). The condition is best envisaged on Eh/pH diagrams (Figs 2–4), although it must be borne in mind that an Eh can only truly be ascribed to individual redox pairs rather than to the entire solution (Thorstenson 1984). The energy contrast is variably composed of an electrical potential difference (ΔE) between redox partners (e.g., H₂/H⁺ and FeII/FeIII) and a chemical potential difference (e.g., ΔpH) (Fig. 2) (Jones 1988). The Eh potential of this particular pair could contribute about one volt to the overall system. But we assume here a mean ΔE of about 300 mV, energy potentially to be exploited in the synthesis of organic monomers.

Even more remarkable is the fact that the chemical potential difference (ΔpH) proves to be similar to what life scientists term protonmotive (*pmf*) or chemiosmotic force (Mitchell 1961, 1979). This force, measured in millivolts, drives the energy transduction system in all cells. It ultimately drives polymerization of organic monomers. But while life usually generates such a force from electrons and/or protons acting on the cell membrane, in this Hadean system the protons were already concentrated on the outside of the membrane (Fig. 1). One pH unit equals about 60 mV (Garrels & Christ 1965, and as read from the H₂O/H₂ slope on the inset on Fig. 3). From Figure 3, we can estimate a total potential available across the membrane. The hydrothermal solution is estimated to have a pH of 9 (Macleod *et al.* 1994), and the ocean has a pH of *c.* 3.5 (Fig. 3). The pH contrast therefore has the potential to contribute about 330 millivolts to a protometabolism if the temperature has equilibrated either side of the membrane. This natural ‘protonmotive force’ exceeds that required to activate the energy currency in the envisaged culture chamber, a currency that can be used to do chemical, osmotic and mechanical work (Thauer *et al.* 1977).

One of the most important ways the protonmotive force is used is in the reformation of diphosphate from monophosphate. The diphosphate is an agent for organic synthesis and polymerisation *via* the elimination of the constituents of water from the reacting components in living systems (Wood 1977, 1985; Baltscheffsky & Baltscheffsky 1992). (Another agent is the complex adenosine triphosphate (ATP)). We speculate (see below) that diphosphate played a similar role to ATP within the relatively hydrophobic environment obtaining in the iron monosulphide membrane:



Should dissipative structures not occur at black smokers too, perhaps as fossilized temporal morphologies around the springs? In 1993, Fouquet *et al.* described what they termed a beehive structure or diffuser growing around chimneys at the Snake Pit sulphide deposit on the Mid-Atlantic Ridge at 23°N which, while not predictable, does satisfy this general expectation (see Fouquet *et al.* 1993, figs 4 & 5 and also Koski *et al.* 1994). Rickard *et al.* (1994, fig. 1) reported another example of a ‘beehive diffuser’ from the Broken Spur hydrothermal deposits 6° further north along the same ridge. These researchers described the beehive diffuser as a ribbed mass of layered anhydrite associated with pyrite and iron oxides surrounding a chalcopyrite-anhydrite wall to the chimneys. The beehive moves up the chimney as that edifice grows, dissolving at the base as it develops and reorganizes at the top. The chalcopyrite and pyrite precipitate from the diffusing hydrothermal solution, while the inversely soluble anhydrite and the iron oxide zones delineate the inflow of the entrained and initially cold, but heating seawater, sucked into the system by the *venturi* effect. The beehive diffuser is not the ‘supreme’ example of a self-organizing process but the interplay of the hydrothermal solution and seawater does generate a complex structure capable of reproducible (though not replicable) growth, a self-assembling heat exchanger. As the sulphate content of the Hadean ocean was probably appreciably lower than today (Grotzinger & Kasting 1993; Habicht & Canfield 1996), it is unlikely that beehive structures were generated at 4.2 Ga. It follows that the initially attractive idea of the building blocks of life being generated and harboured at the interface between sulphides and sulphate layers in a beehive structure may be a false trail.

Yet bacteria growing at black smokers, including some in these ‘beehives’, constitute the base of the food chain in the chemosynthetic oases that occupy portions of oceanic spreading centres (Stetter 1996). What role do bacteria play in geochemical cycling?

Clues from the nature of unicellular (prokaryotic) life

While genetics offers the key to the evolutionary relationships of present-day life, understanding biochemical evolution is important and could contribute more to understanding the development of earlier, simpler life-forms whose metabolic processes would have been more dependent on their geochemical environments. Life processes are exceedingly intricate. Here we consider only the key metabolic reactions, and attempt to use these to demonstrate that life’s emergence can be explained as a continuum from the Earth’s inorganic geochemical processes through to organic biochemical processes.

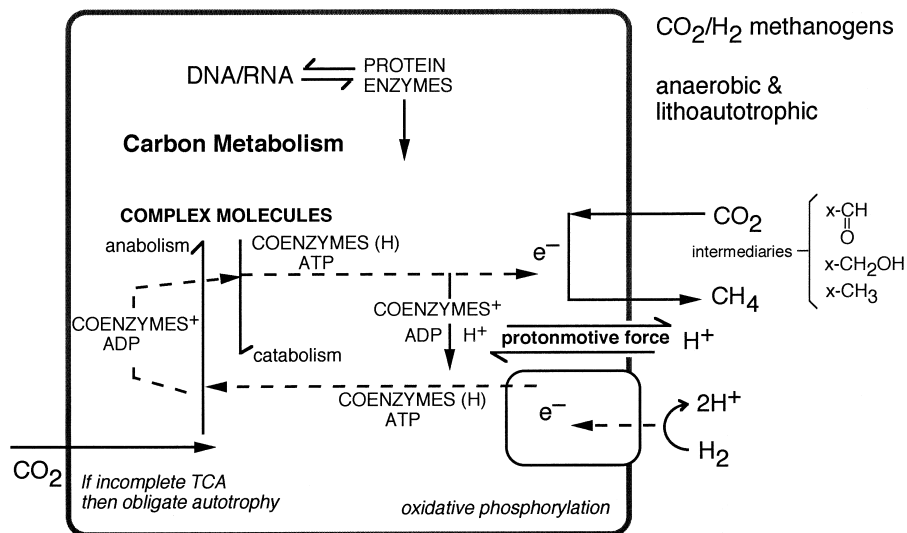


Fig. 6. Probably one of the simplest and most primitive metabolic process, that of a methanogen using hydrogen as an electron source and CO₂ as carbon source and electron acceptor. Intermediaries after Thauer *et al.* (1977) who suggest the x cofactor is -S-CoM which is HS-CH₂-CH₂-SO₃⁻ and is found in all methanogens. An hydrogenase, F420 which is unique to methanogens (Thauer *et al.* 1977), provides electrons from hydrogen.

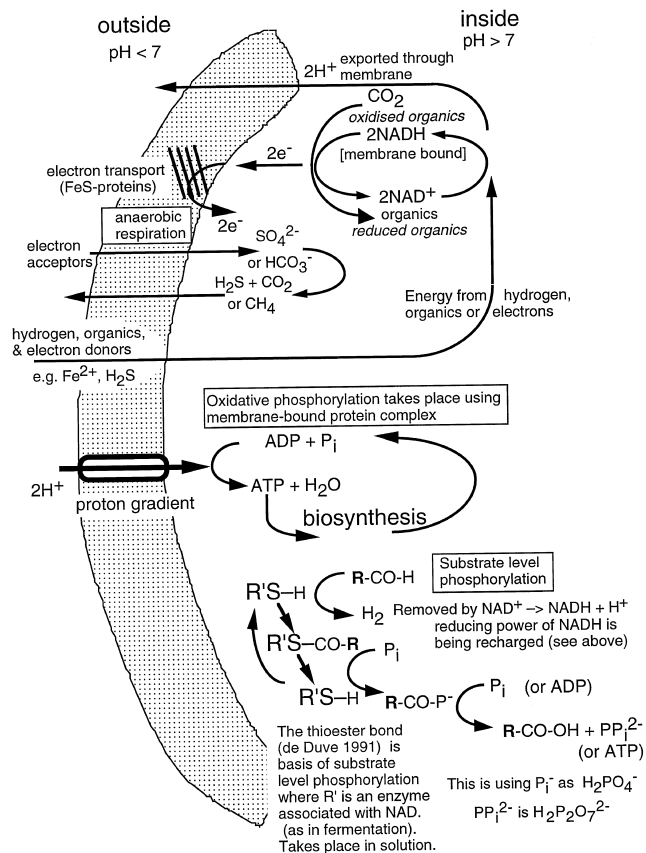
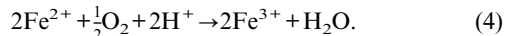


Fig. 7. Cartoon illustrating aspects of the two membrane-related bacterial energy pathways: substrate level phosphorylation (as in fermentation) which usually takes place in solution but may be membrane-associated; and oxidative phosphorylation (as in respiration) which is a membrane process.

Many reactions in the prokaryotic cell (Figs 6, 7 & 8) are driven by the protonmotive force (chemiosmotic gradient) across the cell membrane (Mitchell 1961, 1979; Anthony 1988; Jones 1988; Heberle *et al.* 1994). The cell maintains an internal fluid of higher pH, with a higher concentration of solutes (water activity of about 0.8, comparable to that of NaCl-

saturated water) and a lower redox potential than the external fluid, which has a low to moderate pH and a low content of dissolved components (water activity >0.9, cf. seawater=0.98). Cell pH is normally slightly above 7 but spans the range 6.5 to 8.5. The reason for this is that, for a biological catalyst (enzyme) to function optimally, it should be protonated, a state attained for enzymes around a pH of 7 to 8 (Kotyk 1989). These states are usually held independently of the conditions pertaining in the ambient environment. However, there is at least one exception to this which has a bearing on our hypothesis. The ferrous-iron oxidizing bacterium *Thiobacillus ferrooxidans* uses the natural protonmotive force of low pH waters to make adenosine triphosphate (ATP) (Fig. 9), although in doing so protons entering the cell must be consumed (Brock *et al.* 1994, p. 593). Oxidation of Fe²⁺ by this bacterium is a proton-consuming reaction:



Generally, for prokaryotic cells, 'life' involves the maintenance of pH, redox, chemical and water-activity contrasts across the cell membrane (Figs 7 & 8) (Darnell *et al.* 1986).

