

## 4 Limits for Life

In the past few decades, our concept of a potentially habitable world has broadened from the narrow range between Venus and Mars to literally cover most of the Solar System (Fig. 4.1a,b). Three primary, interrelated developments have contributed to broadening our concept of a habitable world. First, there has been an explosion of research on the ability of microorganisms, especially bacteria and archaea, to grow in extreme environments with respect to temperature, salinity, acidity, radiation, and pressure (Tables 4.1–4.4). Environments that would have been considered inhospitable for life a few decades ago have been found to be thriving with life (e.g., hydrothermal vents and seemingly solid, frozen permafrost and sea ice). Second is a better understanding of a wide range of environments within which liquid water can exist (Fritsen and Priscu 1998; Psenner and Sattler 1998; Gaidos et al. 1999; Vogel 1999; Price 2000; Marion et al. 2003b). Third, our knowledge of the metabolic basis of terrestrial life has broadened to include many biochemical schemes that are completely independent of photosynthesis (Gaidos et al. 1999; Chyba 2000; Chyba and Hand 2001). When the Space Age began almost half a century ago, our understanding of potential extraterrestrial habitats was tied to planetary surface environments where photosynthesis is possible. The third development has decoupled our concept of the limits on potential life from Sun-warmed planetary surface conditions (Fig. 4.1); now we perceive possible habitats in any subsurface porosity, solid/fluid interface, or watery medium having the right aqueous conditions and a possible source of metabolic chemical energy.

Today in the planetary sciences, the first step in the search for life is often the search for liquid water. The current mantra of astrobiology and both NASA and ESA's broader exploration programs is "follow the water," which is as much a matter of following the heat as it is of following the water. Geophysics has become a cornerstone of astrobiology, as there has been increased recognition of geothermal and tidal heating in maintaining warm, wet conditions in planetary crusts and oceans that otherwise would be frozen (compare Fig. 4.1a and 4.1b).

The two Solar System bodies beyond Earth that have elicited the most interest as potential habitats for life are Mars and Europa because both have clearly been impacted by aqueous processes. Considering the expanse of time subsequent to the Solar System's origin, conditions conducive to the occur-

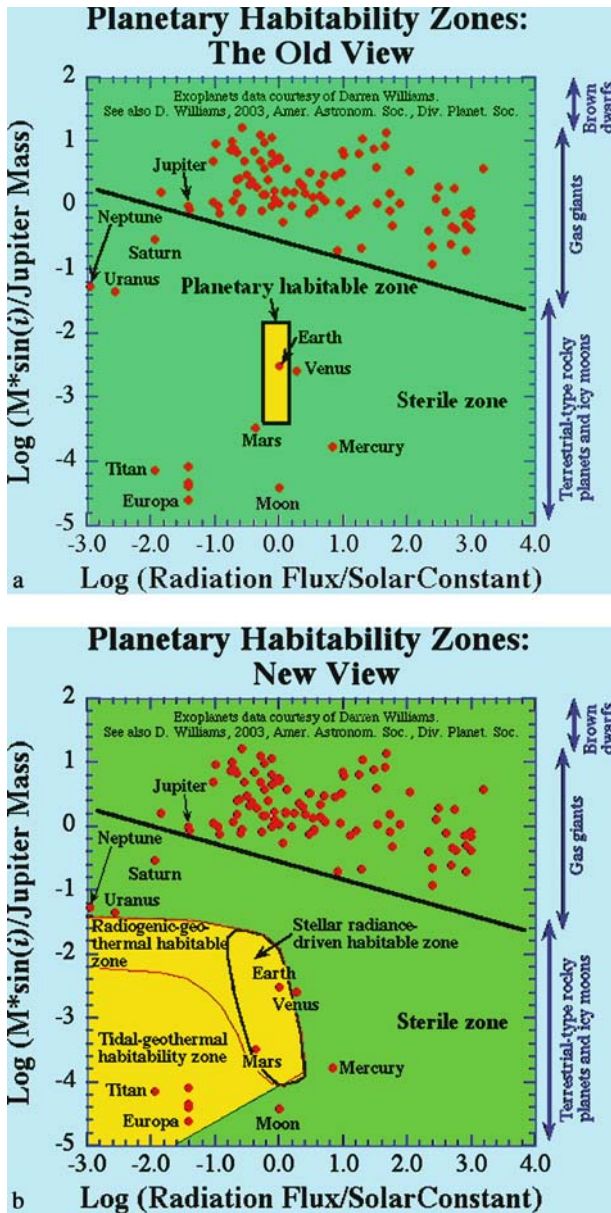


Fig. 4.1. Planetary habitability zones. a Old view. b New view. Reprinted from Kargel (2004) with permission

rence of liquid water and aqueous solutions have existed in some asteroids, many icy satellites, comets, and the gaseous envelopes of the giant planets, in addition to Venus, Earth, and Mars. Nobody would argue that a perihelion

**Table 4.1.** Summary of limits for biological activity at high and low temperatures. Reprinted from Marion et al. (2003b) with permission

Factor	Type of environment	Limits	References
High temperatures	submarine hydrothermal vents	110 to 121 °C	Pledger and Baross 1991; Segerer et al. 1993; Blöchl et al. 1997; Huber and Stetter 1998; Stetter 1999; Madigan and Oren 1999; Stetter 2002; Kashefi and Lovley 2003
	subterranean deep biosphere	110 °C	Pedersen 1993; Fyfe 1996; Stetter 1996
	terrestrial hot springs	103 °C	Kushner 1981; Cometta et al. 1982; Smith 1982; Farmer 1998; Stetter 1999; Farmer 2000; Rothschild and Mancinelli 2001
Low temperatures	ice	-17 to -20 °C	Vogel 1999; Rivkina et al. 2000; Junge et al. 2001; Rothschild and Mancinelli 2001; Gilichinsky 2002; Junge 2002; Junge et al. 2004
	terrestrial	-17 °C	Friedmann and Ocampo 1976; Friedmann and Ocampo-Friedmann 1984; Schroeter and Scheidegger 1995; Gilichinsky 2002
	deep sea	≈ 2 °C	Yayanos 1995; Sassen et al. 1999; Krumgalz et al. 1999
	troposphere	< 0 °C	Sattler et al. 2001

passage of a comet, which might yield transient aqueous solutions, would yield a habitable niche, and almost as improbable are certain transient aqueous environments on Earth, such as stratospheric clouds of aqueous acid aerosols. Likewise, a brief flash of wet conditions on the surface of Mars is not likely to be one where life could arise, though it could be an environment where dormant life could be reactivated. So far there has been comparatively little consideration given, outside of science fiction and some loose but serious science, to potential habitats in the gas giants, though this could be more due to our ignorance of these extremely alien environments than for solidly

