

Mini-Review Article

Is H₂ the Universal Energy Source for Long-Term Survival?

R.Y. Morita

Department of Microbiology, College of Science and College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, OR 97331-3804, USA

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ABSTRACT

This review revisits anabiosis (cryptobiosis or latent life); but more specifically with the discrepancy (time factor) between the finding of viable bacteria in ancient material and the racemization of amino acids and depurination of DNA that would have contributed to their death. The omnipresence of H₂ in the biosphere since life began, its ability to penetrate the microbial cell, its low energy of activation, its ability to form protons and electrons in the presence of Fe(II), and its (including electrons and protons) role in many biochemical reactions make H₂ the best candidate as the energy of survival for microbial cells. Although the concentration of H₂ in most environments is below the threshold level for microbial growth, the surviving cells have a long period of time to carry out the necessary metabolism to offset the racemization and depurination processes. This paper explores a hypothesis that explains this discrepancy.

Introduction

Survival is part of living. The biological pressure for survival is paramount for the continuation of the species. Obtaining energy (food) is the main objective for all forms of life in order to survive. Many organisms formed alternative ways to survive when energy was scarce or nonexistent. Anabiosis, also known scientifically as “latent life” and “cryptobiosis,” has been revisited lately by Cano and Boruchi’s [16] rediscovery of Gilippe’s [37] bacteria in amber and by the identification of microorganisms on material associated with the iceman [51]. Recently, Lambert et al. [67] isolated *Staphylococcus succinus* from Dominican amber, estimated to be

25–40 million years old. Because of this situation, it is time to revisit the concept of anabiosis, especially when so much interest is focused on detecting extraterrestrial life. Higher forms of life are known to undergo anabiosis and, if higher forms can do it, microbes can do it probably better. In 1990, Postgate wrote an article entitled “The microbes that would not die” [105] and, in 1994, he wrote a layman’s version of anabiosis in his book entitled *The Outer Reaches of Life*, [106] especially in Chapter 16 (“Immortality and the Big Sleep”). The time limits on microbial cryptobiosis have become an open question again, according to Gest and Mendelstam [cited by Postgate, 102]. Anabiosis, in many cases, involves starvation-survival coupled with anhydrobiosis

and cryobiosis. These conditions are certainly met with ancient samples taken from the Arctic and Antarctic ice cores.

The concept of anabiosis is not discussed in the current microbiology literature to any degree, and probably the Russian microbiologists are currently the main users of this term. For instance, Vorobyova et al. [124] attributes the survival of bacteria for some millions of year in permafrost to the anabiotic state of the isolates. One of the mysteries the authors bring up is the explosive growth of microorganisms when the frozen material is thawed, a fact that could not be explained by cryptic growth. However, I suggest that this explosive growth may be due to fragmentation (reductive division) of the cells due to the starvation-survival process.

An energy of survival was postulated and the energy of maintenance was relegated to a clone of a pure culture in a test tube, where one addresses the survival of that clone and not survival of the species [88]. Within any clone, there are survivors since all the cells do not die off at the same time. These survivors are the ones found in ancient material that are depleted of an energy source(s) [61, 88], but the fact is that the racemization of amino acids and depurination of DNA does take place over time [9, 103]. Amino acid racemization and depurination of ancient DNA is dependent on the presence of water, the temperature, and the chelation of certain metals to the protein [9 and references therein; 103 and references therein]. Hence, survival of DNA in warm climates is limited to a few thousand years and to 105 years in cold climates. According to Poinar et al. [103], once amino acids are isolated from the metabolic process, racemization takes place. The same is probably true for depurination. The question is how the organisms survive through geological time without being killed by the racemization of amino acids and depurination of DNA (other cellular damages are implied when depurination and racemization are mentioned). Unfortunately, we do not know how much racemization or depurination must take place before an organism expires. When amino acids are tightly bound in proteins and the purine tightly bound in DNA within the cell, do the calculations concerning the rate of depurination and racemization hold?

At one time, it was thought that the gene sequence and structural information on proteins from hyperthermophiles would provide the strategy for thermostabilization. This was not the case. Using the same trend of thought, one can ask: does the cell with its complex molecular structures, such as strong hydrogen bond network or chelation with certain cellular metals, provide the DNA and amino acids with a

protective mechanism(s) against depurination and racemization?

The survival of microbes in amber may be due to the anhydrous state of the amber matrix [103]. To date there has been no attempt to explain the discrepancy between the racemization and depurination rates with the finding of microbes isolated in ancient energy-poor geological material. This paper addresses this discrepancy in terms of the concept of energy of survival, which takes place so that racemization and depurination processes are minimized or repaired in order that anabiosis can take place for extended periods of time. Thus, energy of survival is the energy that the microbes use in metabolism to offset the depurination and racemization process, and it is not the energy necessary for growth and/or reproduction. Hence, the energy of survival results in an extremely slow metabolism, typically too slow to be measured.

Historical Background

In terms of microbiology, Leeuwenhoek [cited in Keilin, 60] showed that animalcules could be kept dry for several months with no signs of life and then be revived. Keilin [60], in his Leeuwenhoek Lecture at the Royal Society of London, presents an overview of the ideas and facts up to the time of his lecture. The term “anabiosis” (return to life) was introduced by Preyer in 1872 [cited in Keilin, 60] for the resuscitation or resurrection of completely lifeless (reversible state of dormancy) but viable organisms. However, “quiescence” or “dormant” has been used also. The term “quiescence” is used mainly by botanists. Keilin [60] further divides anabiosis into anhydrobiosis (due to dehydration), cryobiosis (due to cooling or freezing), anoxybiosis (due to the lack of oxygen), and osmobiosis (due to high salt concentration). Starvation-survival, as another form of anabiosis, can be added to the list. None of the different categories of anabiosis has been studied to any great extent. Cryobiosis and starvation-survival have received the most attention in microbiology.