Life can be classified phylogenetically, on the basis of ribosomal RNA sequence analysis, into three domains (Woese *et al.* 1990) which have different physiological characteristics: the Bacteria or eubacteria (perhaps the most ancient, yet relatively rapidly evolving, with membranes of phospholipids with ester head groups; Fig. 9); the Archaea or archaeobacteria (slowly evolving but with variable membranes of phospholipids with di- and tetra-ether head groups; Fig. 9; Fewson 1986); and the Eucarya or eukaryotes (cells with a nucleus) (Fig. 10). We are concerned here with the Bacteria and Archaea, which are the simplest life-forms, the prokaryotes (i.e., the bacteria with a small 'b'). Organisms on the lowest evolutionary branches within both these distinct domains only grow in hot water (Fig. 10), i.e., they are extreme thermophiles. Thermophilic Bacteria live at up to 90°C (e.g. Jannasch *et al.* 1988) and thermophilic Archaea at up to 110°C (Huber *et al.* 1989; Fiala & Stetter 1986) and there are now indications that certain bacteria live at much higher temperatures (Deming & Baross 1993), perhaps as high as 169°C (Cragg & Parkes 1994). Woese *et al.* (1990) have proposed that the *universal ancestor* to organisms belonging to these domains was also thermophilic

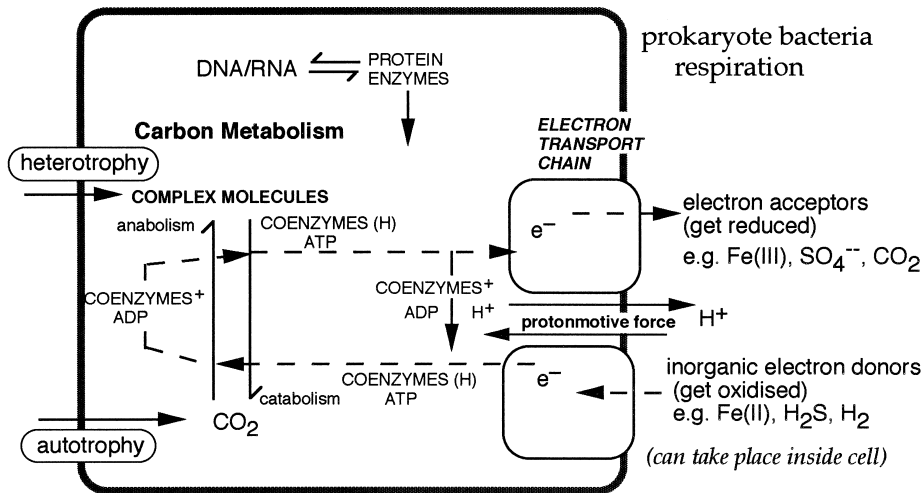


Fig. 8. Main components of respiration and metabolic processes of prokaryote bacteria. Photosynthesis, which contributes to the energy of electrons from inorganic donors, is excluded.

(and see Daniel 1992; Stetter 1992, 1996). Woese & Fox (1977) have termed the universal ancestor the *progenote* (and see Woese 1965; Benner *et al.* 1989). In models for the emergence of life, a major objective is to explain the origin of, and even define, the progenote, the ancestor with the potential to evolve genetically the diverse physiological and metabolic characteristics of all life.

The prokaryotes operate in a chemically varied but limited number of ways (Brock *et al.* 1994). In the case of bacteria that are fuelled by inorganic molecules such as the carbon oxides and hydrogen (the chemoautotrophic bacteria; Table 1), the contribution of respiration to metabolism (Fig. 11) can be considered in terms of an external energy potential between an electron donor (a relatively reduced chemical species) and an electron acceptor (a relatively oxidized chemical species). Hydrogen is the most reduced end-member while oxygen is essentially the most oxidized end-member. A pH contrast between the inside and outside of a cell can provide a potential since H^+ is a relatively oxidized electron acceptor (low pH is comparable to a higher oxidation state; Fig. 2). The larger the difference in redox potential between the redox couple of the participating electron source and that of the sink, the larger the potential energy (Fig. 12). Redox energy, provided by the geochemical environment, is used to generate hydrogen from H^+ via hydrogenase enzymes, and maintain protonmotive force across the cell membrane. Adenosine triphosphate (ATP) (Fig. 9), established as the universal energy currency of life, is produced primarily by the protonmotive force.

Metabolism may be considered as two reciprocal functions: anabolism, the biosynthesis of organic macromolecules brought about mainly by the *condensation* power of adenosine triphosphate, ATP/ADP+inorganic phosphate (P_i), detailed below, and the *reducing* power of nicotinamide adenine dinucleotide phosphate, NADPH/NADP⁺ (Fig. 9); and catabolism, the process in which organic matter is broken down into constituent molecules (which also has the effect of increasing osmotic pressure) mainly by the *oxidizing* power of NAD⁺. These coenzymes are central to metabolic processes in all life and it seems likely that such phosphorous- and nitrogen-bearing organic macromolecules succeeded precursors at a very early stage in evolution (Cairns-Smith 1982). Models for the origin of life must therefore be able to explain the source and early incorporation of both phosphorus and nitrogen.

Of particular interest to us here is the role of iron and sulphur in prokaryotes. They occur together in the most

ancient of all biological catalysts, the ferredoxins (Fig. 13a) (Eck & Dayhoff 1968; Hall *et al.* 1971). These are known as redox catalysts as they can store and transfer electrons. As $[Fe_4S_4]^{2+/+}$ centres, they are ligated to amino acid chains through cysteine ($HOOC \cdot (NH_2) \cdot CH \cdot CH_2 \cdot S^-$). Ferredoxins have a primeval and central role in both anabolism and catabolism. Daniel & Danson (1995) argued that oxidized ferredoxins (i.e., those with centres in the $[Fe_4S_4]^{2+}$ state) were the earliest biological catalysts to mediate catabolism prior to the appearance of NAD(P)H (Fig. 9). These particular ferredoxins catalyse redox reactions at very low potential, i.e., at the hydrogen potential on the H_2/H_2O boundary shown in Fig. 4 (Cammack 1996).

Buchanan & Arnon (1990), reviewing the work of their laboratory, have established the role of reduced ferredoxin in reversing the citric acid cycle, particularly in the net reductive synthesis of pyruvate from acetyl-coenzyme A and the other significant carboxylation, of succinyl-coenzyme A to ketoglutarate (and see Hartman 1975 and Wächtershäuser 1990) (Figs 9, 14 & 15). Coenzyme A (Fig. 9) is the thioester required to prime (derivatize) both the acyl and succinyl groups for their further carboxylation through the sulphide (thioester) bond (Fig. 9). The anabolic contributions of ferredoxins were first unravelled for photosynthetic bacteria but they also have the same part to play in anaerobic anabolism (Buchanan *et al.* 1969). Coenzyme A is a complex thioester but, as de Duve (1991) pointed out, simpler thioesters such as 3-mercaptopropionate (Fig. 9) (Cuevas *et al.* 1985) may be its precursors, and even smaller ones generated in a sulphide rich environment such as ethyl thioacetate ($H_3C \cdot CH_2 \cdot S \cdot CO \cdot CH_3$), derived from acetate ($CH_3 \cdot COO^-$) (Shock 1992) and ethane thiol ($H_3C \cdot CH_2 \cdot SH$) (Heinen & Lauwers 1996), may have been involved at the earliest stages.

Another transition metal important to bacterial metabolism is nickel. This element is required in hydrogenations (Cammack 1988). Volbeda *et al.* (1995) have determined the structure of the nickel-iron hydrogenase from *Desulfovibrio gigas*, a bacterium that reduces sulphate in the presence of molecular hydrogen. In this enzyme a nickel atom is ligated to a protein through four cysteines ($HOOC \cdot (NH_2) \cdot CH \cdot CH_2 \cdot S^-$). An adjacent iron atom is coordinated to two cysteines as well as other ligands, and coupled to this are two $[Fe_4S_4]$ sites and one $[Fe_3S_4]$ site which together act as 'stepping stones' (Cammack 1995) for electrons on their way to their electron acceptor (Volbeda *et al.* 1995). The active catalytic site comprising the

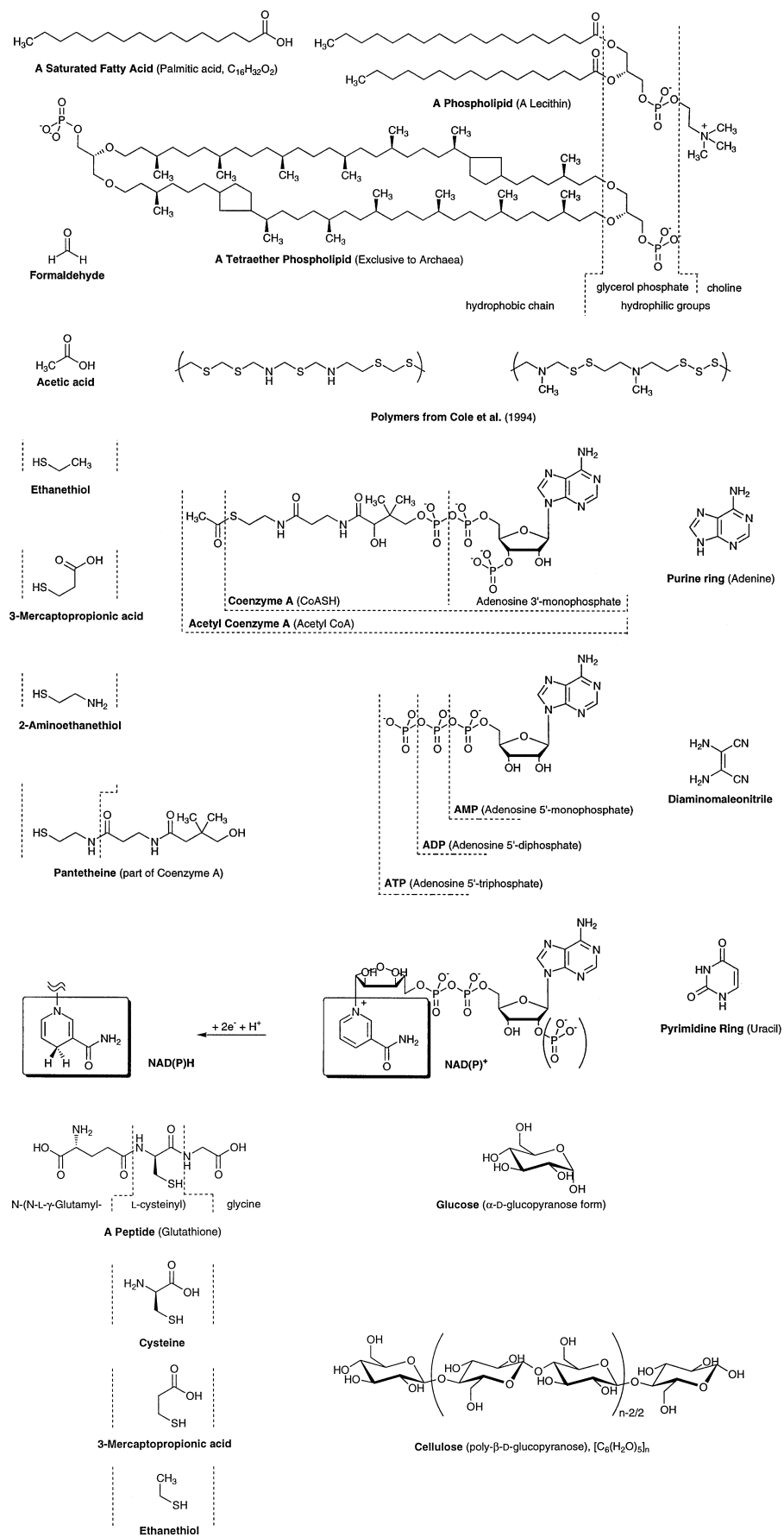


Fig. 9. Structures of organic molecules referred to in the text.

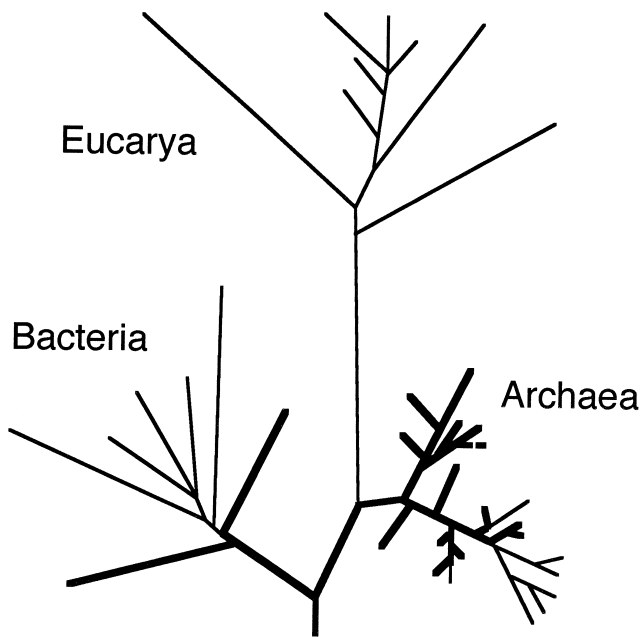


Fig. 10. Position of hyperthermophiles (bold) at the base of the phylogenetic tree (Stetter 1996, modified from Woese *et al.* 1990).

nickel-iron centre cleaves molecular hydrogen to a proton, H^+ , and a hydride, H^- . The proton can be neutralized by reaction with hydroxide. Then reactive atomic hydrogen $H\cdot$ is left as one of the electrons is conducted away from the hydride to an electron acceptor (Cammack 1995). This atomic hydrogen can then contribute to the generation of formaldehyde by reducing the CO_2 bound to the adjacent iron centre.

