

The microbial communities of sulfur caves: A newly appreciated geologically driven system on Earth and potential model for Mars

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ABSTRACT

A handful of investigative teams in several parts of the world are studying abundant biological communities in caves formed by sulfuric-acid speleogenesis. These caves are atypical in terms of origin, chemistry, and ecosystem properties. They prominently display sulfur minerals, characteristic cavity topologies, and notable biological diversity and biological productivity resulting directly from the conditions that produce the caves. Even long-inactive systems still harbor some of these indicators. The microbial and macroscopic ecosystems within sulfuric-acid speleogenetic caves are geologically mediated and maintained. This geological mediation is a theme connecting them with other sulfur-driven ecosystems on Earth, including deep-sea hydrothermal vents, sulfurous near-surface hydrothermal systems, and solfataras. Evidence exists for potentially significant microbial participation in the process of speleogenesis itself. Recent results confirming the high relative abundance of sulfur on Mars, an apparent sedimentary basin with high sulfate concentration, near-surface indicators of ice and water, and trace detection of reduced gases (especially methane) in the Martian atmosphere, possibly deriving from subsurface microbial sources, set the stage for suggesting that sulfuric-acid speleogenetic systems may be useful as astrobiological analogs for hypothetical Mars ecosystems. Unique speleogenetic mechanisms may occur on Mars and could provide subsurface void space suitable for habitation by such hypothetical microbial systems.

Keywords: acid speleogenesis, sulfur, microorganisms, Mars, astrobiology, extraterrestrial caves.

INTRODUCTION

An intriguing speleological story that has unfolded over the past few decades is the role of sulfuric acid in speleogenesis. The idea that this solutionally aggressive acid has produced some of the world's most beautiful and historic caves is revolutionizing our thinking about the interaction of chemistry and biology on speleogenesis (Hill, 2000; Galdenzi and Menichetti, 1995). It has provided the beginnings of a unifying theme that ties together such disparate features as very large cavity size, ramiform maze configuration, massive gypsum deposits, and significant elemental sulfur (Fig. 1). Also, it has linked our understanding of "organic-looking" speleothems like the u-loops in Lechuguilla Cave, New Mexico (Fig. 2), with morphologically similar living examples in modern sulfuric acid caves (Fig. 3).

Accompanying the new sulfuric-acid speleogenetic paradigm, cave microbiologists and others have discovered that unique living systems inhabit these sulfuric-acid speleogenetic caves. The world list of caves fitting these descriptions is short but steadily growing, providing investigators with potential



Figure 1. Sulfur deposits on the walls and ceiling created by H_2S -rich springs below authors D. Northup (left) and P. Boston (right) as they collect samples for stable-isotopic analyses. Image © 2003 by Kenneth L. Ingham. Used with permission.



Figure 2. Lithified u-loop speleothems in Lechuguilla Cave, New Mexico. These have long been speculated to be of biological origin. Image © by Larry McLaughlin. Used with permission.