**Table 4.2.** Tolerances to salinity for microbial activity (adapted from Mazur 1980; Kushner 1981). Reprinted from Marion et al. (2003b) with permission

Tolerances	
Most bacteria/archaea	$a_w > 0.9$
Extreme bacteria/archaea	$a_w \approx 0.70$
Most fungi	$a_w > 0.85$
Extreme fungi	$a_w \approx 0.60$

**Table 4.3.** Summary of extreme pH tolerances. Reprinted from Marion et al. (2003b) with permission

Type of Environment	pH limits	Selected references
Acidic systems acid mine drainage volcanic springs	pH = -0.06 to 1.0 organisms: bacteria, archaea, fungi, algae record pH = -0.06 Picrophilus (archaea)	Bachofen 1986 Schleper et al. 1995 Johnson 1998 Huber and Stetter 1998 Schrenk et al. 1998 Edwards et al. 1999 Robbins et al. 2000
Alkaline systems soda lakes	pH > 11 organisms: bacteria	Bachofen 1986 Zhilina and Zavarzin 1994 Duckworth et al. 1996

**Table 4.4.** Radiation dose giving  $\approx 37\%$  survival for UV and ionizing radiation (adapted from Kushner, 1981; Baumstark-Khan and Facius, 2002). Reprinted from Marion et al. (2003b) with permission

Microorganism	UV radiation ( $\text{J}/\text{m}^2$ )	Ionizing radiation (Gy)
T1-phage (virus)	–	2600
<i>Escherichia coli</i> (bacteria)	50	20–30
<i>Bacillus subtilis</i> (bacteria)	–	33
<i>Deinococcus radiodurans</i> (bacteria)	600	1500–6000
<i>Saccharomyces cerevisiae</i> (yeast)	80	30–150
<i>Chlamydomonas</i> (algae)	–	24
<i>Bodo marina</i> (eukaryote: heterotrophic flagellate)	5000	–
Humans (eukaryote)	–	1.4

based reasons. However, most other places and times of aqueous environments in the Solar System are not out of reason for consideration as life's niches. For example, there is evidence that the moon of Pluto, Charon, may be hot enough to produce liquid water (Vogel, 1999), and there are multiple strong indications that a tidally heated, wet environment exists inside Saturn's moon, Enceladus (Porco et al. 2006; Kargel 2006); these are places where the possibility of life has not been discussed much, but they are as plausible as Mars or Europa as a potential habitat.

A key to life is the stability of liquid water, at least intermittently, for an ill-defined but long period of time; thus, we have to consider places where there is or was adequate heat to maintain H<sub>2</sub>O in the liquid state. Due to the chemical activity of water and its amphoteric and solvent properties, wherever there is introduction of liquid water into previously anhydrous or slightly hydrous systems (such as fresh igneous rocks), there tends to be chemical reequilibration of associated solids; hydrolysis breaks down and transforms silicates and produces water-soluble salts, water-mediated oxidation-reduction reactions transform minerals bearing iron, manganese, copper, sulfur, and other elements; and hydration reactions add crystalline water of hydration to salts and other phases. During that period of phase transitions there is either a state of disequilibrium (diminishing as a stable state is approached) or metastable equilibrium (a thermodynamically unstable assemblage that is kinetically unable to reach a stable state or does so very slowly). It is a remarkable fact that on Earth many such disequilibrated or metastable aqueous environments are exploited by biochemical systems as a source of metabolic energy (Kelley et al. 2002; Huber and Wächtershäuser, 2006). Thus, "follow the water" is not a sure-fire route to finding life or habitable environments, but there is considerable science behind this approach (Furfaro et al. 2007).

The cold, dry surface of Mars today is not an ideal habitat for life. Nevertheless, evidence for a warmer, wetter early Mars has stimulated considerable speculation about the prospects for life on Mars (McKay and Stoker, 1989; Klein et al., 1992; McKay et al., 1992; McKay et al., 1996; Gibson et al., 1997; Shock, 1997; Jakosky and Shock, 1998; Fisk and Giovannoni, 1999; Max and Clifford, 2000; Cabrol et al., 2001; Kargel, 2004).

Europa is a cold, ice-covered moon of Jupiter that might at first seem inhospitable for life. But there is abundant evidence for the presence of a subsurface briny ocean (Khurana et al., 1998; Pappalardo et al., 1999; Kargel et al., 2000; Stevenson, 2000). The putative ocean of Europa has focused considerable attention on the possible habitats for life on Europa (Reynolds et al., 1983; Jakosky and Shock, 1998; Gaidos et al., 1999; McCollom, 1999; Chyba, 2000; Kargel et al., 2000; Chyba and Hand, 2001; Chyba and Phillips, 2001; Navarro-Gonzalez et al., 2002; Pierazzo and Chyba, 2002; Schulze-Makuch and Irwin, 2002; Marion et al., 2003b).

By generally clement Earth standards, the habitats for life on Mars and Europa are likely to be extreme environments. On the other hand, extrater-

restrial life, if it exists, may be well adapted to its environments and would find Earth environments extreme. Nevertheless, we have adapted an Earth-centric perspective and will judge environments by terrestrial life standards (“life as we know it”). In this chapter, we will examine the limits for life on Earth as standards for life beyond Earth. We will examine temperature, salinity, acidity, desiccation, radiation, pressure, and time as potential limiting factors for life as we know it. We are implicitly assuming that the supplies of liquid water, energy, and nutrients are (were), at least, present at some minimal levels to support life on Mars (McKay and Stoker, 1989; McKay et al., 1992; Shock, 1997; Jakosky and Shock, 1998; Fisk and Giovannoni, 1999; Max and Clifford, 2000; Cabrol et al., 2001) and Europa (Reynolds et al., 1983; Jakosky and Shock, 1998; Gaidos et al., 1999; McCollom, 1999; Chyba, 2000; Kargel et al., 2000; Chyba and Hand, 2001; Chyba and Phillips, 2001; Navarro-Gonzalez et al., 2002; Pierazzo and Chyba, 2002; Schulze-Makuch and Irwin, 2002). Other potential limiting factors such as toxic metals and the presence or absence of oxygen or other oxidants are only peripherally examined in this work. For most of the factors examined (temperature, salinity, acidity, desiccation, radiation, and pressure), we focus on “normal” biological activity (i.e., respiration, growth, and reproduction, more or less as we know it); for the factor of time, we examine how much time is required for life to develop on a planet and how long organisms can survive in the dormant state. Then, in Chap. 5, we examine biogeochemical applications of the FREZCHEM model; our treatment includes the geochemistry and potential habitats for life on Mars and Europa. And, finally, in Chap. 6, we examine the search for and future of life in the Universe.