A further biological catalyst or enzyme involved in redox catalysis in highly reduced conditions (i.e., at the hydrogen potential, see Fig. 4) contains tungsten (Kletzin & Adams 1996). In fact, tungsten is a required element in the metabolism of all hyperthermophilic bacteria that live at the base of the evolutionary tree (Stetter 1996) (Fig. 10). In this enzyme a single tungsten atom is linked through four sulphurs to nitrogen-bearing organic rings (two pterin molecules) which are themselves bridged by a magnesium ion (Kletzin & Adams 1996, fig. 7). This enzyme can oxidize aldehyde (Chan *et al.* 1995).

Prokaryotic life processes operate in different redox conditions, in anaerobic and aerobic environments (Table 1, Figs 8, 11 & 12) (Prescott *et al.* 1993). It is likely that bacteria occupy almost all possible aqueous environments on the Earth up to at least $110^\circ C$ at all oxygen tensions, so the commonly considered subdivision into aerobic and anaerobic types is only a convenient simplification.

The proposition that anaerobic life preceded aerobic life, with hydrogen and sulphide available from the beginning and oxygen as a late-comer, is supported by both the geochemical and the biochemical evidence (Goldschmidt 1952; Wald 1964;

Table 1. Classification of bacterial metabolic processes

Electron source	Electron acceptor	Main C-source	Main products
AUTOTROPHIC BACTERIA: energy and carbon from inorganic compounds			
Chemoautotrophic pathway			
<i>Aerobic</i>			
CH_4	O_2	CH_4	CO_2
<i>Anaerobic</i>			
H_2	CO, CO_2	CO, CO_2	Acetate
H_2	CO, CO_2	CO, CO_2	CH_4
H_2	Sulphate, sulphur	CO_2	Sulphide
CO	Nitrate	CO	Nitrite + CO_2
Photoautotrophic pathway (photosynthesis contributes energy)			
<i>Anaerobic anoxygenic</i> (e.g. green and purple sulphur bacteria)			
H_2S , sulphur, thiosulphate	CO_2	CO_2	Sulphur Sulphate
<i>Anaerobic oxygenic</i> (e.g. cyanobacteria)			
Water	CO_2	CO_2	O_2
HETEROTROPHIC BACTERIA: energy from organic matter			
Fermentation pathway (substrate level phosphorylation)			
Organic	Organic	Organic	$CH_4 + CO_2$
Respiration pathway (oxidative phosphorylation)			
<i>Aerobic</i>			
H_2S	O_2	Organic	Sulphate
Organic	O_2	Organic	CO_2
Fe^{2+}	O_2	Organic	CO_2
<i>Anaerobic</i>			
Organic	Sulphate	Organic	Sulphide, acetate, CO_2
Organic	Nitrate	Organic	N_2 , N-oxides
Fe^{2+}	Sulphate	Organic	Sulphide
Organic	Fe^{3+}	Organic	Fe^{2+}
Fatty acid	Water	Fatty acid	Acetate
Acetate	Water	Acetate	$CH_4 + CO_2$
Organic	Sulphur	Organic	H_2S

Note that some individual bacteria can use more than one metabolic process and all can assimilate CO_2 .

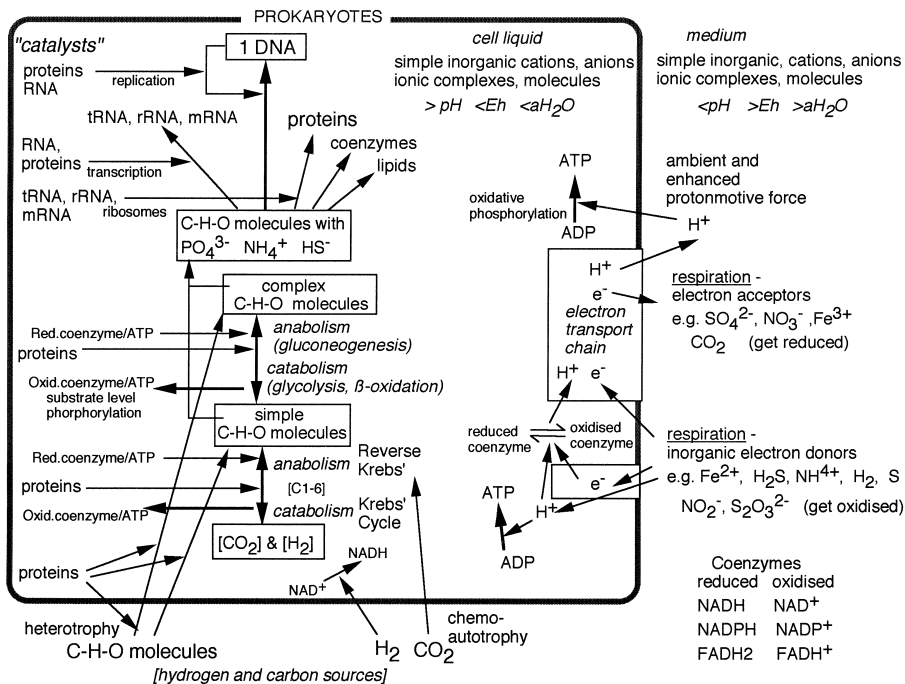


Fig. 11. Details of metabolic processes of the prokaryote cell emphasizing the separation of the ATP/coenzyme controlled carbon metabolism from the energy provision through respiration. By-products of respiration can contribute carbon to biosynthesis. Photosynthesis is excluded.

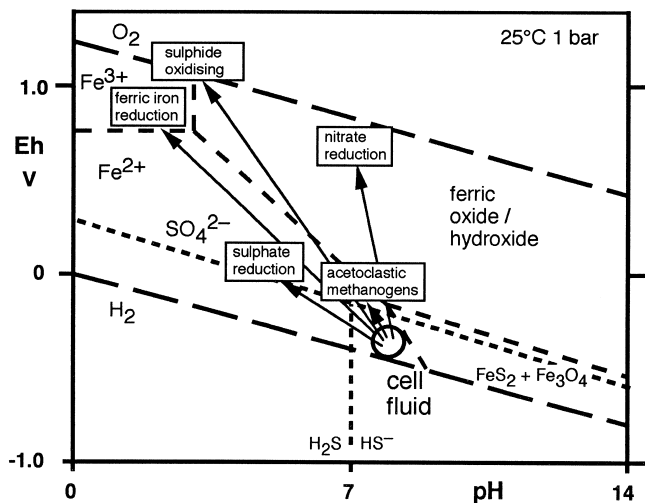


Fig. 12. Redox potential/pH vectors between cellular fluid of bacteria and that of their living environment represented by electron acceptors. The electron acceptors may be out of equilibrium with the ambient environment which may be altered by the activity of the bacteria.

Schopf 1983; de Duve 1997). For example, Brenner (1988) has recorded one possible mutation affecting the amino acids, demonstrating that cysteine [HS · CH₂ · CH(NH₂) · COOH] has probably been replaced by serine [HO · CH₂ · CH(NH₂) · COOH] in some 'modern' proteins. McFadden & Shively (1991) suggested the following evolutionary sequence of bacteria which use carbon dioxide as the sole source of carbon (autotrophs): (i) anaerobic chemolithotrophs (bacteria using inorganic chemicals as energy sources), (ii) anoxygenic photolithotrophs, (iii) cyanobacteria and (iv) aerobic chemolithotrophs. But the redox vector which represents the Eh-pH contrasts between the intracellular fluid and the electron acceptors (Fig. 12) need not have increased systematically, because genetic evolution to different levels of molecular

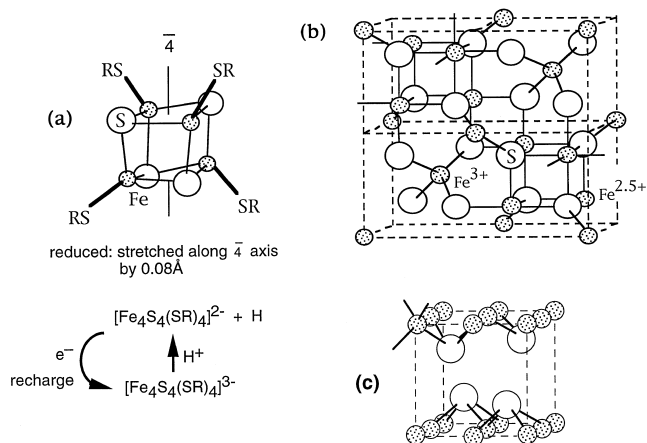


Fig. 13. Similarity in structure of Fe₄S₄ 'thiocubane' unit in protoferredoxins and ferredoxins (a), with the Fe₄^{2.5+}S₄ 'cubane' unit of greigite, Fe₃S₄ (b). All three contain Fe²⁺ and Fe³⁺ cations. Fe₄S₄ is variably distorted in ferredoxins depending on the oxidation state (Berg & Holm 1982). The protoferredoxin would be intermediate in oxidation state between greigite (more oxidized) and mackinawite (more reduced), FeS, which has a layered structure (c) (cf. Figure 4).

sophistication would be required for the diverse oxidized chemical species to participate in life's chemistry (and see Schwartzman *et al.* 1993). Sulphur respiration preceded methanogenesis, and a hyperthermophilic Archaea, *Archaeoglobus*, is a genetically intermediate sulphate-reducer containing some enzymes which are otherwise only known in the methanogenic Archaea (Brock *et al.* 1994).

The electron sources and acceptors, the carbon sources and the products of some metabolic processes in bacteria are given in Table 1, and most of these would have been available as contributors to the first steps towards life. However, only minute amounts of free oxygen were present on the Earth until oxygenic photosynthesis evolved and had time to take effect

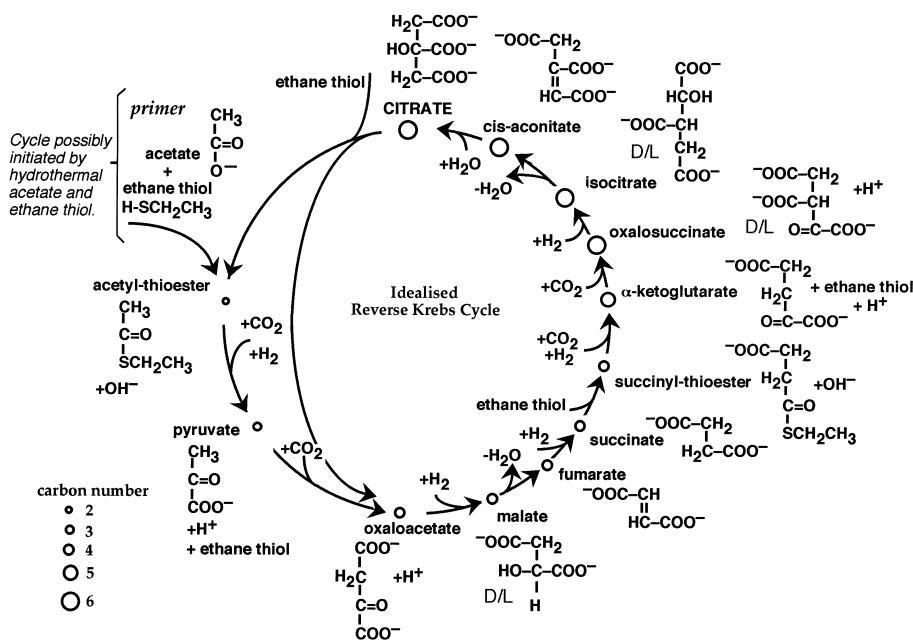


Fig. 14. Reverse Krebs Cycle, modified after Buchanan & Arnon (1990) to show the putative role of thioester and the charge balance based on the driving forces: carboxylation, hydrogenation and hydration/dehydration within the C-H-O system.