The most common microbiological examples of anabiosis are the germination of bacterial spores of *Bacillus* and *Clostridium* formed by starvation, yet microbiologists are reluctant to use the term anabiosis or cryptobiosis, which are terms used frequently when dealing with higher forms.

Living Bacteria in Ancient Geological Material

Bacteria have been found in ancient geological material, both marine and terrestrial. The older data have been re-

viewed by Kennedy et al. [61 and references therein] and Morita [88 and references therein]. This subject was a hotly debated subject during the late 1920s and the early 1930s, when Lipman [71, 72] reported the recovery of bacteria in ancient rocks and coal that were millions of years old. Ghiorse and Wilson [40] published an early review of the microbiology of the terrestrial subsurface. Currently, there are many investigators dealing with the microbiology of the subsurface environment as can be seen in the books published and/or edited by Fliermans and Hazen [31], Chapelle [17], and Amy and Haldeman [4], as well as a special issues of *Geomicrobiology Journal* (vol. 7, No. 1 and 2, 1989), and *FEMS Microbiological Reviews* (vol. 20, 1997). From all the reports of bacteria in deep subsurface environments, there can be no doubt that microbes exist in ancient material. The diversity of various organisms in these environments is also striking. One of the latest documentations concerning microbes in ancient subsurface environments, more specifically the Taylorsville Basin in Virginia, is presented by Onstott et al. [95]. Fredrickson et al. [34] discusses pore-size constraints on the activity and survival of subsurface bacteria. Pore spaces in rocks are sufficient to accommodate bacteria but insufficient space for bacteriovores. Nevertheless, based on the concentration (radioactivity) of U and K in the geological formation, Onstott et al. [95] concluded that the bacterial activity was sufficient to repair the DNA damage by radiation within the time frame of 10 kyr.

Distribution of viable bacteria and their activity in subsurface samples of the Upper Ringold formation (White Bluffs, south-central Washington state) were spatially discontinuous, and, in some samples, no microorganisms or microbial activity could be detected by the methods employed [13]. Nevertheless, these microbes had the ability to maintain some level of metabolic activity over a minimum of 12,000 years in an extreme environment where sediment-associated nutrient levels are very low. The average annual recharge is 15 $\mu\text{m}/\text{year}$; hence, the nutrient flux from recharge must border on the nonexistent (because of the desertlike conditions at the surface). Survival does take place, and it is another example of starvation-survival.

In reference to hypothesized deep subsurface communities, Gold [44] states, "Life is not dependent on solar energy and photosynthesis for its primary energy supply and is essentially independent of the surface circumstances." Furthermore, the biosphere extends down to the upper portion of the Earth's crust [121] and a large portion of the biosphere may exist below the ground surface [44, 99]. In addition, living bacteria have been entrapped within sedi-

ments for most of this time [121 and references therein]. Throughout Earth's history, life has been primarily bacteriological, since higher forms evolved later [109].

"Most current theories for the origin of life have a common theme of chemical evolution, starting with the ordinary constituents of the atmosphere, crust and hydrosphere in some reducing environment, and resulting in chemoautotrophic bacteria-life forms" [80]. The earliest bacteria-life forms probably utilized H₂ as the energy source. Sulfur/hydrogen and Fe(III)/hydrogen autotrophy are used for energy generation in chemolithotrophic archaeobacteria and may represent a primeval mode of life [30, 120, 123 and references therein]. Both Fe(III) and sulfur were abundant in the prebiologic ocean [124 and references therein]. If life did originate in the subsurface (terrestrial or ocean) [10, 24, 44] where energy for life was derived by chemical processes, the common ancestral cell would be a thermophile and probably derived its energy source from H₂. (Nonhyperthermophilic common ancestral cells are also advocated by a few others [36 and references therein]). The cell would then be a thermochemolithotroph and the heterotrophs would have evolved through mixotrophy. Life could have originated in the subsurface since it could have been the most favorable location for the first generation of self-replicating systems [24], because rocks have hydrogen, methane gas, and other fluids percolating upward. The self-replicating systems may have evolved further and progressed upward as well as laterally with subsequent adaptation to lower temperature and other conditions. It would have been very improbable for life to be restricted to the proximity of the surface, since such living material would have to remain viable in the dormant state for very long times and it would not be protected from cosmic rays [24]. The various hypotheses on the origin of life and their evolution do not take into account "(1) the widely diverse set of physical and chemical conditions that would have been necessary for the sequential processes of chemical, biochemical, biological, and ecosystem evolution; (2) a continuous concentrated source of energy to drive these evolutionary processes; (3) a source of biologically important trace elements, such as molybdenum (associated with nitrogen-cycling reactions); or (4) the geologically short time interval between the formation of Earth's oceans and the appearance of microorganisms in the fossil record." The presence of H₂ takes care of item 2 in the foregoing [10].