## 4.1 Temperature

There are three environments on Earth where microbes have been identified with temperature tolerances in a range of 100°C to 121°C, namely, submarine hydrothermal vents, the subterranean deep biosphere, and terrestrial hot springs (Table 4.1). The highest temperature tolerances (110–121°C) are found in microbes from marine hydrothermal vents and the subterranean deep biosphere; high pressures prevent these waters from boiling at 100°C, the normal boiling point of water at 1.01 bar (1 atm) pressure. From terrestrial hot springs, microbes have been isolated that can tolerate temperatures up to 103°C (Table 4.1).

Hyperthermophiles are invariably either bacteria or archaea. Eukaryotes have an upper temperature range of ~50–60°C (Madigan and Marrs, 1997; Nealson, 1997; Nealson and Conrad, 1999; Rothschild and Mancinelli, 2001). Until recently, *Pyrolobus fumarii* (an archaea) had the highest known temperature tolerance of 113°C (Blöchl et al., 1997); this organism has a minimum temperature for growth of 90°C and an optimum temperature of 106°C and is a strict hyperthermophile (Stetter, 1999). Recently, an archaea was isolated

from a hydrothermal vent with a temperature tolerance of 121 °C; this organism is closely related to *Pyrodicticum occultum* and *Pyrobaculum aerophilum* and doubled in cell number after 24 h at 121 °C (Kashefi and Lovley, 2003).

There are several types of environments on Earth where significant water exists at prevalent low temperatures such that ice and liquid aqueous solutions commonly coexist: permafrost, snow, glaciers, lake and river ice, sea ice, and parts of the atmosphere (polar troposphere, global upper troposphere, and stratosphere). In addition, the deep sea floor occurs at temperatures very close to the freezing point of water. For example, temperatures in the oceanic abysses hover around 2 °C at a maximum hydrostatic pressure of 1100 bars (10,660 m) in the Mariana Trench (Yanos, 1995). Table 4.1 summarizes some of these environments. Furthermore, in some permafrost and sea-floor environments, the presence of nonpolar gases under pressure can stabilize a modified form of ice known as gas hydrates even where temperatures are not quite low enough for ordinary ice to form.

There have been a number of reports in recent years demonstrating that some microbes can metabolize, albeit slowly, at temperatures in a range of -17 °C to -20 °C (Table 4.1). These organisms include bacteria, lichens (a symbiotic association of algae and fungi), and fungi (yeasts). Many of these ecosystems are in protected environments such as in aqueous pockets in ice (Priscu et al., 1998; Psenner and Sattler, 1998; Thomas and Dieckmann, 2002) and within rocks (cryoendoliths) (Friedmann and Ocampo, 1976; Friedmann and Ocampo-Friedmann, 1984), where climate is more hospitable than in exposed areas. On the other hand, Price and Sowers (2004) have argued that there is no evidence of a minimum temperature for metabolism (growth, maintenance, or survival). Their extrapolated (from -20 °C) rate at -40 °C in ice corresponds to  $\approx 10$  turnovers of cellular carbon per billion years. If these arguments are correct, then microbes could survive indefinitely, provided they are protected from especially destructive forces such as high temperatures and radiation.

Temperature as well as salinity and other compositional variables have a profound influence on the viscosity of aqueous solutions and many aspects of life. As temperature declines and salinity increases, viscosity increases, diffusion rates decline, metabolic rates decline, motility of motile microorganisms and feeding rates decline, life spans may increase, and rates of evolution decline. All other things being equal, aqueous systems and planets allowing only cryogenic life will have less evolved life than chemical systems and planets that have permitted life to occur for substantial periods at higher temperatures.

An intriguing possibility we raise here for the first time is that there must be particular brine compositions where the net volume change during freezing is zero. That is, the combined effects of ice expansion and contraction effects due to salt precipitation and brine compositional migration toward a more salt-rich residual brine all average out to zero net volume change.

Whereas this would be a very special case to be exactly zero, a rough approximation of zero volume change has been observed in the high-pressure phase equilibrium/volumetric studies of brines by this book's second author and collaborators at Lafayette College (Hogenboom et al., 1995; Dougherty et al., 2007). This condition requires a system to be either at a eutectic or a cotectic crystallization path involving ice plus at least one salt phase. The intrigue here is that these special brine compositions may be uniquely habitable in conditions of intense freezing and thawing of psychrophilic microbial life. At least cellular damage due to expansion and contraction of a cell's environment would be minimized for these compositions, and if other causes of freeze damage (crystal punctures of cells, for instance) can be minimized, these brines might favor psychrophilic life. The same experiments have shown that under some circumstances, brines freeze by production of impressive daggerlike needles (each a potential threat to any cell wall that intervenes), whereas in other cases such quench crystals do not form (Dougherty et al., 2007). Crystal morphology is controlled both by brine composition and the rate of cooling and extent of supercooling. Ultimately, all of these issues come down to thermodynamics, phase equilibria, and kinetic factors related to crystallization and solute diffusion.

## 4.2 Salinity

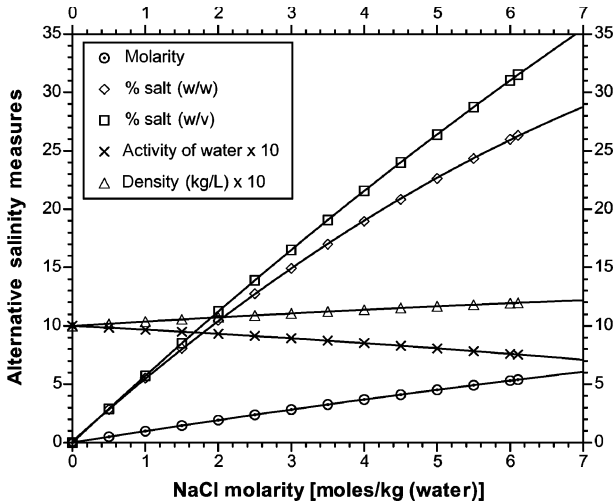
Salinity affects microbial activity, in part, because it controls water availability. The higher the salinity, the more energy an organism must expend to maintain a favorable osmotic balance. Salts, of course, have effects on living organisms beyond water availability. For example, salts can be both a source of essential nutrients as well as a source of toxic heavy metals. Also, sulfate salts appear to be more favorable for life than chloride salts; see the discussion in Sect. 5.1.2 (Aqueous Saline Environments). However, in this section on salinity, the focus will be on salinity as a control on water availability.