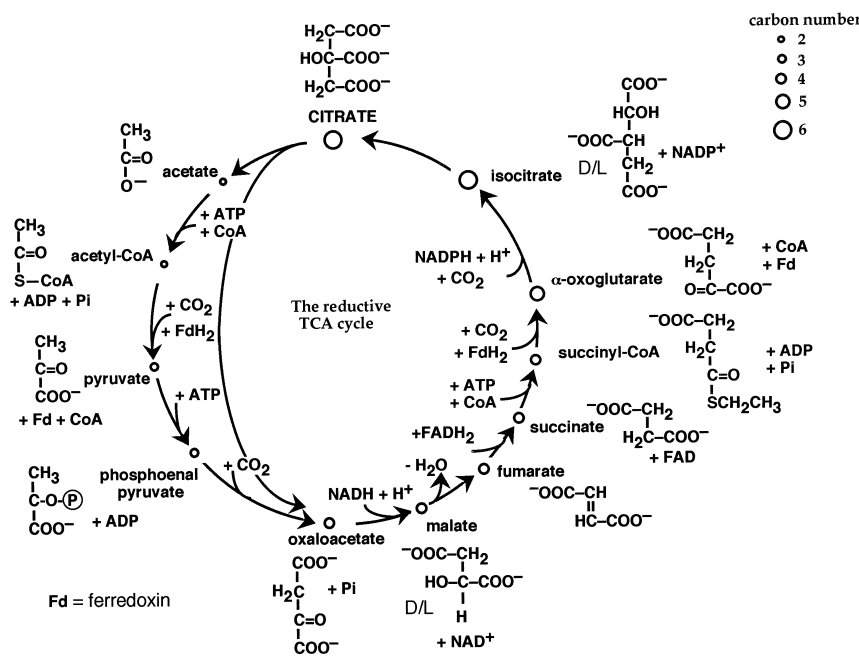


Fig. 15. The reductive TriCarboxylic Acid cycle (citric acid or Krebs cycle), characteristic of green sulphur bacteria (use reduced sulphur compounds as electron donors for photosynthesis). (BIOTOL 1992, p. 185.)

about 2 billion years ago. So the first redox processes could not have involved oxygen as an electron acceptor. We can also consider how bacterial redox processes relate to their state of evolutionary complexity to obtain clues as to the likely reactants for the earliest stages. Important questions to ask are: 'How easy is it to tap the electron source?' and, 'How easy is it to use the electron acceptor?' For example, fixation of nitrogen as ammonia is an intricate biochemical process involving the use of enzymes that contain iron-molybdenum sulphide centres which provide the necessary hydrogen and bond it to the relatively inert dinitrogen (Stiefel & George 1994). It seems likely that this process was a relatively late development in the evolution of life, and that inorganic ammonia was available from the start (see Shock 1992). The use of hydrogen as an electron donor is a primitive trait, whereas use of oxygen as an

electron acceptor, which allows the use of more oxidic donors, is a more evolved trait (Brock *et al.* 1994).

Carbon dioxide is particularly resistant to use as an electron acceptor (oxidizing agent) without specific carboxylating enzymes (McFadden & Shively 1991), yet all forms of life appear to be able to process carbon dioxide (Atlas 1989). Nevertheless, before the development of enzymes, the high partial pressure of carbon dioxide (perhaps tens of bars) at the ocean bottom may have made assimilation easier. We know of only two chemical demonstrations of *abio*genic carbon dioxide reduction in water at low temperature (<200°C) in the laboratory. Sarkar & Das (1992) fixed CO₂ using a reduced tungsten organic complex as a catalyst, and Heinen & Lauwers (1996) generated nanomolar quantities of thiols from CO₂ using FeS as a catalyst. We should note that the FeS used by Heinen &

Lauwers probably contains a small proportion of native iron (our XRD analysis).

Looking towards the lower limbs of the evolutionary tree, the methanogenic archaeobacteria are strongly represented (Danson *et al.* 1992). Yet they are a disparate group. Autotrophic methanogenic bacteria can use hydrogen and CO or CO₂ to obtain energy (Fig. 6), with methane as a by-product (Wolfe 1992; Brock *et al.* 1994). Methanogens that use the catabolism of acetate (CH₃ · COO⁻) can gain energy by what is known as the heterotrophic acetoclastic pathway. Water is the oxidizing agent, and methane and carbon dioxide are the by-products. Organic molecules which act as energy sources are generally intermediate in oxidation state between CH₄ and CO/CO₂, e.g., fatty acids and the more oxidized acetate (Fig. 9). All methanogens can use NH₄⁺ as a nitrogen source (Brock *et al.* 1994). As explained above, the only transition metals that are absolutely required for growth of methanogens are iron, nickel and tungsten (and perhaps cobalt and zinc), elements available in the earliest oceans, or, in the case of tungsten, in the alkaline hydrothermal fluid itself.

Bhatnagar *et al.* (1991) raised the question as to whether the autotrophic methanogens or the heterotrophic methanogens arose first, and noted the similarity of the autotrophic methanogenic pathway to that of the apparently evolutionary unrelated eubacterial acetogens. It therefore seems likely that organisms with relatively distant genetic relationships can have common roots for their metabolic processes. However, it is the basis of the metabolic process that is important, e.g. the key enzymes, and not necessarily the electron donor and acceptors. So the classification of metabolic processes in Table 1 is only a pointer to evolutionary relationships. Recalling the role we assume for iron monosulphide in the first membranes, there is one other suggestive primitive feature of some methanogens, that certain of them contain polyferredoxins with up to twelve [Fe₄S₄]^{2+/-} centres (Reeve *et al.* 1989; Steigerwald *et al.* 1990; Hedderich *et al.* 1992; Cammack 1996). These relatively evolved ferredoxins allow electron transfer from one Fe₄S₄ centre to the next, perhaps passing electrons through the membrane (Reeve *et al.* 1989). The polyferredoxin may be mimicking an earlier 'abiotic' configuration, where such centres were sequestered by the sulphur- and nitrogen-bearing polymers constituting a portion of the early membrane (Kimoto & Fujinaga 1990; Cole *et al.* 1994) (Fig. 9), a configuration important to methanogenesis.

Life as a chemical process

The cell membrane (Fig. 7) can be thought of as *the* fundamental biochemical barrier, but this may be considered in two ways.

Life's chemical processes *could* be viewed as the exploitation of energy sources to maintain the cell membrane and hence the longevity of the cell and its descendants. Cell construction and maintenance requires the genetically controlled biosynthesis of the major membrane constituents, i.e. the various lipids, as well as more complex, renewable molecules, such as proteins, which sit within or on the membrane and control chemical processes across it (Fig. 7). This approach sees the life-process as the controlling agent, leaving 'life' difficult to define and understand. However, membrane *assembly* is not entirely controlled genetically, e.g., when cells divide, the membrane is shared and the membrane area may be expanded by addition of lipids present as vesicles or micelles within the cells (e.g., Kell & Glasser 1993).

An alternative approach is to view life's chemical processes, such as the construction and maintenance of the cell membrane, and the various energy transfer pathways, as a *result* of the attempt of the chemical components of the internal fluid and external fluid to attain chemical equilibrium. Thus chemical and electrochemical contrasts, in particular the pH contrast, between the inside and outside of a cell, maintained by external redox disequilibria, can drive the cell's chemical processes. So chemical equilibrium is *inhibited* by the existence of the intervening membrane, and secondarily by the *dislocation* of ionic reactants in complex organic substrates. In the words of Williams (1961, p. 3) 'It may well be that the achievement of a separation of activated reagents in space plus restricted diffusion provides the fundamental distinction between biological chemistry and test-tube chemistry'. This latter view is at one with the organic- and iron-sulphide membrane protocell model presented below for the emergence of life.

Model for the emergence of Life

In the model presented here, the 'life' process developed at a submarine hot spring site. The process was dominated at first by inorganic iron sulphide precipitates, but was rapidly taken over by organic molecules (cf. Cairns-Smith 1982). The chemistry of C+H+O+N+P now dominates life processes (Wald 1962), and these elements are the main components of all biological molecules such as amino acids, proteins, carbohydrates, fatty acids, lipids, RNA and DNA, as well as the non-protein coenzymes (e.g., ATP, NADPH; Fig. 9). Nevertheless iron and sulphur were structurally important at an early stage, and continue to be essential in metabolism.

Why should these particular elements and not others (e.g., Al and Si) be involved in the constant chemical cycling that constitutes life? It has often been speculated that a silicon-based life might exist somewhere in the Universe. But, as with other elements in the third period of the p-block, the silicon-silicon bond cannot survive attack by water, oxygen or ammonia, vital constituents of life. By way of contrast, carbon-carbon, carbon-nitrogen and carbon-oxygen bonds are all stable in the presence of water, and are therefore potential components of natural polymers (Shock & Schulte 1995). Also, as carbon forms double bonds with oxygen in carbon dioxide, this molecule achieves an octet of electrons in the outer shell, and therefore independence. Carbon dioxide is readily accessed from air or aqueous solution.

Iron (± Ni, W) and sulphur

Ferrous iron in silicates in the oceanic crust is the major ultimate source of electrons in hydrothermal solutions. These electrons are carried to the ocean mainly in molecular hydrogen. In the absence of molecular oxygen, ferric iron is a major sink for such electrons. Moreover, given its two valence states, iron can, in various circumstances, accept, store and transfer electrons. These properties are central to the emergence of life.

Wächtershäuser (1988) has argued for the formation of pyrite, on oxidation of FeS by hydrogen sulphide with the generation of hydrogen, as the primeval energy source for the origin of a (surface) metabolist. If such a reaction were to be of significance in this context (for example, deep in the hydrothermal mound), the particular iron sulphide entity involved in the reaction could take no further part in protometabolism. This is because pyrite has a complex structure which involves

ferrous iron ligated to six sulphur pairs, $\text{Fe}(\text{S}_2^{2-})_6$, making the mineral difficult to nucleate and very difficult to reduce (Finklea *et al.* 1976).

We prefer to consider iron sulphide precipitates which can conduct electrons, and in which the valence of iron can be readily switched. Mackinawite and the more oxidized greigite (Fe_3S_4) have closely related crystal structures (Krupp 1994) (Fig. 13). Sulphur atoms are closely packed cubically along (111) in greigite, although the equivalent sub-lattice is somewhat distorted in mackinawite (Krupp 1994, fig. 5; and see Lennie *et al.* 1995). Krupp suggested that oxidation from the ferrous state in mackinawite to the ferric state in greigite is probably facilitated by electronic conduction along the iron bands in the mackinawite, iron which is metallically bonded (Vaughan & Ridout 1971). As the ferrous iron loses electrons, the reduced radius of the ferric iron allows their diffusion, and mackinawite is transformed to greigite (Krupp 1994). Two thirds of the iron in greigite is in the ferric state. For the reverse (reduction) process to take place would again require some redistribution of the newly reduced iron.