Hydrogen-Driven Subsurface Ecosystems

However, not all microbes in the deep subsurface are in the starvation-survival mode, mainly because they have energy

more than sufficient for the energy of survival. These lithotrophic microbial ecosystems are driven mainly by inorganic energy sources, such as H_2 , CH_4 , and H_2S , etc. This ecosystem is not based on photosynthetic system. The oxygenic photosynthetic system was preceded by the lithotrophic microbial ecosystem, since fossil bacteria are found before oxygenic green plant photosynthesis evolved on Earth. The lithosphere can extend kilometers below the Earth's crust [39]. Examples of these ecosystems are presented by Stevens and McKinley [122], Stevens, [121], Parkes et al. [99], and Pedersen [102], as well as by the many studies on the microbiology of the hydrothermal vents [58, 59]. Methanogenic Archaea and homoacetogenic bacteria have been isolated in deep granitic rock aquifers [64, 100, 101]. In other words, bacteria have been isolated in ancient deposits where there is no apparent energy source [ex. [2, 16, 49, 124]. Thirty-five percent of the bacterial samples obtained from subsurface sediment (260 m below the surface in South Carolina) were capable of growing on H_2 [33]. Thus, it is possible, through syntrophy, that other organisms are provided with the necessary energy to remain viable but in a state of anabiosis. A review of chemolithoautotrophy is presented by Stevens [121].

The H_2 necessary to support microorganisms in the deep basals of the Columbia River basin, described by Stevens and McKinley [122], was hypothesized to be generated abiologically from the reaction of basalt and groundwater. However, Anderson et al. [5] produced evidence that the amount of H_2 produced by the basalt reacting with groundwater at an alkaline pH was not environmentally relevant. They further suggest that the H_2 produced could not be sustained over geologically significant time frames.

Survival Mechanism

In all probability, many of the organisms found in ancient material are in the starvation-survival state since their source of energy has been depleted by microbes themselves [87] and most environments on Earth are oligotrophic, even soil. There is a great diversity of microbes in any ecological environment; it is a rare exception when only one or two species are found in any environment. When an organism is stressed under one set of conditions, cross-protection usually occurs against other stresses. For example, the stress of starvation cross-protects against osmotic stress [57], against heat stress [57], and against high and low temperature [41, 42] due to the formation of stress proteins. (For an overview,

see [88].) Thus, it is possible that when organisms are starved (starvation-survival) and then desiccated by natural means, their ability to survive may be enhanced. Mesophiles are found in permafrost. These mesophiles were probably in the starvation-survival mode before they were frozen—mainly because their immediate environment was oligotrophic.

In nature, survival is the normal mode for most organisms and growth occurs only occasionally. For most microorganisms in nature, the period of no growth is much longer than the period of growth. If we are to search for extraterrestrial microbial life, conditions for the growth of microorganisms certainly do not appear to be optimal, if we assume extraterrestrial life is similar to life as we know it on Earth. Thus, extremophiles are being examined as the possible forms of extraterrestrial microbial life in outer space [82]. Nevertheless, the conditions on other planetary bodies in our solar system are harsh, and we must consider anabiosis.

One generally thinks in terms of organic matter as the energy source for the majority of microbes in nature. With such a large number as well as so many different physiological types of microbes in any environmental sample (there are exceptions), it does not take long before the energy source (organic matter) becomes scarce. As a result, Williams [127] termed soil “ultra oligotrophic” and soil has more organic matter than seawater. Morita [88] reviewed the organic matter content of various environments in detail.

Kieft and Phelps [63], in a paper titled “Life in the slow lane: activities of microorganisms in the subsurface,” state that the difference between potential and *in situ* activity of microbes is mainly due to methods. Laboratory microcosm estimates of microbial activity are often orders of magnitude higher than *in situ* rates [18, 102]. This is to be expected when the effects of perturbation, bottle (surface) effects, and addition of small amount of energy (primer effect), etc. are taken into consideration. Kieft and Phelps [63] give examples why the calculated rates of activity are not the *in situ* rates. Calculated rates of microbial activity are averaged over time and distance; whereas the actual rates of activity are temporally and spatially heterogeneous [3, 11, 89]. These slow rates of metabolism in the subsurface are supported by Chapelle and Lovley [18] and Phelps et al. [102]. This slow rate of metabolism will be directed primarily toward survival rather than growth [20]. Phelps et al. [102] suggested that many laboratory values overestimate the *in situ* rate by factors 10^3 to 10^6 over what geochemical models and knowledge of groundwater flows would substantiate. There is sup-

port for the emerging theory that individual microorganisms survive in some subsurface environments for millennia while carrying out cellular metabolism at a glacially slow rate [63]. Krumholz [65] concludes, that, in the lithotrophic microbial ecosystems, subsurface microorganisms appear to have developed mechanisms for survival so that anabiosis can take place. Phelps et al. [102] estimate that the average doubling time of the microbial biomass in Atlantic Coastal Plain subsurface sediment is on the order of centuries. One must also ask, when active bacteria are reported from ancient material, whether we have broken the suspended animation state by perturbing the sample, giving it a substrate or electron acceptor, different conditions from its *in situ* environment, etc.

Organic Matter as Energy of Survival

Microbial communities can include loose and tight associations among microbes and various terms have been applied to these associations, such as consortia, syntrophic associations, synergistic associations, mixed culture, or assemblages [118]. Slater [118] proposed seven different categories based on different biological mechanisms: “(1) provision of specific nutrients by different members of the community, (2) the alleviation of growth inhibition, (3) interactions which result in the alteration of individual population growth parameters, therefore producing a more competitive and/or efficient community performance, (4) a combined metabolic activity not expressed by individual populations alone, (5) cometabolism, (6) hydrogen transfer reactions, and (7) the interaction of several primary (i.e., dominant) species.”