Because salinity has been studied by scientists representing many disciplines, measures of salinity vary widely among disciplines. Chemists tend to prefer concentration units of molality [mol/kg (water)], while physiological microbiologists often report salinities as salt% (wt/wt) [g/100 g (soln)], salt% (wt/vol) (g/100 ml), or as the activity of water ( $a_w$ ). The activity of water is probably the best measure of salinity as it relates directly to the osmotic gradient controlling flows of salts and water into and out of organisms.

This diversity of measures of salinity is especially a problem in cross-disciplinary work, where alternative measures from other disciplines are often unfamiliar. The equations needed to convert from one measure to another are

$$m \left( \frac{\text{kg}(\text{water})}{\text{kg}(\text{soln.})} \right) \rho = M, \quad (4.1)$$





**Fig. 4.2.** Alternative measures of salinity for pure NaCl solutions at 25 °C and 1 atm pressure. Reprinted from Marion et al. (2003b) with permission

$$\frac{\sum_i m_i MW_i}{10} \left( \frac{\text{kg(water)}}{\text{kg(soln)}} \right) = \% \text{salt}(w/w), \quad (4.2)$$

$$\% \text{salt}(w/w) \rho = \% \text{salt}(w/v), \quad (4.3)$$

$$\frac{\text{kg(water)}}{\text{kg(soln.)}} = \frac{1000}{1000 + \sum_i m_i MW_i}, \quad (4.4)$$

where  $m$  is the molality [= mol/kg (water)],  $M$  is the molarity [= mol/L],  $\rho$  is the density (= kg (soln.)/L), and  $MW$  is the molecular weight (g/mol). The density of solutions ( $\rho$ ), which is needed for some of the above conversions, can either be measured experimentally or estimated with a model (e.g., FREZCHEM, Eq. 2.88).

Figure 4.2 depicts several measures of salinity for pure NaCl solutions at 25 °C and 1.01 bar total pressure calculated using the FREZCHEM model and Eqs. 4.1 to 4.4. NaCl solutions were used in this example because this salt is often used as a background salt in physiological studies (Madigan and Oren, 1999; Kaye and Baross, 2000). For salts other than NaCl or at temperatures other than 25 °C, Eq. 4.1 to 4.4 must be relied upon to make conversions or see Marion (2007).

Table 4.2 outlines the approximate salinity limits for biological activity of bacteria/archaea and fungi. Salinity is one of the few limiting factors for life where a eukaryote (fungi) has a higher tolerance than prokaryotes (bacteria/archaea). To place these limiting  $a_w$  values in perspective, the  $a_w$  for seawater is 0.98. Most prokaryotes and fungi can tolerate much higher salinities than seawater (Table 4.2).

### 4.3 Acidity

Acidity is typically quantified using the pH scale

$$\text{pH} = -\log_{10}(a_{\text{H}^+}), \quad (4.5)$$

where  $a_{\text{H}^+}$  is the hydrogen ion activity. The activity of a single ion cannot be measured unambiguously (Pitzer, 1995); therefore, assumptions must be made in defining pH. One convention is to use a chemical thermodynamic model to estimate the activity coefficient of  $\text{H}^+$  ( $\gamma_{\text{H}^+}$ ). Then, given an experimental measurement of the molal concentration ( $m$ ), one can calculate the activity as follows:  $a = \gamma m$ . This is how most geochemical models work. Another assumption, most frequently used in calibrating pH standards, is the MacInnis convention (Harvie et al., 1984). In this case, the assumption is made that  $\gamma_{\text{K}^+} = \gamma_{\text{Cl}^-}$  in all solutions of the same ionic strength. This allows one, indirectly, to estimate  $\gamma_{\text{H}^+}$  and to define  $a_{\text{H}^+}$ . The reason for raising this issue is that pH values calculated with these two conventions can lead to very different pH values in extremely high acidities ( $\text{pH} < 1.0$ ). [See Marion (2002) for a fuller discussion of this issue.]

There are many studies demonstrating that a wide range of organisms can tolerate pH values  $< 1.0$ . For example, bacteria, archaea, fungi, and algae have all been demonstrated to tolerate pH values  $\leq 1.0$  (Table 4.3). The current record holders are *Picrophilus oshimae* and *Picrophilus torridus* (archaea), which can grow at a pH of  $-0.06$  (Schleper et al., 1995). Unfortunately, in these acid studies, it is not always clear which of the above two pH conventions was used. For example, the Schleper et al. (1995) study, which reported the lowest pH value of  $-0.06$ , only indicates that  $1M$  HCl was used as a reference for pH 0; but there is no clear indication which convention was used to calibrate their pH electrode. On the other hand, the Iron Mountain acid studies (e.g., Schrenk et al. 1998; Edwards et al. 1999; Robbins et al. 2000) clearly use the MacInnis convention in calibrating electrodes at low pH (Nordstrom et al. 2000).

Invariably, at least on Earth, high acidities are associated with high concentrations of heavy metals because strong acids are highly effective in dissolving primary minerals and releasing heavy metals into the environment (Krishnaswamy and Hanger 1998; Robbins et al. 2000; Lopez-Archilla et al. 2001; Fernandez-Remolar et al. 2003). Therefore organisms that tolerate strong acidity also tolerate high levels of heavy metals.

There are fewer studies of high alkalinities ( $\text{pH} > 10$ ) than of high acidities ( $\text{pH} < 1.0$ ) probably because high alkalinities are more rare in nature. Nevertheless, there are reports of organisms tolerating pH values  $> 11$  (Table 4.3), and maybe even as high as 12.5–13 (Bachofen 1986; Duckworth et al. 1996).

## 4.4 Desiccation

The desiccating power of the atmosphere is generally measured by relative humidity (RH), which is related to the activity of water ( $a_w$ ) by

$$a_w = RH/100. \quad (4.6)$$

Just as  $a_w = 0.6$  is considered the lower limit for biological activity in saline solutions (Table 4.2),  $RH = 60\%$  is considered the lower limit for biological activity under dry atmospheric conditions (Kushner 1981; Dose et al. 2001).

A clear distinction must be made between biological activity and survival under desiccating conditions. Some organisms can survive 99% loss of water with  $a_w \sim 0$  (Mazur 1980). *Bacillus sphaericus* spores survived 25 million years of desiccation in amber through a process called anhydrobiosis (Fischman 1995). Bacteria, fungi, plants, and insects have been shown to survive extensive periods of dehydration (Rothschild and Mancinelli 2001). A clear distinction must also be made between conditions allowing biological activity and conditions allowing the origin of biological activity. If this distinction exists, the boundaries of these conditions are not known because the genesis of life is inadequately understood.