A colloidal iron monosulphide (mackinawite) membrane is likely to have been precipitated spontaneously at the interface between the exhaling, alkaline, bisulphide-bearing hydrothermal solution and the iron-bearing Hadean ocean (Fig. 4). Mackinawite $[\text{Fe}(\text{Ni})_{1+x}\text{S}]$ crystallites are more likely to have been precipitated than siderite, because at the pertaining pH, sulphur activity, temperature and pressure, iron sulphide is relatively insoluble. (Nevertheless, considering the phase relations demonstrated in Fig. 4, we can imagine the reduced iron acting as a binding site for CO_2 introduced from the ocean through the membrane.)

Bubbles comprising this iron sulphide membrane could have been hydraulically inflated over the hot seepages, where they encapsulated the reduced alkaline hydrothermal solution (Russell *et al.* 1989, 1993). As the bubbles became distended, they weakened and failed, and daughter bubbles were generated. Thus the redox and pH front remained at the growing surface of the sulphide mound (Fig. 1). Bubbles furthest away from the feeder veins would have been disadvantaged, unless the structure of their membranes particularly disposed them to supporting an osmotic pressure. This osmotic pressure would have been induced by the generation of abiotic, charged, organic molecules, especially the carboxylates (Shock 1996). Contiguous compartments (Fig. 5a) generated by budding of the iron monosulphide membrane (Fig. 5b), would contain fluid mixes at slightly different Eh and pH conditions, and harbour different reactants and products, as energy cascaded from one chemical and electrochemical level to another. These kinds of possibilities were elegantly considered for other types of inorganic membrane by Cairns-Smith (1982, 327 and 351–356). As discussed below, organic synthesis would have been catalysed by the iron (nickel) monosulphide, which, unlike fine metal and oxide/hydroxide catalysts, cannot be poisoned by sulphidation.

Iron monosulphides such as mackinawite can contain up to 20% nickel (or cobalt and copper), probably tetrahedrally bonded between the sulphur-sulphur layers (see Fig. 13c) (Vaughan 1970). The FeS membrane considered here may have only adsorbed a few per cent of nickel, enough to force the cleavage of the hydrothermal hydrogen at the nickel site with the production of a transient hydride. The left-over proton would be neutralized with hydroxide in the protocytoplasm. The electrons could be transported through the membrane along the iron layer situated along (001), conducting

toward the final electron acceptor such as Fe(III) on the outside of the membrane. The atomic hydrogen could then hydrogenate the CO_2 molecules bound on adjacent iron sites.

Were thiolates to have been generated in the bubbles as we surmise (Russell *et al.* 1994; Cole *et al.* 1994; and see Heinen & Lauwers 1996), the $[\text{Fe}_4\text{S}_4]^{2+}$ cubane structure comprising a proportion of greigite could have been bonded to such ligands (rather than ferric iron and sulphur), and a protoferredoxin generated (Bonomi *et al.* 1985; Müller & Schladerbeck 1985; Holm 1992; Cammack 1996) (Fig. 4 and compare Fig. 13a with 13b). In this case the iron-iron distance shortens from 3.5 Å to *c.* 2.7 Å while the sulphur-sulphur distance remains at *c.* 3.5 Å (Bertini *et al.* 1994 and references therein) (Fig. 13), and is not further distorted on reduction to $[\text{Fe}_4\text{S}_4]^+$. Fe(III) in the low potential protoferredoxins would have been an electron acceptor within the iron sulphide compartments. Where multiples of such structures existed (cf., the polyferredoxins of Reeve *et al.* 1989), electrons could have been translocated through the membrane to the outside where protons and Fe(III) in the ocean could accept them. This mechanism would have displaced the less controlled conduction along (001) in mackinawite mentioned above. Thus there is a certain congruence between the iron sulphide chemistry discussed further below with the biochemistry of these two elements, a congruence missing from the Wächtershäuser hypothesis where pyrite is merely a waste product and not intrinsic to biochemical processes (de Duve 1997) (Fig. 4).

There is some experimental evidence to support the catalytic activity that we imagine for the protoferredoxins. Tezuka *et al.* (1982) have demonstrated the electroreduction of carbon dioxide catalyzed by iron-sulphur $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ clusters to produce formate (HCOO^-), oxalate ($^- \text{OOC} \cdot \text{COO}^-$) and CO, though in non-aqueous conditions. Protoferredoxins have about 80% of the activity of the true ferredoxins (Christou & Garner 1980; Bonomi *et al.* 1985; Holm 1992). Here is one of the links to life as we know it, because every living prokaryote contains such iron sulphide proteins within their membranes. For example, the eubacterium *Thermotoga neapolitana* (Jannasch *et al.* 1988) is an obligate thermophilic (80°C) anaerobe that metabolizes sulphur, depends on tungsten for optimal growth, and generates H_2 , CO_2 and organic acids (Fiala & Stetter 1986). The 4Fe-4S ferredoxins in this organism are comparable with those in certain thermophilic archaeobacteria, and they probably evolved from a single 4Fe-4S cluster (Darimont & Sterner 1994).

Once these protoferredoxins had self-assembled, then more complex varieties may have formed, involving not only nickel but tungsten as well (Adams 1992). Depending upon their ligands the redox mid-points of both these metals can lie at low redox potentials, i.e. approximating to the hydrogen potential (Fig. 4). Individual nickel and tungsten atoms, in conjunction with proteins containing Fe_4S_4 (and in one case Fe_3S_4) centres, variously comprise enzymes that catalyse electron transfer reactions at extremely low redox potentials (Cammack 1988, 1996; Adams 1992; Volbeda *et al.* 1995). The redox switch would have operated by gain and loss of electrons as Ni(II) or W(VI) converted to Ni(I) or W(IV) and back, the Fe_4S_4 centres providing the electron pathway to the electron sink (Volbeda *et al.* 1995; Kletzin & Adams 1996). Both hydrogenation and aldehyde oxidation take place at such low potentials. During hydrogenation, nickel-iron hydrogenase cleaves hydrogen as an electron is lost to the Ni(II) centre. An adjacent iron centre is the binding site for the carbon oxides which are reduced to simple organic molecules (Cammack 1995). In a contrasting

reaction, the tungsten enzyme catalyses the oxidation of aldehyde (Chan *et al.* 1995; Kletzin & Adams 1996). In these early times, the nickel may have been supplied from non-sulphurous springs whereas tungsten would have been provided by the hydrothermal solution as WS_3^{2-} , WS_4^{2-} or WO_4^{2-} (Nekrasov & Konyushok 1982).

Although abiotic assemblies of nickel-iron-sulphur hydro-genases are yet to be synthesized in aqueous conditions, Sarkar & Das (1992) have generated a functional model of a tungsten enzyme in the laboratory, $[W^{IV}O(S_2C_2(CN)_2)_2]^{2-}$, using simple organic ligands. Given this work, we imagine tungsten to have been ligated in the earliest membrane through carbon/sulphur/nitrogen ring complexes of the form generated by Cole *et al.* (1994). So, because of the likely self-assembly of this and other protoferredoxins, we argue that the improved membrane had the capability both to anabolize and catabolize organic molecules. The laboratory demonstration of such behaviour is a requirement of the hypothesis.

As commented above, the sulphur bond of an organic sulphide is used in group transfer (de Duve 1991), for example, in the priming of the reverse citric acid cycle (see below) (Figs 14 & 15). Oxidation of the organic sulphide also generates dimers through the disulphide bond, behaviour likely to lead to further modification of membrane properties (Russell *et al.* 1994, fig. 8). In this reaction, in the first protocell, the electron would also have been transferred *via* the iron layer in the mackinawite crystallites, and later *via* the Fe_4S_4 centres comprising the protoferredoxins.

Several other d-block elements (e.g., Co, Zn and Mo) can be sequestered by organic sulphides and may have been significant in earliest life, but such ligation was not an option available for the lithophile p-block element, aluminium.

Carbon and hydrogen

Carbon dioxide in the ocean and hydrogen in the hydrothermal solution would both have been available at the ocean floor seepages, at concentrations of about 300 mmol in a litre of the respective fluids (Evans *et al.* 1994; Wiebe & Gaddy 1934). Both the hydrogen and the carbon dioxide may have been concentrated by adsorption in the membrane. Hydrothermal hydrogen was probably the primary fuel that energized emergent life. (The localized emission of hydrogen generated during the formation of pyrite, envisaged by Wächtershäuser (1988), would by contrast have been minimal; Shock *et al.* 1995, pp. 151–152).

So that we may appreciate redox relationships between organic molecules, they are arranged in order of oxidation state Williams (1961). A simplified energy scale of some carbon chemicals used for present-day life processes can be constructed, with hydrogen at the base (provider of energy as electrons) and oxygen at the top (energy/electron sink), all in equilibrium with H_2O . The sequence (see Figs 9 & 16) is: $(H_2)CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow CH_3 \cdot COOH \rightarrow CO \rightarrow CO_2(O_2)$. Only neighbours can coexist in equilibrium, except for the possibility of further intermediates. Thus CO_2 and H_2O are in equilibrium with carbonic acid and bicarbonate on the Earth's surface in oxic and anoxic environments. However isomers, polymers and metastable states add to the complexity of organic systems: formaldehyde H_2CO , acetic acid CH_3COOH , and monosaccharides $(CH_2O)_n$ (e.g., glucose $C_6H_{12}O_6$), as well as $CH_4 + CO_2$, $CO + H_2$ and $C + H_2O$, all have the same C:H:O ratio, i.e. CH_2O (Fig. 16). This is the approximate chemical composition and oxidation state of organic life since glucose,

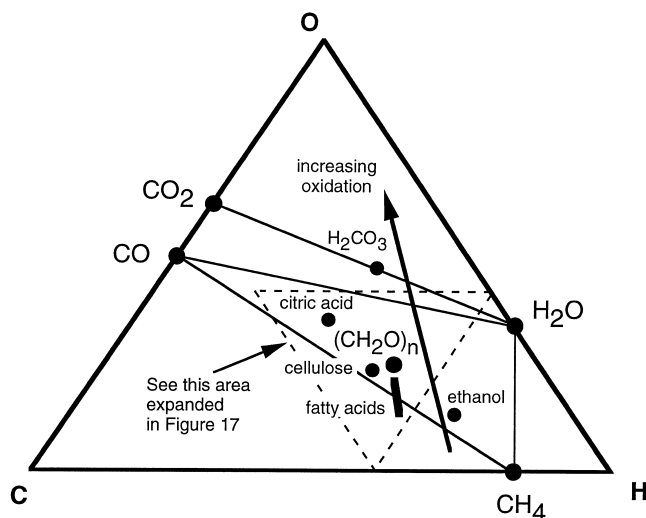


Fig. 16. C-O-H phase diagram showing low temperature stability fields. $(CH_2O)_n$ (formaldehyde, acetic acid, glucose) represents the approximate composition and oxidation state of the organic carbon of present day life. (Citric acid $COOHCH_2COHCOOHCH_2COOH$, ethanol CH_3CH_2OH .)

polymerized by condensation (non-redox loss of H_2O) as the polysaccharide cellulose $[C_6(H_2O)_5]_n$ (Fig. 9), is so abundant. It is also the central point of organic redox, where the carbon oxidation state is zero (Williams 1961). The sequence: formaldehyde, acetic acid, glucose, is one of increasing bond strength. This indicates that redox, although the most fundamental, is only one of the influences on organic reactions, and that isomerism, polymerization, hydration/condensation (driven by the protonmotive force) as well as molecular conformation (stereochemical shape), also have roles to play in metabolism.