Chapelle and Lovley [18] demonstrated that deep aquifer microbes metabolize organic matter at an extremely low rate. The results of Krumholz's et al. [66] indicate that the main energy source of bacterial communities living in shales (Cretaceous, 90–93 million years old) was organic matter, and that the microbial activity was greatly reduced because of the restrictive pore size. Krumholz et al. [66] and Krumholz [65] examined sands and muds alternately deposited during the Cretaceous period and suggest that the microbial activities are sustained by heterotrophic processes. Organic material slowly diffuses through shale pore throats that are, on average, much smaller than those of sandstones (<0.2 μm dia. for shales and up to 13 μm for sandstone). Slow diffusion of nutrients and of microbes themselves through these pores may limit the growth and metabolism of shale-bound organisms [34]. Ground shale, when added to sandstone, promoted microbial activity, whereas shale samples alone

did not reduce sulfate or produce acetate [66]. It was proposed that complex organic matter diffused from the shale to the sandstone where it is acted upon by fermentative and syntrophic bacteria to form fatty acids and hydrogen, which, in turn, were responsible for the formation of acetate from CO₂ by acetogenic bacteria or sulfide from sulfate by sulfate-reducing bacteria [66]. Thus, most microbial activity occurred at the sandstone–shale interface. Unfortunately, the extent to which perturbation of the samples played a role in these results was not discussed. When organic matter was laid down with the original microflora, most of the bioavailable molecules have been utilized, leaving the more recalcitrant organic molecules.

Nevertheless, Krumholz et al. [66] suggest that there are large areas of undiscovered biomass growing on ancient nutrient-rich deposits within isolated subsurface sedimentary rocks. Such a model would account for the long-term survival of microbial communities in the subsurface and justify searching for microbial life in unexplored environments. The question is, just how rich is “nutrient-rich”? The total organic carbon (TOC) was approximately 0.75% w/w in the Whitewater Arroyo shale and 1.5% in the Clay Mesa shale. Just how much of the material is bioavailable to the microorganisms when, during the diagenesis of sediments, the indigenous microbes would have utilized the labile components of the TOC readily, leaving the more recalcitrant material? This recalcitrant material is generally polymeric and requires energy to synthesize the necessary exoenzymes to degrade it into monomers so that the organic matter can be transported into the cell. Many cellular transport mechanisms also take energy. What is the source of such energy? Hence, the concept of bioavailability of the organic matter comes into question. (For a discussion of bioavailability of organic matter for microbial use, see reference [88].) When divided by millions of years, the metabolic rate would be negligible. Additionally, the metabolic utilization of the organic matter would take place more readily when the geological material was first deposited.

However, the possibility that slow degradation of recalcitrant organic matter might be the source of energy of survival for some bacteria, so that the organisms can repair proteins and DNA, should not be ruled out. When considering carbonaceous compounds, typically present at low concentrations in the environment and in mixtures of a high complexity, the turnover times may be as short as 2 h and as long as 5,000 years [50]. Many compounds in geological material are sequestered so that the microorganisms cannot access them (for an overview of the subject see [88])

Hydrogen, the Energy of Survival

In this paper, H₂ is stressed as the most important and dominant source of energy for cells to offset the depurination of DNA and the racemization of amino acids as well as other cellular damages that occur when cells are deprived of a readily available energy source. I do not want to rule out the possibility that organic matter, CH₄, or some other source may play a role also.

H₂, in its free elemental state, occurs terrestrially in relatively small quantities compared to other elements. Therefore, sampling for H₂ requires special precautions and the detection and quantification require specialized instrumentation. Some metal surfaces catalyze the production of H₂ in aqueous solution and therefore must be avoided during sample collection. H₂ is also a very dynamic compound. No adequate sample storage or preservation procedure has been found, mainly because of its penetrating powers [19]. When H₂ is considered as the energy of survival, we are dealing with concentrations below the threshold level needed for growth and reproduction (see section below); otherwise, hydrogen-driven microbial ecosystems may result when sufficient H₂ is available.

Hydrogen Production

Abiological. Abiogenic H₂ and CH₄ are produced in the Earth's crust, and Apps and van de Kamp [6 and references therein] present an excellent review of the hydrogen and methane produced in the subsurface environment. Basically, these authors list six possible mechanisms by which crustal H₂ is generated: "(1) reaction between dissolved gases in the system C-H-O-S in magmas, especially in those with basaltic affinities; (2) decomposition of CH₄ to carbon (graphite) and H₂ at $T \geq 600^\circ\text{C}$; (3) reaction between CO₂, H₂O, and CH₄ at elevated temperatures in vapors; (4) radiolysis of water by radioactive isotopes of uranium, thorium, and their daughters, and potassium; (5) catalysis of silicates under stress in the presence of water; and (6) hydrolysis of ferrous minerals in mafic and ultramafic rocks." Hydrogen is found in the upper mantle (ca. 300 to 680 km in depth) and diffuses upward toward the atmosphere. Process 1 has received much attention in current literature where the focus is on the hydrothermal vents and subduction zones of the ocean as well as the H₂-driven ecosystems mentioned above. H₂ resulting from radioactivity (process 4 above) may result from alpha bombardment of gaseous hydrocarbons [133].

Production of H₂ from method 5 above is discussed also by Stevens and McKinley [122 and references therein]. Gold [43] and Gold and Soter [44] maintain that H₂ and CH₄ should be chemically stable in the upper mantle and that it is migrating into the crust. Because of this situation, H₂ is present everywhere, and in many environments in very low concentrations. Most sites where H₂ and CH₄ can be generated abiologically are also those where no structural traps are present, and "the mechanism of hydrogen generation and escape from the Earth's atmosphere, which may have been operative for about 1×10^8 years must have progressively oxidized the magma ocean even though it was buffered by iron-containing minerals and carbon and its gaseous oxides" [6]. Venting of liquids and gases (energy for microbial life) from the Earth's mantle beneath the crust is not limited to cracks in the ocean floor and is widespread [52]. Process 6 occurs in geological material (ultramafic rocks) and is termed "serpentinization" by geologists; the subject is reviewed by Moody [86].