Examples of especially dry environments on Earth include the Atacama Desert of northern Chile and the Dry Valleys of Antarctica. Dose et al. (2001) exposed spores, conidia, and cells of several microbes to 15 months of desiccation in the dark at two locations of the Atacama Desert. *Bacillus subtilis* (bacteria) spores (survival  $\sim 15\%$ ) and *Aspergillus niger* (fungi) conidia (survival  $\sim 30\%$ ) outlived other species. *Deinococcus radiodurans* (bacteria) did not survive the desert exposure because they were readily killed at RH between 40% and 80%, which occurred during desert nights (Dose et al. 2001).

## 4.5 Radiation

Two types of ionizing radiation can limit life, and either can do its deadly work in long-term, low doses or short-term, intense doses: (1) short-wave electromagnetic radiation (ultraviolet, x-ray, and gamma-ray parts of the spectrum); and (2) high-energy corpuscular radiation (energetic electrons and protons especially, and heavier ions). Major sources of lethal radiation doses include (a) cosmic phenomena (gamma ray bursts, nucleosynthesis due to supernovae, stellar/solar fusion, and stellar/solar photospheric reradiation), (b) planetary magnetospheric phenomena (including trapped solar wind and sputtered, ionized material released from planetary surfaces by interaction with cosmic EM and corpuscular radiations), and (c) radioactive decay in planetary objects due to long-lived radionuclide decay and short-lived cosmogenic nuclide decay (those nuclides generated partly by neutron activation). Ultimately, all of these radiation sources trace back to nucleosynthetic

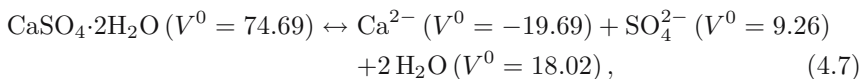
processes in stars and heavier objects. The surface of Europa, for instance, would be deadly to frozen life on time scales of seconds to days (due to energetic magnetospheric ions), while the cosmic-ray-shielded deep permafrost of Earth would be deadly to frozen organisms on time scales of hundreds of thousands to millions of years due to gamma radiation caused by long-lived radionuclide decay.

Resistance to one form of radiation does not necessarily convey protection from other forms. Almost all organisms are prone to UV damage because the macromolecules that propagate genetic information (DNA) absorb UV radiation. For example, the experiments in the Atacama Desert cited in the previous section were done in the dark (shade). Direct exposure to UV radiation in these experiments killed all organisms within hours (Dose et al. 2001).

Table 4.4 depicts resistance to UV and ionizing radiation for several microbes. *D. radiodurans* is well known to have a high resistance to ionizing radiation. This resistance to radiation is thought to have evolved initially as a resistance to desiccation. The mechanism for conveying this resistance is believed to be due to their ability to quickly repair DNA damage (Kushner 1981; Smith 1982; Bachofen 1986; Jawad et al. 1998; Rothschild and Mancinelli 2001). Other mechanisms to protect organisms from UV radiation include the development of iron-enriched silica crusts (Phoenix et al. 2001) and self-shading (Smith 1982). Also, both water and ice are effective in absorbing UV radiation (Baumstark-Khan and Facius 2002).

## 4.6 Pressure

Pressure affects physics, chemistry, and biology. Chemical reactions that lead to a decrease in volume are favored by pressure. For example, the dissolution of gypsum at 0 °C is as follows:



where  $V^0$  is the molar volume ( $\text{cm}^3/\text{mol}$ ) at infinite dilution:

$$\Delta V_r^0 = V_{\text{Ca}}^0 + V_{\text{SO}_4}^0 + 2V_{\text{H}_2\text{O}}^0 - V_{\text{CaSO}_4 \cdot 2\text{H}_2\text{O}}^0 = -49.08 \text{ cm}^3 \text{ mole}^{-1}. \quad (4.8)$$

In this case,  $\Delta V_r^0$  is negative, which implies that pressure will cause the reaction to shift to the right causing a dissolution of gypsum. Another important reaction is the stability of water ice and liquid water at subzero temperatures under pressure:

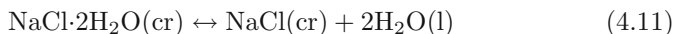


which has a  $\Delta V_r^0 = -1.63 \text{ cm}^3 \text{ mol}^{-1}$  at 0 °C. In this case, pressure will cause a melting of ice (to reduce the volume) with a consequent lowering

of the freezing point (Fig. 3.3). Another reaction that will be considered in Chap. 5 is



which has a  $\Delta V_r^0 = 16.31 \text{ cm}^3 \text{ mol}^{-1}$  at  $0^\circ\text{C}$ . In this case,  $\Delta V_r^0$  is positive, which will cause the reaction to shift to the left, favoring the precipitation of  $\text{MgSO}_4 \cdot 12\text{H}_2\text{O}$  at a higher temperature. Also, we showed previously that the equilibrium temperature for the reaction



increases with pressure ( $\Delta V_r^0 = 5.10 \text{ cm}^3 \text{ mole}^{-1}$ ; Fig. 3.20). These four examples of pressure on chemical reactions are sufficient to demonstrate that the effect of pressure is dependent on the volumetric properties of the individual constituents, which makes every chemical reaction highly individualistic.

High pressures can occur in both deep-earth and deep-sea environments, but there are some fundamental differences between these two systems. In the deep sea, hydrostatic pressures on organisms is simply  $p = \rho gh + p_a$ , where  $p_a$  is ambient atmospheric pressure at the surface (generally about  $1 \text{ atm} = 1.010325 \text{ bars} = 0.101325 \text{ MPa}$ ),  $\rho$  is the density of seawater (about  $1026 \text{ kg m}^{-3}$ ),  $g$  is surface gravitational acceleration (about  $9.8 \text{ m s}^{-2}$ ), and  $h$  is the depth (m). For example, for 2 atm pressure (1 atm above sea level pressure), the depth is 10 m. In the deep earth, the confining pressure could be atmospheric with organisms growing in air pockets, such as caves or soil pore spaces, or in the water-filled pore spaces of deep-sea sediments, where the pressure then is hydrostatic pressure for that depth below sea level. For depths beneath the land surface or sea floor greater than about 1 to 2 km, porosity tends to become choked off, and pressure equilibrium with the atmosphere or ocean is disrupted, so hydrostatic pressure tends increasingly to become lithostatic. Life may occur at even higher pressures within deep aquifers and crustal brine pockets, where the organisms may be subjected to both hydrostatic and lithostatic pressures. Unfortunately, the actual pressures under which these deep-earth microbes grow are poorly documented (Pedersen 1993). Another fundamental difference is that deep-sea environments decrease in temperature with increasing depth (within the sea itself), while deep-earth (continental and oceanic crustal) environments increase in temperature with increasing depth.