Thermodynamically metastable (i.e. kinetically stable) organic molecules on the Earth's surface are protected within living systems (Williams 1961); organic matter and methane cannot exist in equilibrium with free oxygen (Fig. 16). In anoxic conditions the thermodynamically stable molecules are H_2O , CO , CH_4 and CO_2 . Shock (1992) also considered the possible organic molecules in hydrothermal systems as 'metastable', so although they can form at medium hydrothermal temperatures, they are thermodynamically unstable relative to H_2O , CO , CH_4 and CO_2 .

The notion that carbon dioxide is likely to be reduced to organic compounds at hydrothermal temperatures below $250^\circ C$ has been widely argued (Corliss *et al.* 1981; Hall 1986; Ferris 1992; Shock 1992, 1996). In particular, Shock (1992, 1996) has carefully calculated the metastabilities of carboxylates, ketones and alcohols as inorganic carbonate in seawater mixes with hydrothermal solutions, with their fugacities buffered by both quartz-magnetite-fayalite (QFM) and the more oxidized pyrite-pyrrhotite-magnetite (PPM) mineral suites. The fugacity of carbon dioxide is taken as 10 bars, the presumed atmospheric pressure in the Hadean (Walker 1985; Kasting *et al.* 1993). We should note that this is a conservative value as it is likely that leakage of high pressure CO_2 from the mantle could have come close to saturating the deep ocean for short periods (cf. Zhang 1996). Shock (1996) demonstrated that the possible synthesis of organic molecules between 50° and $250^\circ C$ is sensitive to the fugacity of the hydrothermal solution, which must be buffered by QFM for all inorganic carbon to be converted to organic molecules. This is still a

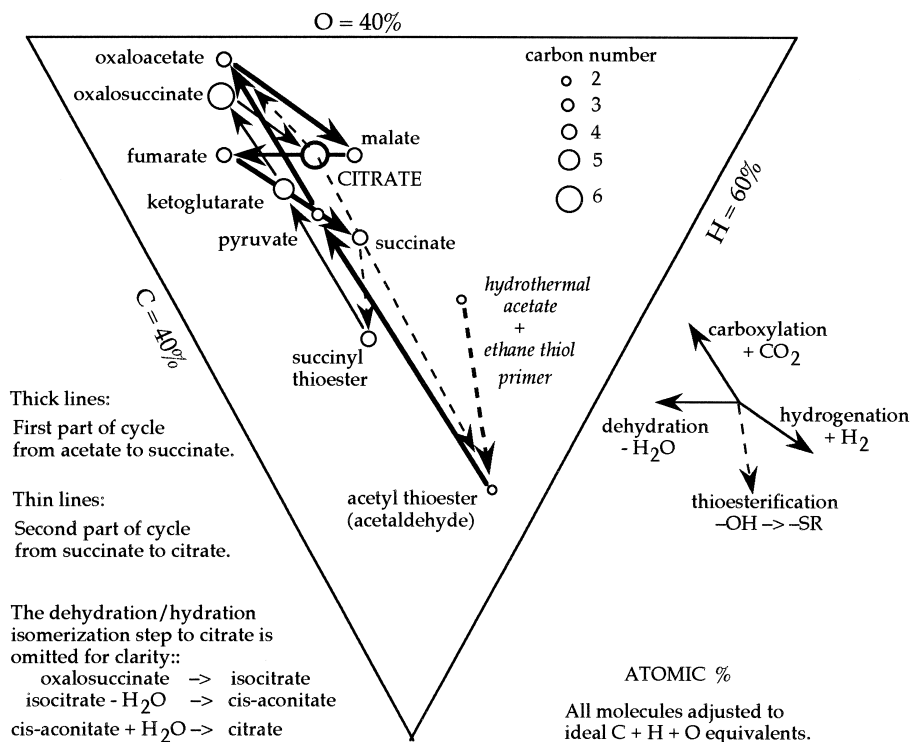


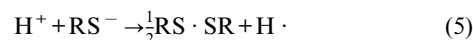
Fig. 17. Reverse citric acid cycle within C-H-O diagram. The iron sulphide protocellular micro-environment on the ocean floor at the hydrothermal/seawater interface, provides the four chemical forces (carboxylation, dehydration, hydrogenation, thioesterification) necessary to drive the cycle, by a progressive increase in carbon number, to the molecule citrate. The cycle is primed by thioesterification (using hydrothermal ethane thiol) of hydrothermal acetate to acetyl thioester. Thioesterification is a replacement of an hydroxyl (-OH) group by -SR driven by a condensation; since -SR is nominally replaced by H for plotting above, the thioesterification step appears as the loss of one oxygen atom (e.g. acetate→acetaldehyde). The cycle consists of two congruent angular paths which spiral towards citrate because in such a presentation the distance represented in atomic % by a molecular change decreases with increase in carbon number. At the 'top' of the cycle, citrate disproportionates in the presence of thioester to oxaloacetate and acetyl thioester, and the cycle repeats in a positive feedback loop.

conservative choice, since 4.2 billion years ago the redox state of the mantle was probably two or three $\log f_{\text{O}_2}$ units below that of the quartz-fayalite-magnetite buffer (Arculus & Delano 1980). Remarkably, Shock (1996) demonstrated that at temperatures below about 150°C, the longer chained polymers (dodecanoate is the longest chain considered so far) will theoretically be most represented of all the organic molecules, and that they could be generated at no energy cost.

Based on Shock's (1996) calculations, we surmise that organic molecules could have been generated from the carbon oxides 4.2 billion years ago in a strongly reduced hydrothermal system: (i) in the descending limbs of the hydrothermal system, as surviving carbonic acid in the Hadean seawater was reduced during reactions with Fe(II); (ii) where high temperature carbon oxides derived by mantle degassing became entrained in the relatively cool hydrothermal solutions; (iii) where the hydrothermal solution mixed with the carbonic seawater by entrainment immediately beneath the hot spring; (iv) at the point of exhalation of the hydrothermal solution with the seawater (Fig. 1). Off-setting the generation of organic molecules in the hydrothermal solution itself, is the likelihood of much of the inorganic carbon being lost to carbonation reactions (e.g., Griffith & Shock 1995). Given these assumptions, Shock (1992) has argued that, of the common organic molecules, only acetate, ethanol and aldehyde were delivered to the exhalative site. The only activities of aqueous carbon-bearing species that attain significant values are acetic acid (the feeder to the citric acid cycle) with a value approximating

100 μmoles (see Shock 1992, fig. 2), and ethanol. It is these two species that we use in our model (Figs 1, 7–9, 14, 16 & 17), although we assume alcohols to be partially replaced by thiolates such as ethane thiolate ($\text{CH}_3\text{CH}_2\text{S}^-$) in the sulphide-rich milieu (cf. Kaschke *et al.* 1994; Heinen & Lauwers 1996). Bearing in mind the presence of zeolites and clays within the basaltic and komatiitic wall-rocks, we surmise that any insoluble organic molecules formed deep within the hydrothermal system would not have been continuously delivered to the spring, and that charged organic molecules would have been delivered only sparingly.

The model therefore envisages the delivery of hydrogen, acetate, bisulphide, ammonia, and possibly some nucleic acid bases such as adenine and uracil (Ferris 1992), from the hydrothermal fluid to the iron sulphide mound and membranes (Figs 1, 9 & 17). Ethane thiol and hydrogen, perhaps in a more active state, would also have been generated in the membrane by the protonmotive force. In this process we imagine protons from the Hadean ocean oxidizing organic sulphide to disulphide in the protocyttoplasm *via* $[\text{Fe}_4\text{S}_4]^{2+/+}$ centres ligated to the ethane thiol in the membrane (Russell *et al.* 1994, fig. 8):



Carbonic acid for carboxylation also permeated through the membrane from the Hadean ocean to bring about the kind of mixing required by Shock's calculations. The initial

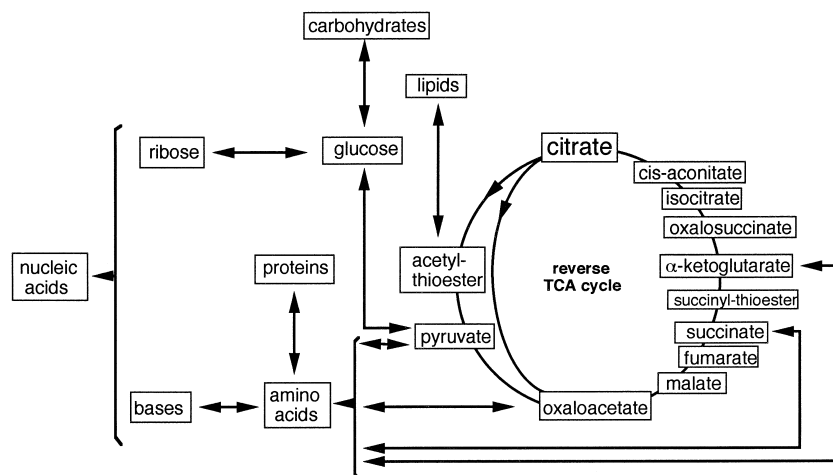
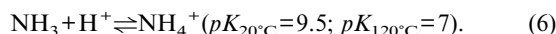


Fig. 18. Biosynthesis based on the central role of the reverse TriCarboxylic Acid cycle (citric acid or Krebs cycle). After Buchanan *et al.* (1969).

polymerizations depended on the formation of a thioester of acetate (Figs 14, 15). From this active molecule, two main polymerizations are likely (Fig. 18). The first produces the stable fatty acids with high numbers of carbon atoms within the chains (Shock 1996). This type of polymerization relies on hydrogenations and would be expected to take place in iron sulphide compartments closer to the hydrothermal feeders (where hydrogenations would be the main reaction). The second is the pathway to unstable citrate which requires carboxylations as well as hydrogenations, and is more likely to take place in compartments at the surface of the mound (Figs 1 & 14). Once generated, citrate breaks down to oxaloacetate and acetate again, and a positive feedback or autocatalytic cycle begins. This is the citric acid (tricarboxylic acid, or Krebs) cycle driven in reverse by the high CO_2 partial pressure (Hartman 1975; Wächtershäuser 1990) (Figs 14, 15, 16 & 17). The generation of the di- and tri-carboxylates increases the osmotic pressure within the surface compartments, a pressure that would act on the inorganic membrane to cause distension and budding (Russell & Hall 1995). The fresh iron monosulphide, 'doped' with nickel (Vaughan 1969, 1970; Morse & Arakaki 1993) and/or protoferredoxins, would have catalysed these polymerizations.