CH₄ and H₂ can also be produced by thermocatalysis of organic matter when hydrothermal fluids interact with organic rich sediments [38, 126].

Biological. The latest review on the microbial production of H₂ is given by Nandi and Sengupta [90], but was first reported by Popoff [104], followed by Fitz [35] and Hoppe-Seyler [55]. The early history on microbial H₂ production was presented by ZoBell [132]. In the early reports it was suggested that H₂ was produced in river or pond mud, lake mud, marine mud, soil, swamps, dead leaves, horse manure, human feces, septic tanks, sewage sludge, retting flax, oils, and elsewhere in nature. However, virtually all of the hydrogen produced is converted to methane within the soil before it escapes into the atmosphere [22]. The amount of H₂ in aquifers varies between 20 and 100 nM, whereas 10-nM concentrations are rarely exceeded when organic matter is fermented [65].

H₂ is produced by anaerobes including *Clostridia*, methylotrophs, methanogenic bacteria, rumen bacteria, Archaea, facultative anaerobes (e.g., *Escherichia*, *Enterobacter*), aerobes (e.g., *Alcaligenes*, *Bacillus*, *Klebsiella*), photosynthetic bacteria, and cyanobacteria. Many anaerobic fermentative bacteria dispose of excess reducing power by evolving gaseous hydrogen [48]. CO₂ and H₂ are produced when organic materials are decomposed under anaerobic conditions; hence, swamps, rice fields, waterlogged soils and the rumens of cattle are the habitats in which major amounts of H₂ are

produced. Other than the foregoing, the more common organisms and mechanisms will not be mentioned here, but only the unusual organisms and mechanisms.

Production of H₂ is not restricted to anaerobic environments; it can be produced in well-aerated soils. H₂ production by nitrogenase, a key enzyme of nitrogen fixation, occurs as well in root nodules [28, 110]. This process is the major source of hydrogen in soil [22].

Many heterotrophic hyperthermophiles in the absence of S⁰ produce H₂, that, if allowed to accumulate, will inhibit their growth [84]. Chemoorganotrophic anaerobic moderate thermophilic eubacteria (*Clostridium thermocellum*, *Clostridium thermohydrosulfuricum*) produced H₂ and CO₂ as fermentation products. *Thermoanaerobacterium thermosulfuricans* and *Thermoanaerobacter ethanolicus* also produce H₂ and CO₂ as fermentation products.

Occurrence and Distribution of Hydrogen

H₂ can be expected to be ubiquitous on Earth above the upper mantle [13, 44, 45] and, mainly because of its permeation property, it will diffuse through iron, glass, or polymers [8, 12, 32, 93]. H₂ is adsorbed as a molecule and, for glass and polymers, the rate of permeation varies directly with the pressure [93]. However, in the presence of iron, “H₂ is adsorbed as a molecule, but the competing attraction of the iron surface renders its H–H bond weaker. Dissociation results, as shown by the dependence of the rate of hydrogen permeation through iron on the square root of the pressure. The hydrogen then goes through as isolated atoms or proton and recombines on the other side” [93]. This indicates no dissociation of the gas on the surface; hence, H₂ penetrates these materials as molecular H₂. Gaseous H₂ will also penetrate steel (causing hydrogen embrittlement, etc.) and other metals, even at low temperatures [68]. For more specific details on permeation of gases through solids, see Norton [93].

Terrestrial Environment. H₂ occurs in igneous rocks and volcanic gases [21]. An early review of hydrogen in rocks is presented by ZoBell [133]. Only a small proportion of the H₂ reaches the aerobic layers of soil, mud, and water [128, 129, 132]. Analysis of 230 Franciscan rock and mineral samples (including San Luis Obispo ophiolite) indicates that H₂ occurs in the Franciscan form (Coast Ranges, California) [81]. Abiogenic CH₄ and H₂ are also found in significant quantities in mines in crystalline rocks in the Canadian and Fen-

noscandian shields [73], in Zambales ophiolite (Philippines) [1], and in mantle source rocks in Oman [91]. These gases are generated by hydrolysis of meteoric waters circulated through fractures in mafic and graphitic and metamorphic rocks [6]. CH₄ and H₂ gas seeps or significant groundwater concentrations of these gases have been reported in numerous localities with no clear relation to organic sources [1 and references therein], and in many cases these seeps are associated with mafic–ultramafic rocks. H₂ is also found in salt beds and freshwater lakes and sediments. Thus, we find H₂ in all subsurface environments above and including the upper mantle.

Ocean and Hydrothermal Vents. The surface waters of the ocean have concentrations of H₂ and CO far greater than atmospheric equilibrium would predict [14 and references therein]. Surface water concentration of these gases show variations between 0.18 and 1.96 nM [53, 111, 113, 114], but the waters are supersaturated. This H₂ probably originates from microbiological processes, especially in nutrient-rich water masses [113]. Although the sources of variations in H₂, CO, and CH₄ are not completely understood, they appear to be mediated by biological production and consumption processes [112]. The solubility of H₂ is 1.69 cm³/100 cm³ of water at 27°C. Although the predominant gases emitted by magmas at the midocean spreading centers and ocean island plumes are CO₂, H₂O, CO, SO₂, and H₂S, H₂ and CH₄ are present. Typical concentrations in surface water are 0.2 to 1.5 nM, and ambient deep water concentrations are approximately 0.2 to 0.4 nM [14, 53, 54]. H₂, like CH₄, is stable in oxygenated seawater, and the primary mechanism for its removal from the hydrothermal plumes appears to be microbial oxidation [125].