Microorganisms have been isolated from the Mariana Trench in the Pacific (10,660 m depth) where pressures reach 1100 bars (110 MPa; Yayanos 1995; Kato et al. 1998; Abe et al. 1999). Two bacteria similar to *Moritella* and *Shewanella* are apparently obligately barophilic with optimum pressures for growth occurring at 700 bars (70 MPa) and no growth below 500 bars (50 MPa; Kato et al. 1998). These Mariana Trench organisms grow at a temperature of  $2^\circ\text{C}$ .

There are some archaea associated with deep-sea hydrothermal vents that can survive at pressures as high as 890 bars (89 MPa; Pledger et al. 1994). The

high pressure of hydrothermal vents has a compensatory effect that allows stabilization of molecules, which allows growth at elevated temperatures up to 121 °C (Table 4.1).

Microorganisms have been found growing at depths of 2.8–4.2 km beneath the land surface (Pedersen 1993; Fyfe 1996; Kerr 1997). Microbes at 4.2 km grow at a temperature of 110 °C. Temperature, rather than pressure, is probably the most important growth-limiting factor for deep-earth microbes (Pedersen 1993; Fyfe 1996). Organic biopolymers and complex cellular structures tend to be destroyed at elevated temperatures, and apparently elevated metabolism and cellular repair activity does not compensate for the rates at which critical bonds are broken; hence, cells cannot repair thermal damage beyond a point.

Organic-rich shale-hosted zinc-lead sulfide and barite Red Dog deposits in the Brooks Range (Alaska) contain fluid inclusion bearing sphalerite veins (Leach et al. 2004). Many of the inclusions have salinities ranging from 14 to 19% NaCl equivalent, based on melting analysis. It is thought that these brines originated by formation of a hypersaline (~30% NaCl) evaporitic marine brine on tidal carbonate flats; then, after burial, this brine mixed with more dilute aquifer waters. This saline brine then presumably participated in the origin of the ore rocks. The fluids became trapped in the sphalerite at depths of 2.4 to 7.4 km (pressures of 650 to 2100 bars) and temperatures ranging from 383 to 453 K (Leach et al. 2004). Only the lowest formation temperatures of these inclusions are consistent with any form of hyperthermophilic life. The rocks later were subjected to pressures of up to 3400 bars and temperatures of up to 487 K, according to analysis of a different set of aqueous and coexisting methane fluid inclusions.

These findings for the Red Dog ore deposits, typical of similar zinc ore deposits around the world, would indicate that the original evaporitic marine environment was followed progressively by burial and increases in pressure and temperature, with a sequence of microbial life forms possible until eventually only life's organic, broiled remains could exist in the rocks. This type of burial-induced biological succession and biochemical metamorphism, starting with common evaporitic deposits in basins of high primary productivity and hypersaline conditions, is very common throughout Earth's history and is a key aspect of petroleum and gas genesis. In fact, indigeneous ecological communities of thermophilic bacteria and hyperthermophilic archaea are thought to inhabit hot petroleum reservoirs (L'Haridon et al. 1995; Magot et al. 2000). Culture experiments have shown that *Ferroglobus placidus*, an obligate hyperthermophile, is able to reduce Fe<sup>3+</sup> in anaerobic oxidation of a wide range of aromatic hydrocarbons (Tor and Lovley 2001).

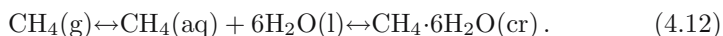
Most petroliferous deposits originally were laid down under warm to hot arid climates and tectonic situations conducive to rapid burial, such as rift basins. One can envisage a complete ecological succession during burial start-

ing with the inhabitants of the original depositional waters and evolving to these bizarre petroleum hosted, deep subsurface ecosystems.

Inland cold-climate basins that are both evaporitic and biologically productive are much rarer than warm-climate evaporitic basins in Earth history. Such basins now exist in western China. Cold- and warm-climate evaporitic basins are expected to be very different in several respects. Key differences should affect both the phase equilibria of precipitated and metamorphosed salts and the sustainability of chemolithoautotrophic biological activity at depth during burial. Primary precipitated salts in cold basins would tend to include a rich variety of highly hydrated salts, which then would undergo a cascading sequence of incongruent dissolution and dehydration events as the assemblage is buried and heated. In hot-climate basins, many of these salt hydrates are omitted right from the start. Primary productivity may be lower in the colder basins, but if there is a subsurface biochemical energy source, life may be maintained to greater depths owing to cooler surface temperatures and overall cooler geotherms. Rather than characteristically burning out at about 4 km burial depth and 383 K, microbial life in cold-climate depositional basins on Earth might be sustained to as deep as 6 km, if thermal conductivity, heat flow, and other factors besides surface temperature are the same.

On Mars, where surface temperatures are much lower than on Earth and temperature gradients as a function of pressure are comparable (reduced heat flow is compensated by reduced gravity), life probably can exist to much higher pressures and slightly higher temperatures and far greater depths than in Earth's crust. The lower gravity also means that, all other things being equal, pore space compaction occurs less rapidly as a function of depth on Mars than on Earth. Thus, we would not be surprised if hyperthermophilic life existed to depths of >10 km on Mars, if it exists there at all. Because of the slow cycling of water, energy, and nutrients at depth, metabolic activity is believed to be extremely slow at great depths on Earth (Kerr 1997), and this would be a limitation deep in Mars' crust as well.

Another phenomenon found under pressure at depth in terrestrial oceans and in permafrost is gas hydrate deposits. Hydrates of natural gases such as methane ( $\text{CH}_4 \cdot 6\text{H}_2\text{O}$ ) and carbon dioxide ( $\text{CO}_2 \cdot 6\text{H}_2\text{O}$ ) form on Earth beneath low-permeability strata under high pressure and low temperature (Kvenvolden 1993; Sloan 1998; Blunier 2000):



The stability of these solid-phase compounds is a function of pressure, temperature, and matrix salt composition (Sloan 1998; Marion et al. 2006). Gas hydrates could be important sources of high-energy carbon (Carney 1994). On Earth, gas hydrate deposits can sustain complex chemosynthetic communities (Sassen et al. 1999; Fisher et al. 2000). There is speculation that gas hydrates may be present on Mars and Europa (Kargel et al. 2000; Max and

Clifford 2000). Many metallic ore and evaporite minerals contain methane, though not in hydrate form. For instance, the Red Dog zinc ores mentioned above contain methane inclusions in association with saline inclusions. In many instances, microscopic methane and carbon-dioxide-rich fluid inclusions form clathrates within their tiny hydrous envelopes. Some salt deposits are so enriched in high-pressure free gas inclusions and/or gas hydrates that mining, crushing under boot, or other means of physical disturbance causes popping or even explosive decrepitation.