Nitrogen

The first abiotic synthesis of a biological molecule, urea ($\text{NH}_2 \cdot \text{CO} \cdot \text{NH}_2$), was effected by Wöhler in 1828, simply by heating ammonium cyanate (NH_4CNO). Whether urea was available in the hydrothermal solution is unknown, but Shock (1992) has calculated that nitrogen would have been present as a component of ammonia, stable in the alkaline hydrothermal springs. Ammonia could have come from the mantle via degassing during magmatism and/or partial dissolution of ammonium-bearing minerals such as feldspars. Atmospheric fallout would also have contributed any ammonia which survived photo-oxidation. Some of this could also have entered early cells by the passive diffusion of NH_4^+ across the membrane and its pH dependent dissociation to NH_3 and H^+ , just as in some present-day bacteria (Drews & Imhoff 1991):



Based on their experimental work, Hennes *et al.* (1992) have argued that amino acids would have been generated

within the hydrothermal system (but see Shock 1992). Be that as it may, amino acid synthesis and condensation to short peptide chains within the developing iron sulphide protocells, would have been an essential step preceding the evolution of reproducing cells that were capable of surviving remotely from a hydrothermal system. Anyway, given that the Hennes experiments were carried out under ten atmospheres of CO_2 with H_2 buffered by PPM, they better simulate the possible reactions in the protobiotryoidal 'culture chambers' than they do the hydrothermal system *sensu stricto* (see also Marshall 1994). Hydrogen cyanide (HCN) was a necessary ingredient in Hennes *et al.*'s (1992) experiment, and they speculated that it was likely to have been present in the hydrothermal fluid (Ferris 1992).

It is possible, though undemonstrated, that adenine and other nitrogen-containing bases such as uracil could have been generated by self-condensation, especially as such condensations would have been favoured in the alkaline conditions obtaining in the hydrothermal system feeding the growing sulphide mound (Ferris 1992; cf. Oró & Kimball 1961, 1962) (Fig. 3). In the most reduced parts of the Hadean crust, HCN is likely to have been present (Ferris 1992) and to have made its way to the convecting solutions feeding the ocean floor springs and seepages (Fig. 1). Once in this mildly alkaline solution it would have self-condensed to diaminomaleonitrile (Sanchez *et al.* 1967), an intermediate in the formation of the purine ring and required for the generation of a nucleotide base (Ferris & Orgel 1966) (Fig. 9). The formation of this intermediate is accelerated in the presence of formaldehyde (Schwartz & Goverde 1982). Alternatively, adenine may have been generated within the protobiotryoid from oxaloacetate, ammonium and hydrogen.

Phosphorus

Equation (7) illustrates a fundamental reaction in life processes since it transfers 270 millivolts of electrochemical energy or 'proticity' (cf. electricity; Mitchell 1979) for molecular biosynthesis (Thauer *et al.* 1977) (Fig. 2).



Phosphate is extremely insoluble in alkaline solutions in the presence of calcium (Arrhenius 1987), but occurs as water-soluble polyphosphates in acid solutions *via* the partial

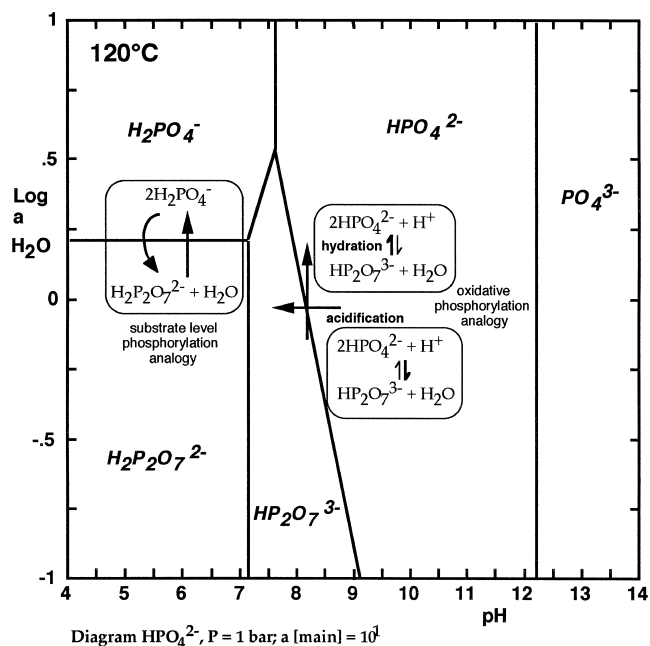


Fig. 19. This diagram, computed using Geochemist's Workbench (Bethke 1992a, b; 1996), illustrates schematically how high energy (stable at lower pH, lower water activity and higher temperature) polyphosphate can form by polymerization of low energy monophosphate (note the high activity of phosphate used in this diagram). The polyphosphate has the capacity to drive dehydration polymerizations on its hydration by the reverse reaction. This is analogous to the ATP/ADP energy cycle of life which takes place within bacterial cells at a water activity of about 0.8 and pH about 7.5. Phosphorylation can take place in two ways. In oxidative phosphorylation the dehydrating power of ATP is renewed by the protonmotive force (acidification). In substrate-level phosphorylation ATP is renewed by removal of H and OH by an NAD associated enzyme and is therefore possibly a later development.

hydrolysis of volcanic P_4O_{10} (Yamagata *et al.* 1991). However, it would therefore have been available as phosphate in the mildly acidic Hadean ocean, even in the presence of iron (Fig. 20). Phosphate could have been adsorbed as diphosphate onto the membrane where, mediated by a thioester (de Duve 1991) and protected from calcium ions by the sequestering tricarboxylic acids, it could have provided anabolic energy through rehydration (Fig. 19).

This is similar to how adenosine triphosphate (ATP) works as it converts to adenosine diphosphate (ADP) and more rarely to the monophosphate (AMP) in living systems (Berger 1973). The monophosphate could then have been oxidatively rephosphorylated to the diphosphate by the protonmotive force operating across the membrane. If rehydration of inorganic diphosphate (PP_i) took place in the interior of the protocell on reaction with alkaline hydrothermal solution, it would just have released thermal energy. Only if it were coupled to a dehydrating condensation would it be useful in synthesis (de Duve 1991, p. 7), for example, in the polymerization of the simplest amino acid, glycine, to the dimer (eqn. 3). In this overall reaction, OH is removed from the carboxyl group of one monomer, and H from the amine group of the other monomer, a dehydration which perhaps could take place in the environment of the iron sulphide membrane where the activity of water would have been relatively low (cf. Wächtershäuser 1992). Mechanistically, an orthophosphate group released

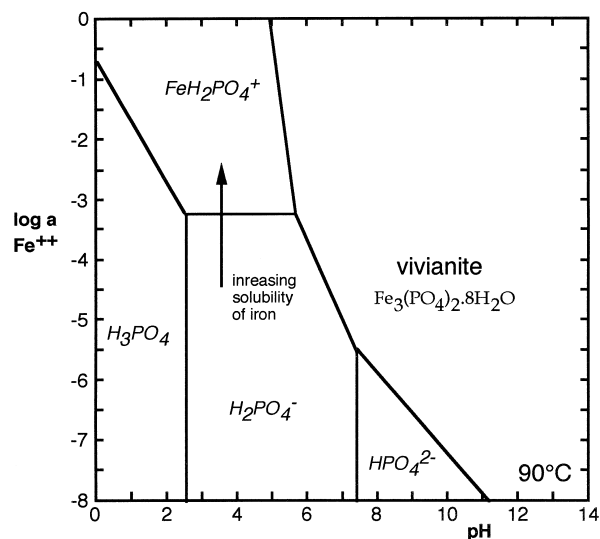


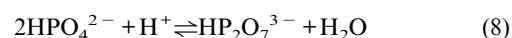
Fig. 20. Diagram constructed using Geochemist's Workbench (Bethke 1992a, b; 1996) illustrating how iron can have a high solubility (high $\log a \text{Fe}^{++}$) in relatively acid seawater when solubility in presence of phosphate is controlled by vivianite, $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$, which is insoluble in alkaline solution.

from the di- or pyrophosphate replaces the hydroxyl, and the entirety is then transferred to a hydrogen site of an adjacent amine where orthophosphate is released along with a hydrogen ion as the amino acids dimerize.

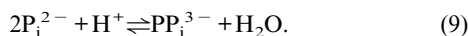
Wood (1977, 1985) and Thauer *et al.* (1977) have pointed out that while the free energy of hydrolysis of inorganic diphosphate ($\Delta G^{\circ}_h = -21.9 \text{ kJ mol}^{-1}$) is less than that of ATP ($\Delta G^{\circ}_h = -31.8 \text{ kJ mol}^{-1}$) under physiological conditions, it is high enough to provide an energy source for most synthetic reactions such as that figured in equation (3). Lipmann (1941) called the phosphate to phosphate bond a 'high energy' bond because of its potential to do biochemical work, and its significance in dehydration reactions. Strictly speaking though, it is a relatively weak bond.

The processes shown in Fig. 19 represent entirely inorganic models for, but possible evolutionary precursor reactions to: the oxidative phosphorylation (also possible biotically with inorganic phosphate) of ADP to the coenzyme ATP by protonmotive force; and the possible later development of substrate level phosphorylation of ADP, through the removal of H and OH by NAD in association with an enzyme (Fig. 9).

It is because the ATP reaction, involving as it does a complicated organic 'tail', could not have been part of the first steps, that we follow Wood (1977) and Baltscheffsky & Baltscheffsky (1992) in envisaging inorganic monophosphate re-dimerizing to the diphosphate in the membrane (where it might concentrate on the surface of mackinawite) as protons translocate from the outside to the inside of the iron monosulphide bubbles (Figs 1 & 19). The re-dimerization of the monophosphate could have taken place abiotically because the iron monosulphide membrane separated fluids of contrasting pH values, and the mean pH coincided approximately with the value at which inorganic pyrophosphate is in equilibrium with monophosphate:



or



For diphosphate in ocean water to be used directly in this way, it would have had to have been in the millimolar rather than the micromolar range it is today (de Duve 1991, 1997), a possibility considered likely by Arrhenius *et al.* (1993).

The involvement of phosphate at this early stage in metabolism probably locked in this component as a structural item in the phosphate-based coenzymes, as well as in the information system of RNA and DNA. Phosphate provides a good example of how aspects of cell metabolism are prefigured in the geochemistry of the hydrothermal environment.

Protometabolism

Shock & Schulte (1995) demonstrated that, while the mixing of seawater with hydrothermal solution buffered by pyrrhotite–pyrite–magnetite in the crust leaves CO_2 in the carbonate field, mixing of seawater with highly reduced hydrothermal solution buffered by quartz–magnetite–fayalite favours the formation of metastable organic molecules between *c.* 50° and 200°C. Carboxylic acids dominate, though alcohols and ketones are prominent above *c.* 130°C. Also, as the temperature of the mixture falls, larger organic molecules are favoured. So we may imagine that if the oxidation state of the hydrothermal fluid is increased, for example by reaction with Fe^{3+} in the membrane, then inorganic carbonate species again become more stable. As Shock & Schulte (1995) pointed out, it follows that ‘fluid mixing could drive the geochemical equivalent of a metabolic system’. We suggest that the site where the transition was made from a geochemical metabolism to a biochemical metabolism was in the catalytic iron monosulphide occupying the interface between the hydrothermal solution and Hadean ocean water. All that is required for the successful encapsulation of this process is that anabolism should exceed catabolism. It follows that protometabolism, like metabolism itself, was not restricted to the two dimensions of a growing pyrite crystal (Wächtershäuser 1990), but operated in three dimensions coupled to large scale hydrothermal convection.

The differentiation of protoanabolism from the hydrothermal system, and its full unification with metabolic processes operating in the primitive cell, probably had to await the development of the DNA/RNA code.