H₂, when sampled for, was found in the first hydrothermal vent system discovered [67]; H₂ was produced as well as consumed by the indigenous microbes [70]. Since then, many other hydrothermal vents have been studied, and H₂ has been found emanating from these vents. The H₂ concentrations found in the plumes may be low relative to those existing in hydrothermal fluids away from the vent because of the rapid oxidation by bacteria of H₂ [131]. The concentration of H₂ in mmol/kg of hydrothermal fluids was 0.16 to 0.42 for the Endeavor hydrothermal site, 0.20 to 0.53 for the San Juan de Fuca hydrothermal site, 0.23 to 1.7 for the 21° East Pacific Rise, 0.47 for the 11° East Pacific Rise, and 0.14 for the 13° East Pacific Rise [116]. Alteration of basalt by seawater would produce relatively high dissolved H₂ and

H₂S concentrations [115 and references therein]. Further reaction would result in the formation of pyrrhotite–magnetite, which would produce even higher H₂ and H₂S concentrations [116]. There are many data concerning the occurrence of H₂ in the vents of the ocean, for additional information see Karl [57].

Atmosphere. The chemistry of the atmosphere and the atmospheric composition is kept close to homeostasis largely as the result of the Earth's biosphere [22, 74]. Various concentrations of hydrogen in the air (0.5 to 1.0 ppm) have been given [93 and references therein], but these values can vary depending on where the samples are taken. Where there are no local sources of H₂ concentrations are close to atmospheric equilibrium [131]. Naturally, at higher altitudes, the concentration increases. In clean air, H₂ is more abundant than CO [47]. There is free H₂ in the atmosphere and in natural gases from various parts of the world. Samples taken 10 m above the ocean surface gave a mean value of 0.65 ± 0.02 ppmv in the mid-North Atlantic, and higher values were obtained near Africa [53]. Also, in volcanic gases, it ranges from less than 1% to 73%. At the CEPEX site, an average of 0.56 ppmv of hydrogen was found. H₂ is also expelled from volcanic gases, and its origin is the oxidation of magnetite to hematite [7]. It appears that the hydrogen concentration in the atmosphere is increasing at a rate of 3.2 ± 0.5 ppbv (10⁹) per year due to anthropogenic sources [62].

Permafrost. In ice cores taken from depths between 99 and 2,191 m through the Antarctic ice sheet near Byrd Station, Epstein et al. [27] reported the δ hydrogen (D/H) ratios only, but it demonstrated conclusively that hydrogen is present.

Microbial Growth with Hydrogen as the Energy Source

De Saussure [25] first observed the disappearance of H₂ from soil. Later, Immerdorff [56] proved that it was a biological process. Liebl and Seiler [69] noted that both CO and H₂ are destroyed at the soil surface and six different types of soil were capable of taking up H₂. There is an equilibrium between the production and the consumption of H₂ in soil [69]. It is well known that many soil organisms can utilize H₂ [26]. There are many genera and species of Knallgas bacteria, both gram-negative and gram-positive, and soil is considered the dominant sink for atmospheric H₂ [108]. The variations in H₂ concentrations in various environments are attributed to the presence of H₂-utilizing bacteria. In addition to the microbial utilization of H₂, soil hydrogenase can function aerobically and anaerobically.

Many organisms use molecular H₂ preferentially as an electron and energy donor and compete for the hydrogen available [107]. Photolithoautotrophs, photolithoheterotrophs, chemolithoautotrophs, and chemolithoheterotrophs all use H₂ as electron donors. All the species of *Hydrogenomonas* are capable of utilizing H₂ for energy, but there are various species in other genera that are gram-positive (*Arthrobacter*, *Bacillus*, *Mycobacterium*, *Nocardia*) and gram-negative (*Alcaligenes*, *Ancylobacter*, *Aquaspirillum*, *Derexia*, *Flavobacterium*, *Paracoccus*, *Pseudomonas*, *Rhizobium* and *Spirillum*) that are capable of utilizing H₂ as an energy source. These thermophilic genera capable of growth on H₂ are *Hydrogenobacter* and *Aquifex*. These organisms have a membrane-bound hydrogenase, the electrons generated are passed through an electron transport chain, and ATP is generated by proton pumping and membrane-bound ATPases. It should also be noted that lateral transfer of the hydrogenase gene can occur, since the hydrogenase gene is sometimes located on conjugative plasmids.

A genus that can oxidize both hydrogen and carbon monoxide is *Carboxydobacteria*, but *Alcaligenes eutrophus* and various species of *Azotobacter* and *Hydrogenophaga* also have the same ability. Acetogenic bacteria, mainly in the genera *Acetobacterium* and *Acetogenium*, utilize H₂. *Clostridium acetocum* also has the same ability. Cooxidation of substrates generally does not furnish cells with the energy needed for growth and metabolism.

Strict H₂-oxidizing bacteria oxidize H₂ under aerobic conditions without CO₂ fixation; hence they are different from other aerobic (*Acetobacter*, *Azotobacter*, *Enterobacteriaceae*, etc.) and anaerobic bacteria (*Desulfovibrio*, *Clostridium aceticum*, *Acetobacterium woodii*, *Methanobacterium thermoautotrophicum*).