Recently, it was demonstrated in a diamond anvil cell that *Shewanella oneidensis* and *Escherichia coli* strains remain physiologically and metabolically active at pressures of 680 to 16,800 bars for up to 30 h (Sharma et al. 2002). At pressures of 12,000 to 16,000 bars, living bacteria resided in fluid inclusions in Ice VI crystals and continued to be viable when pressure returned to 1 bar. However, only 1% remained alive; whether this constitutes viability or survival under pressure is contentious (Couzin 2002). Nevertheless, it demonstrates that pressure may not be much of an impediment for some life forms, and that even the deep ocean of Ganymede might be suitable for life.

What do first principles say about the limits to life imposed by pressure? Life needs a polar aqueous solution. The polarity of water goes to zero beyond megabar pressures; it eventually undergoes metallization (Fig. 4.3). That kind of extreme “megabar water” is almost surely unsuited for life, though it may be liquid at the right temperature conditions. It is not known what value of polarity is needed for water to act as a life-giving medium or whether some other polar volatile, such as ammonia, might take on properties at very high pressures that would help it substitute for water as a life-giving medium. The physics of water at hundreds of kilobars to megabar pressures and the high-pressure suitability of water for life over part of this pressure range is almost completely unexplored territory. Whether there exists some range of depths and pressures within the gas giants to serve as an abode of exotic life is really unclear; it would appear that Jupiter and Saturn probably have adiabats that are too hot and atmospheric dynamics that are too strongly convective (Fig. 4.4). Uranus and Neptune might potentially be of greater astrobiological interest because the pressure conditions in the thermally acceptable zones are not exorbitant (Fig. 4.4), and these planets’ atmospheres are probably more stable than the larger gas giants. The deep molecular “oceans” (or ice layers) of water, ammonia, and methane believed to exist in Uranus and Neptune are subject to pressures in the range of several hundred kilobars to several megabars (Hubbard et al. 1995). The top of this icy domain could be a habitat for unfamiliar types of life, but conditions there are so uncertain and biochemistry so unknown that we have to leave this as a speculation. Much more likely, temperatures are so high that complex organic molecules do not exist, and instead these layers truly are simple molecular mixtures. We



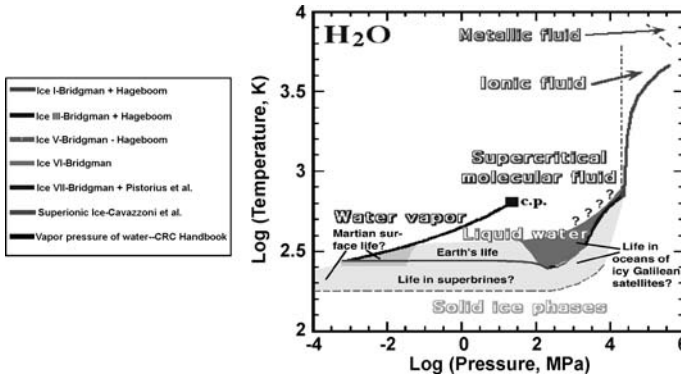


Fig. 4.3. The stability of water as a function of temperature and pressure. Reprinted from Kargel (2004) with permission

can make more definitive statements about the possibilities of life at a few tens of kilobars and lower pressures relevant to icy satellites.

Water at tens of kilobars resembles water at ordinary pressures in many respects. It has a strongly polar molecular structure, solvent and amphoteric acid/base properties, and thermal expansivity and bulk modulus properties similar to water at ordinary pressures, although boiling points and freezing

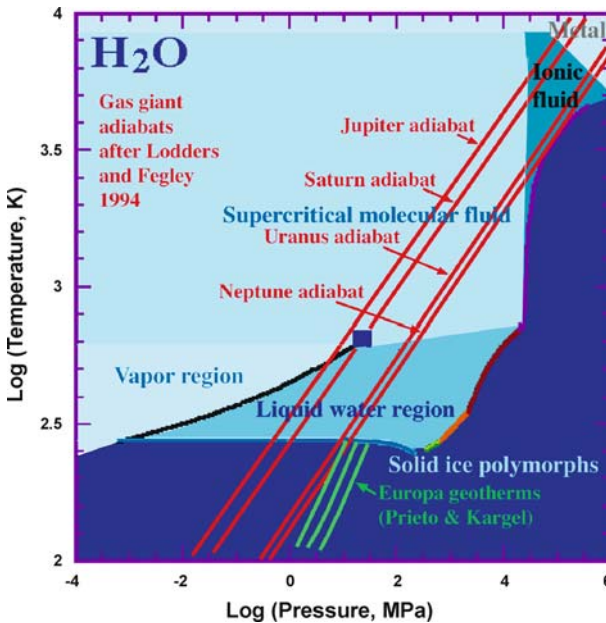


Fig. 4.4. Searching for life by mapping water

points shift considerably and the structure of equilibrium ice phases and the sign of the volume change upon freezing all are different from the case of water at low pressure. For pressures of ordinary relevance to icy satellites, asteroids, and planetary crusts (Fig. 4.3), excluding impact shock pressures, it would seem that pressure itself is not a life-limiting factor. In fact, pressure extends the conditions where liquid aqueous solutions may exist (by elevating boiling points and depressing melting points of ice 1h); in the range of hundreds of bars, this is a key to the viability of hyperthermophiles, and we expect this aspect to become even more significant into the range of several kilobars. However, the basic properties of water do change with pressure, and these changes affect thermodynamic phase equilibria, reaction rates, and the functioning of biological systems. Gas-filled vacuoles also change their volume and density, and gas solubilities in cellular fluids change with pressure, and with it, mineral equilibria (such as carbonate precipitation boundaries) shift. For many microbial species, it is just necessary to have life adapted and biochemistry “calibrated” to the relevant pressures. This happens all the time in hot springs, hydrothermal systems, and marine environments, where a given individual may be subjected to changes in pressure ranging from a few bars to a few hundred bars. The ability of individuals to adapt to pressure changes has been investigated in the laboratory, and this adaptability has its limits (Couzin 2002; Sharma et al. 2002), but pressure itself does not appear in any way to be a life-limiting factor over the range of pressures encountered in Earth’s hydrosphere and upper crust.