Miniaturization and the organic take-over

Organic take-over, by the synthesis of long-chained fatty acids ($\text{CH}_3 \cdot [\text{CH}_2]_n \cdot \text{COO}^-$) at 100–150°C (Shock 1996) and more complex molecules (with mixed hydrophobic and hydrophilic characteristics) involving nitrogen and sulphur (Fig. 9) (Kimoto & Fujinaga 1988, 1990; Cole *et al.* 1994) within the iron monosulphide culture chambers, would have led to an immediate scaling down of compartments through an order of magnitude or so. As these organic polymers were generated, they could have assembled themselves on the interior of the inorganic membrane. They could also have formed into smaller bubbles or micelles within the iron sulphide compartments. Although small concentrations of such organic molecules, acting as a surfactant or detergent, would have added flexibility to the iron sulphide bubbles, larger concentrations would have weakened the inorganic membrane during distension, and the colloidal iron monosulphide membrane would have been lost as organic polymers took over the role of

keeping the two fluids of contrasting redox apart. The organic polymers would have moved through the porous filter that constituted the interior of the iron sulphide mound, to build up eventually on the outside surface of the mound, forming a layer of reproducing vesicles. This is the link with the coacervates of Oparin (1957) and the liposomes of Luisi’s group (Oberholzer *et al.* 1995) outlined in the introduction. However, iron and sulphur are still retained in the membrane, in enzymes, in all prokaryotes. One reason for this is their affinity for organic molecules, i.e., the stability of organic sulphides and the thiophilic and oxyphilic nature of both ferrous and ferric iron.

Some of these predominantly organic vesicles may have been naturally inoculated with free nucleobases, as well as nucleobases attached to a peptide backbone (PNA), rather than the complex sugar phosphates that provide structure to RNA and DNA (cf. Nielsen 1993; Oberholzer *et al.* 1995). Perhaps the nucleobases and peptide polymers were generated in the FeS compartments in the hydrothermal mound, but how this happened remains entirely obscure. Tentatively, we assume that polymerization of the bases *via* peptide links was aided by the mineral sulphide surfaces in the membrane (Wächtershäuser 1992). Perhaps an interim stage in chemical selection and replication was provided by molecules generated in the system, through random catalytic interactions, which had the effect of propagating their own chemical compositions (Kauffman 1993; Lancet *et al.* 1996). Whatever the initial system of replication, colonies of these ‘minimal organic cells’ would have resembled iron sulphide microbialites (Russell 1996*b*). Such a morphology is likely to have arisen as the sulphides were not only precipitated by these metabolising protocells, but also because the mucilaginous surface would have trapped sulphide particles (cf. Burne & Moore 1987).

The far-from-equilibrium conditions in which these geochemical processes and mechanisms operated would have been widespread for a limited period of Earth history, and would have provided ample opportunity for such a unique sequence of events leading to the minimal cell, the common ancestor of all life on this planet. Although coupled to a long-lived hydrothermal system, the actual gestation period for organic synthesis and the self-assembly of organic protocells capable of fledging and replication from within the iron monosulphide hatcheries would have to have been rapid, and may have taken weeks or months, rather than the millions of years normally assumed for the emergence of life.

Discussion of uncertainties

There are still difficulties with this hypothesis, mostly to do with concentrations of both the organic and inorganic phases for the supposed development of compartments. For example, in our simple experiments, it has been necessary to increase the concentrations of Fe(II) and HS^- up to 25-fold (i.e., to 500 mmol) in the two solutions, in order to achieve the morphologies broadly comparable to those found in the Tynagh orebody, although membranes do form at 20 mmol.

The concentrations of organic molecules estimated to be available in the hydrothermal solution (Shock 1992) were also appreciably lower than required for most experimental syntheses of complex molecules (Hennet *et al.* 1992; Cole *et al.* 1994), although we have argued that the iron monosulphide membrane would have acted to concentrate these (cf. Leja 1982). Moreover the relative proportions of organic molecules derived from: (i) the early crust, (ii) in Fischer–Tropsch-like

reactions (Ferris 1992), or (iii) generated within the iron sulphide compartments themselves, are not known. Even though organic molecules, generated in or gleaned from the crust, were required to prime earliest life, eventually life 'discovered' how to generate organic molecules straight from carbon dioxide. Therefore we still need to puzzle out the precise makeup of the 'natural' catalysts that would bring about the generation of the fatty acids, ketones and alcohols revealed by Shock (1996) as the likely metastable products of the mixing of hydrothermal waters with ocean. Another possibility is that the more easily reduced carbon monoxide was the source for the first organic molecules, and that the early biological catalysts that successfully reduced this molecule subsequently evolved to fix carbon from the dioxide.

These uncertainties highlight an old controversy between those who see life beginning with organic molecules as both fuel and material, i.e., those believing in heterotrophy (e.g., Oparin 1938) and those who believe life emerged from purely inorganic substances, i.e., those advocating autotrophy or lithotrophy (e.g., Hartman 1975). It may appear that we have rather hedged our bets over this dispute, but overall we favour an autotrophic origin of life. Doing so requires that we consider the entire hydrothermal system as a pre-living entity and that evolution brought about a miniaturization of scale from kilometres to millimetres and eventually to micrometres (Russell *et al.* 1994). Yet we cannot exclude the possibility that some exotic organic molecules were required for the first functioning of a metabolizing system as suggested by Deamer (1985).

We would like to emphasize that the equilibrium diagrams calculated using Geochemist's Workbench (Bethke 1992a, b, 1996) have been designed to make specific points about the nature of processes we envisage rather than to give accurate geochemical conditions. Computation of such diagrams requires the use of exact geochemical values which are given by the programmer, and it is very difficult to express uncertainty. For example, we have chosen 90°C for the Hadean ocean water, 150°C for the hydrothermal solution and 120°C for the mixing environment. Gas fugacities and activities of species can also be fixed or controlled, but pressure is not an independent variable; it depends on temperature. The diagrams can aid the simplification of complex relationships and they can be applied to predict the directions that chemical processes may take in very complex systems. They can also be used to guide experiment. Care must be taken in using the diagrams for other than the specific points that they are designed to make.

Conclusions

The origin of life may be seen in the context of a chemically energetic open system coupled to a hydrothermal convection cell. The kind of medium temperature submarine seepage identified as the likely site for life's emergence, provides all the ingredients as well as the reducing power to generate a protometabolist. The model for the emergence of life outlined here sees the key molecular components (da Silva & Williams 1991) originating rapidly and coming together at an early stage. The main sources, processes and mechanisms are as follows.

(i) The spontaneous precipitation of a nickeliferous iron monosulphide membrane at the interface between an alkaline, off-spreading centre, submarine hydrothermal seepage and the

rather acid ocean. This membrane inhibited the flow of chemical and electrochemical energy between the two fluids (Russell *et al.* 1988, 1989).

(ii) The ocean provided positive ions and ionic complexes, particularly Fe(II) and Ni(II), initially derived from black smokers and sulphur-deficient hot submarine springs (Macleod *et al.* 1994). Fe(III) was generated from Fe(II) by photo-oxidation (Braterman *et al.* 1983).

(iii) The ocean also provided the phosphate and carbonic acid to the semi-permeable iron monosulphide membrane, as well as protons (activated by Ni to H⁺ in the membrane) (Arrhenius *et al.* 1993; Grotzinger & Kasting 1993; Russell *et al.* 1994).

(iv) The alkaline spring supplied reduced sulphur, hydrogen, ammonia, aldehyde, acetate and possibly cyanide and tungsten complexes (Shock 1992; Ferris 1992; Macleod *et al.* 1994; Nekrasov & Konyushok 1982).

(v) Assimilation of carbon dioxide and hydrogen at low Eh (c. 550 mV), medium hydrothermal temperatures (100–150°C), high CO₂ partial pressure (>10 bars, high pH ~9) (Shock 1992, 1996; Shock & Schulte 1995; Shock *et al.* 1995) was catalysed by mackinawite (Fe(Ni)_{1+x}S) crystallites and then protoferredoxins of the general formula [Fe₄S₄(S · CH₂ · CH₃)₄]²⁻³⁻, co-assembled from Fe₄S₄ clusters and ethane thiol generated in the membrane (Bonomi *et al.* 1985; Heinen & Lauwers 1996). Fe(III) in the ocean was probably the terminal electron acceptor.

(vi) A tungsten centre is especially important in catalysing aldehyde oxidation. Ligated to sulphur, both Fe(II) and W(IV) could be switched to Fe(III) and W(VI) and back again respectively, close to the water/hydrogen phase boundary. This behaviour augmented catalytic activity rendering Fe, W and S indispensable to hyperthermophilic archaeobacteria. These elements would therefore have been required by the earliest metabolist (Cammack 1996; Kletzin & Adams 1996).

(vii) Contiguous FeS compartments acted to isolate constituents in slightly differing pH and Eh conditions which controlled mutual accessibility of reactants and products (cf. Cairns-Smith 1982, pp. 311, 353).

(viii) The simple amino acids were generated within the iron sulphide compartments as carboxylic acids were aminated on reaction with hydrothermal ammonia and possibly cyanide (Hennet *et al.* 1992).

(ix) Organic sulphides were instrumental in the transfer and addition of organic monomers or small polymers, one to another, in particular organic syntheses (de Duve 1991).

(x) The naturally occurring protonmotive force of c. 300 mV was harnessed *via* the generation of inorganic diphosphate, to polymerize fundamental organic molecules such as amino acids by condensation (dehydration) reactions in the membrane. Diphosphate may also have been regenerated in the membrane by thermal cycling (cf. Muller 1985, 1995).

(xi) Once long-chained carboxylic acids (Shock 1996) and organic polymers (in part consisting of sulphur and nitrogen) (Cole *et al.* 1994) had reached high concentrations in the colloidal FeS bubbles, they either coated the interior of the membrane or organized themselves as micelles in the iron monosulphide compartments. These 'abiotic' polymers then took over the role of separating the two contrasting fluids (cf. Oparin 1957; Oberholzer *et al.* 1995).

(xii) As a result of this organic take-over there would have been a miniaturization through an order of magnitude or so, but the processes of translocation of molecules and production of energy would have remained somewhat similar.

(xiii) At this stage, although the hydrothermal solution would no longer have been channelled directly into the interior, hydrothermal hydrogen could have diffused through the organic membrane comprising the now individual cells. Additional hydrogen could have been generated by protoferredoxins, by the natural protonmotive force from the protons in the acidic ocean.

(xiv) Batteries of iron monosulphide $[4\text{Fe-4S}]^{1+/2+}$ centres would have been retained, in the sulphur-bearing organic polymers comprising this new membrane, as electron transfer conduits to the outer surface (cf. polyferredoxins).

(xv) Following the development of sets of molecules which could catalyse the further synthesis and replication of a metabolizing molecular ensemble (Lancet *et al.* 1996), self-replicating peptides (Lee *et al.* 1996), the PNA (peptide nucleic acid) (Nielsen 1993), and then the generation of the RNA and DNA required for individuation of organic cells, would have been rapid in this milieu. Nevertheless, the precise way that this system evolved to what Dyson (1985) has called a 'second origin of life' remains obscure.

(xvi) Once the first cells developed and became autonomous, they were free to colonize black smoker chimneys.

(xvii) Iron, sulphur and phosphate, inorganic components of earliest life, remain centrally implicated in metabolism.

E. Shock, M. Adams, T. Brown, G. Cairns-Smith, D. Daia, R. Daniel, C. de Duve, E. Jagger, J. Kusel, A. Lennie, J. Parkes, J. Sherringham, B. Simoneit, D. Turner, D. Vaughan, R.J.P. Williams and R. Woodall were generous with their time and knowledge in discussions. E. Shock and R. Daniel critically read an earlier version of the manuscript. The support, generosity and hospitality of GEOPIG is gratefully acknowledged. Western Mining Corporation kindly supported fieldwork. We thank the Natural Environment Research Council for financial support through grant numbers GR3/7779 and GR3/09926.

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Received 7 September 1994; revised typescript accepted 30 September 1996.
Scientific editing by Stewart Molyneux.