Archaea as well as bacteria can be found in the hyperthermophiles. Stetter [119] provides the following hydrogen energy-yielding reactions occurring in the chemolithoautotrophic hyperthermophiles: (1) 2H₂ + O₂ → 2H₂O (*Pyrobaculum*,* *Sulfolobus*,* *Acidianus*,* *Metallosphaera*,* *Aquifex*), (2) H₂ + NO₃⁻ → NO₂⁻ + H₂O (*Pyrobaculum*,* *Aquifex*, *Acidianus*,* *Stygiolobus*, *Pyrodictium*,* *Thermoproteus**), (3) H₂ + S⁰ → H₂S (*Pyrobaculum**), (4) 4H₂ + SO₄²⁻ → S²⁻ + 4H₂O (*Archaeoblobus**), and (5) 4H₂ + CO₂ → CH₄ + 2H₂O (*Methanopyrus*, *Methanothermus*, *Methanococcus*). (The genera marked with an asterisk are facultative heterotrophs.)

In anaerobic sedimentary environments, only microbes catalyze the oxidation of H₂ coupled with the reduction of nitrate, Mn(IV), Fe(III), or carbon dioxide [78], with Fe(III)

in subsurface environments [76]. In anoxic groundwater the terminal electron acceptor was Fe(III), sulfate, or methanogenesis [77]. However, in these situations, there are sufficient quantities of hydrogen that the reactions can take place. When reduction of Fe(III) occurs, the formation of Fe(II) results which can help break the H–H bond of H₂. The earliest life of the three domains (Archaea, Bacteria, and Eucarya) were based on inorganic nutrition. Photosynthesis and use of organic compounds for carbon and energy metabolism came comparatively later during their evolution [98]. One does not have to look very far to find environments based on lithotrophy (especially H₂ as energy source); the intestinal tracts of animals (including man) are excellent examples. The occurrence of methane is usually a result of H₂ metabolism. H₂ metabolism is a dominant theme among organisms isolated from geothermal environments or deep aquifers [100, 122]. Some Archaea (sulfate reducers, purple nonsulfur bacteria, methanogens), living intracellularly with eukaryotes, serve as metabolic sinks for H₂ [29, 98].

The metabolism of inorganic compounds (lithotrophy) is widely distributed phylogenetically, intermixed with organotrophic organisms [98]; hence, organotrophy arose many times from photosynthetic or lithotrophic organisms, and many bacteria can switch between modes of nutrition, carrying out photosynthesis in the light and lithotrophy or organotrophy in the dark. Even hyperthermophilic methanogens can utilize H₂. Pace [98] states (1) “Bacteria that are closely related by molecular criteria can display strikingly different phenotypes when assessed in the laboratory through the nature of their carbon and energy metabolism”, and (2) “the superficial metabolic diversity of these types of Bacteria belies their underlying close evolutionary relatedness, giving no hint of the close similarities of their basic machineries.”

Microbial communities contain populations of hydrogen utilizing microorganisms as well as H₂-producing microorganisms. H₂ is a major product of fermentation of organic matter in anaerobic environments, as well as of the serpentinization of rocks, yet it does not accumulate often. This nonaccumulation is likely due to the many microbes that have the ability to utilize H₂ either directly or indirectly.

Interspecies transfer of H₂ should also be considered in discussions of mechanism of utilizing H₂. H₂ generated by one species is transferred to another species. Since the latter species utilizes all the H₂, none is liberated. The exchange of H₂ between different organisms [117] occurs mostly in anaerobic environments. Some must live symbiotically with H₂-utilizing bacteria such as methanogens, acetogens, and

sulfur reducers. Furthermore, various inorganic substances [oxygen, nitrate, MnO₂, SeO₄, CrO₄, Fe(OH)₃, HSeO₃, H₂AsO₄, H₃AsO₃, and SO₄²⁻] are electron acceptors, which when coupled with hydrogen oxidation can provide various amounts of free energy. For more detailed information concerning this subject, see Newman et al. [92].

H₂ transfer is extensively discussed by Wolin [130] in terms of bacteria involved in fermentations. H₂, a major product of fermentation of organic matter, does not accumulate because of the presence of H₂-utilizing bacteria (hydrogenotrophs), which may or may not require anaerobic conditions. H₂ is known to be an important intermediate for most anaerobic food chains, except denitrification. Therefore, it serves as a link between fermentative and respiratory processes, known as interspecies transfer. When there is an abundance of H₂ or other inorganic energy source, good growth of microbes occurs, as is found in the hydrothermal vents [70], certain subsurface environments [122], or in deep ocean sediments [97]. Elsewhere, where the inorganic energy source becomes extremely limited, survival takes place.

There are two primary metabolic schemes in Archaea that rely on hydrogen as the main energy source where the main electron acceptor is carbon dioxide and the end product is methane. Methane from hydrothermal sources shows signs of being produced by thermogenic processes. However, methane from cold seeps has the characteristics of being bacterially formed [96]. This is the first metabolic scheme. The second scheme is when a sulfur compound serves as the terminal electron acceptor. Isotopic analysis [98] of methane present in the outer few kilometers of the Earth's crust or on the surface is the result of the production by methanogenic Archaea [98]. The thermoacidophilic Archaea, which are hyperthermophiles that live at temperatures up to 113°C, live on a diet of H₂, CO₂, and elemental sulfur and “exhale” hydrogen sulfide. The dominant metabolic theme among organisms isolated from geothermal settings or deep aquifers [100, 122] is the use of hydrogen.