Adaptations over pressure ranges of many kilobars or tens of kilobars may be difficult for many individuals because of large thermodynamic shifts, but the properties of water probably do not change so much that biology would be impossible. For a given set of conditions where a liquid-water phase exists, organic chemistry probably will tend to find the right equilibria where biochemistry may get rooted. If one biochemical reaction pathway is not stable for a certain set of conditions, then another probably will be. If so, the type of life that emerges and its major biochemical pathways will be suited to the prebiotic chemistry that prevails for a certain set or range of conditions. We expect that there are limits to this biochemical flexibility to adapt to or evolve with the pressure variable, but the pressure limits to life at least are broad and probably extend beyond the science community’s observational and experimental limits so far explored.

One way that pressure must affect life is by its control of the freezing point of water and the viscosity of water and aqueous solutions near the freezing point (or at any temperature) and, hence, pressure control on ionic mobility and reaction rates, including metabolism. This in itself does not limit life, but it helps to set the pace of life’s genesis, evolution, and life span, and that may affect the viability of life in the face of unstable or dynamic conditions.

A major discontinuity in the behavior of water occurs at about 2100 bars, where the equilibrium ice phase changes from ordinary ice 1h, which is less

dense than water, to ice III, which is more dense. The volume expansion on freezing below 2100 bars is a severe problem for many species because growing ice crystals in freezing cells rupture cell walls both by the expansion of cell contents and by crystal penetration through the cell walls. Cellular damage during freezing ought to be reduced or eliminated at pressures above the ice I/ice III transition. Freezing, however, may still kill cells by rupturing their cell walls with growing crystallites. Thawing at these elevated pressures, on the other hand, may be traumatic due to the sudden expansion. Freezing at any pressure may cause cell damage by exclusion and forced precipitation of solutes, which may isolate nutrients away from the cellular domains where metabolism would utilize them once thaw conditions occur.

## 4.7 Time

Two aspects of time have a bearing on the possibility of extraterrestrial life. (1) How much time is required for life to develop on a planet? (2) How long can life survive in the dormant stage in isolation from conditions normally considered vital for life such as cycling of liquid water, energy, and nutrients? There are further higher-order issues that pertain to time. Does the time required for biogenesis and evolution depend on the composition of the chemical system? Do these critical time spans depend also on the temporal and spatial stability of composition? How do the physical nature and biochemical processes of life depend on the stability (dynamical time constants pertinent to changing conditions) of a chemical system? For instance, are acidic solutions characteristic of stratospheric clouds fundamentally hostile to any type of life, or are they hostile because of the dynamic state? Where a salt pan's composition admits certain types of life, might frequent flushing by fresh water render that life nonviable? How do diurnal fluctuations in solar ultraviolet radiation affect the viability of habitats in surface soils? These questions are clearly relevant to the day-to-day viability of life in certain terrestrial habitats, but even more so it seems that dynamic conditions would affect biogenesis. The effects are not necessarily all deleterious to life, as dynamic conditions, including oscillating compositions, can drive biochemical energy gradients, which may be useful to life; the life simply has to avoid being killed by a zeroing or reversal of a favorable gradient.

The Earth began forming about 4.6 billion years (Ga) ago. The first 600 to 800 million years (Ma) of Earth's existence have been erased by the constant early bombardment of asteroids and comets (Arrhenius and Lepland 2000; Delsemme 2001; Ehrenfreund and Menten 2002; Wharton 2002). The earliest geologic evidence for life on Earth dates to 3.5 to 3.8 Ga (Schopf and Packer 1987; Mojzsis et al. 1996; Ehrenfreund and Menten 2002; Stetter 2002; Wharton 2002). Based on this evidence, it has been argued that life on Earth developed rapidly within about 200 to 300 Ma. During this interval, the Earth evolved from a hot dry rock to a cool wet world. Evidence suggests that the

rain of asteroids and comets brought to Earth water, organic molecules, and gases that are key ingredients for the establishment of life (Delsemme 2001; Horneck and Baumstark-Khan 2002). According to present knowledge, the time necessary for life to develop might require hundreds of millions of years.

This model of the evolution of life on Earth does not preclude the possibility that life arrived on Earth fully formed from another body [the Panspermia Hypothesis (Horneck and Baumstark-Khan 2002; Wharton 2002; Napier 2004; Wallis and Wickramasinghe 2004)]. In this case, only a short-term temporary abode would be necessary for life to become established.

Another, perhaps more important, question is: How long can life survive in the dormant state on a planet even under hostile conditions? A number of reports in recent years have suggested that microbes can survive in the frozen state (in ice or permafrost) for periods ranging from thousands to 3 million years (Soina et al. 1995; Stone 1999; Christner et al. 2000; Gilichinsky 2002). On an even longer time scale (25 to 40 Ma), viable microbes, similar to *B. sphaericus*, have been isolated from bees encased in amber (Fischman 1995; Cano and Borucki 1995). The longest reputed record for survival goes to a *Bacillus* spp. that has been isolated from halite crystals believed to be 250 Ma (Vreeland et al. 2000). In that study, only 2 of 53 salt crystals had viable bacteria, suggesting that survival is a rare occurrence. However, Hazen and Roedder (2001) have argued that in the absence of primary growth features in the specific halite crystals studied, the age of these crystals and their fluids must remain in doubt. In a reply to these concerns, Powers et al. (2001) defended their crystal and fluid inclusion ages.

Earlier, under “Temperature,” we discussed the work of Price and Sowers (2004), who argued that there is no lower temperature limit for microbial activity, which implies that microbes could survive indefinitely, provided they are protected from especially destructive forces such as high temperature and radiation. All claims of exceptionally long-dormant life in frozen terrestrial environments have been challenged on the basis of low-temperature metabolism, which must continue, very slowly, despite the microbe’s state of suspended animation. A further challenge has been raised on the basis of radiation damage due to radiolysis from long-lived radionuclides; without functioning means to repair damage to DNA and other cellular molecular apparatus, the mounting toll of radiolytic damage would render any life dead within the amount of time claimed for these dormant periods. Hence, cell debris resembling cells might survive in a frozen state for millions or billions of years, but the cells themselves would have resisted resuscitation after hundreds of thousands of years even under optimum conditions. One possibility, however, is that sluggish metabolism in frozen microorganisms, coupled with resupply of nutrients and reductants or oxidants, might take place in unfrozen brine films, and possibly even molecular repair activities might occur slowly in frozen microorganisms. However, it seems to us that the challenges to frozen life are pretty severe.

Life in our Solar System could have started rapidly if the Panspermia Hypothesis is correct and life was seeded to Earth (and Mars and Europa?) from outside, or it may have taken hundreds of millions of years. At this time, only the crudest boundaries can be placed on the time for life to develop or the survival time for life after environmental conditions become hostile, but it may be on the order of hundreds of millions of years.