Coliforms can use H₂ as an energy source, but cannot use it for growth. This may be a vestige of the first microorganisms on Earth that used H₂. It is possible that most organisms still have this primitive mechanism and use it for survival over geological time periods.

Threshold Levels of Hydrogen for Growth and Reproduction

Lovley [75] demonstrated that there was a threshold below which *Methanobacterium formicicum* was not able to con-

sume H_2 in pure culture. Dissolved H_2 concentrations were found to be 1.5 to 2.5 ppm in systems where microbial sulfate reduction is the terminal process and 8 to 12 ppm in systems where methanogenesis is dominant [78]. H_2 uptake threshold was 385 ppm for acetogenesis, 64–98 ppm for methanogenesis, 6–10 ppm for sulfate reduction, and >1.0 ppm for fumarate reduction [46]. The threshold values are related to the free energy available from the oxidation of H_2 coupled to the reduction of various terminal electron acceptors. However, in methanogenic sediments, the steady-state concentrations of H_2 are 8–12 ppm [78], but these values are much lower than the lowest hydrogen thresholds measured in pure cultures of methanogens [46].

The steady-state H_2 concentrations in naturally occurring anaerobic environments appear to be related to the dominant terminal electron acceptor sink [46, 78, 79, 85, 94]. Thus, under iron-reducing conditions, the steady state hydrogen concentrations are 0.2 nM, and 1 to 1.5 nM under sulfate-reducing conditions. Under methanogenic conditions, the concentrations are 7 to 10 nM. It has also been shown that the thresholds decrease with temperature and, as a result, psychrotrophic homoacetogens can utilize H_2 to partial pressures as low as mesophilic methanogens [23 and references therein].

Many hydrogen-consuming anaerobes have anomalously low thresholds below which hydrogen consumption does not occur; and this low threshold is the result of thermodynamic limitations. The low-affinity system is typical of aerobic Knallgas bacteria, which grow with hydrogen as an energy source, but have a threshold for hydrogen that is too high to allow the oxidation of atmospheric H_2 [22 and references therein]. However, Knallgas bacteria apparently do contribute to the oxidation of H_2 in the vicinity of H_2 -producing root nodules of nitrogen-fixing legumes [22]. In soil, apparently two different kinetics can be observed. One operates at relatively high gas (CH_4 , H_2 , and CO) concentrations and consists of well-known bacteria that use the gases for growth; the other operates at trace gas concentrations. The latter situation is due to either bacteria or soil enzymes that have not been isolated [18]. Soil hydrogenase can also oxidize the H_2 at atmospheric concentrations.

Hydrogen Metabolism

H_2 is recognized to be an excellent energy source for bacterial growth. In ecosystems where it is plentiful, hydrogen-driven ecosystems can result; However, this paper concerns itself where the amount of H_2 is below the threshold level for

growth and metabolism. Thus, the rate of H_2 metabolism needs to generate only a sufficient amount of energy to offset or prevent the processes of racemization, depurination, and other decompositions, and not for growth and reproduction. As a result, the rates are too slow to be measured in the laboratory.

There is much literature on hydrogen, protons, and electrons in the various metabolic processes in biochemistry, and it will not be repeated here because of its voluminous size. What will be mentioned are those items that are needed to stress the theme that H_2 is the energy of survival so that anabiosis can take place. It should be remembered that $H_2 \rightarrow 2 H^+ + 2 e^-$ is bidirectional and that the energy of activation for H_2 is low. However, the formation of protons and electrons in this equation is not restricted to the activity of hydrogenase. As previously mentioned, it can result when H_2 comes in contact with an iron surface [93]. [Fe(III), as previously noted is an electron acceptor in many microbial reactions.] Protons are released from hydrous manganese dioxide when heavy metal ions are adsorbed [83]. In turn, these protons can be used by the ATPase proton pump to drive ATP synthesis. These protons and electrons are also implicated in myriads of biochemical reactions of the cell, and proton jumping does occur.

Hydrogenases obtained from various microorganisms all show remarkable similarity in molecular properties, but are very diverse in their molecular mass, cofactor composition, and spectroscopic properties and may reflect different functions of the enzyme in different organisms [15 and references therein]. They are found in many species of aerobic and anaerobic bacteria. Hydrogenases are subdivided into those that produce H_2 and those that consume H_2 ; thus, the term "hydrogenase" refers not to a single enzyme, but to a family of enzymes. For more information concerning the hydrogenases, see Cammack et al. [15].

Because of its permeability, H_2 is ubiquitous in all material in the upper mantle and above. Because of its permeability, active transport mechanisms of the cell membrane need not be implicated, and active transport does take energy, which is already in short supply in ancient geological material. Unlike recalcitrant organic matter, no exoenzymes are needed to catalyze it into monomers so that active transport can take place. In addition, the H bond network provides a natural route for rapid H^+ transport. Nevertheless, over geological time there is a sufficient amount of H_2 to offset the racemization and depurination processes. As an energy source to overcome racemization and depurination only (no growth or reproduction), the cell has retained its

ability to utilize H₂ (protons and electrons) in all the oxidoreductase reactions where the removal of two hydrogen atoms is involved in the majority of biological oxidations. Hydrogen is an integral part of many biological compounds and it can be passed along a pathway to eventually yield energy. There are all types of hydrogen acceptors and all the metabolic processes of the cell where they are involved such as the half reactions. The process of obtaining energy from H₂ is too slow to be measured in the laboratory, since the system has centuries to carry out the reactions and the rate may rely on the diffusibility of H₂ into the ecosystem.

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