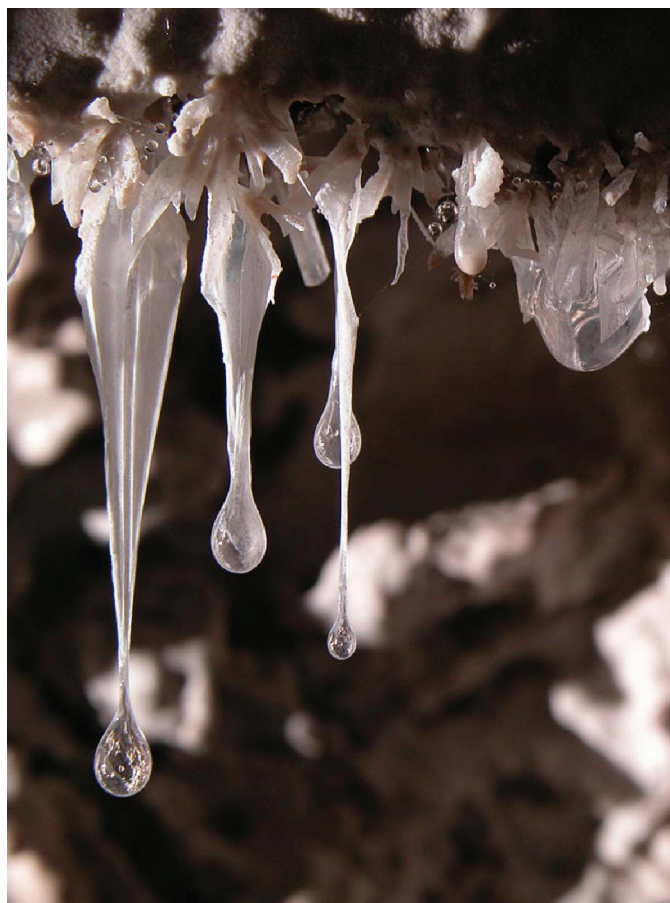


Figure 3. Strings of microbial slime communities in Cueva de Villa Luz, Tabasco, Mexico. These microbial communities are composed of thiobacilli and other organisms that oxidize the H_2S to gain energy to support their metabolism. Two processes going on simultaneously include conversion of wall carbonate to sulfuric acid-soaked gypsum paste, while in situ mineralization of gypsum, selenite, and elemental sulfur occurs within these hanging slime communities. Image © 2003 by Kenneth L. Ingham. Used with permission.

analogues for interpreting their particular study sites in this larger context (Table 1). The fortuitous interplay between sulfide, water, and life in the subsurface of Earth can also provide a potential model for extraterrestrial microbial ecosystems, notably for Mars, which can assist us in the astrobiological search for life beyond our planet (Boston et al., 1992, 2001).

BRIEF HISTORY OF THE SULFURIC-ACID SPELEOGENESIS IDEA

The concept that sulfuric acid plays a role in speleogenesis within carbonate rocks first appeared in English-language literature in the late 1960s. Morehouse (1968) declared that sulfuric acid dominated the dissolution process in Level Crevice Cave, Iowa. Oxidation of massive and disseminated pyrite and marcasite in the limestone bedrock by epigenic (“formed from above”) waters provided the acid. Jagnow (1979) suggested this process to explain the spectacular caves in the Guadalupe Mountains of southeast New Mexico and west Texas after studying a compelling example at the Queen of the Guadalupe Cave, which lies immediately under a gossan (a surface weathering feature produced by oxidation of pyrite to secondary iron oxides). Epigenic sulfuric-acid speleogenesis has been demonstrated in the Xingwen Karst of China (Bottrell, 1993; Bottrell et al., 2001), and several workers (Krothe and Libra, 1983; Moncaster et al., 1992; Lowe et al., 2000) advocated the process as a significant contributor to conduit inception in caves later enlarged by epigenically derived carbonic acid.

Egemeier (1973, 1981) began a conceptual shift amongst North American speleologists from strictly epigenic to hypogenic (“formed from below”) sources for sulfuric acid by his clear documentation of the process in action at Lower Kane and Hellspont Caves in north-central Wyoming. He demonstrated that H_2S in oxygen-depleted water rapidly oxidized to form sulfuric acid (H_2SO_4) upon entering the caves through subterranean springs. The H_2SO_4 and limestone bedrock, in turn, quickly combined to form nearly ubiquitous sub-aerial gypsum weathering surfaces. The cave streams and back-flooding from the adjacent Big Horn River both dissolved and removed any gypsum that fell into the water. Egemeier (1971, 1987) also suggested that hypogenic sulfide-rich groundwater was responsible for the genesis of Carlsbad Cavern and possibly other caves in the Guadalupe Mountains. He postulated a strictly abiological process, not considering any possible role for bacteria in oxidizing hydrogen sulfide in the cave atmosphere.

The concept of hypogenic sulfuric-acid speleogenesis, particularly in the Guadalupe Mountains, began a transformation in thinking toward mixing models that combine reduced, hypogenic, sulfide-rich brine solutions with oxidized, epigenic fresh water (Queen et al., 1977, Queen, 1994; Davis, 1980; Hill, 1981, 1987). Petroleum reservoirs were the suspected source of the sulfidic waters in both the Wyoming and Guadalupe Mountain caves.

The 1980s brought growing awareness and interest in sulfuric-acid speleogenesis. Investigators recognized that rising, mineralized water formed the caves in the Frasassi Gorge (Galdenzi, 1989, 1990; Galdenzi and Menichetti, 1989) and many other large karst systems in Italy (Galdenzi and Menichetti, 1995). The lowest levels of Grotta del Fiume–Grotta Grande del Vento Cave System are actively forming from rising, sulfidic waters entering vadose passages as described by Egemeier’s model. Discoveries beginning in 1986 in Lechuguilla Cave near Carlsbad Cavern, revealed extensive evidence of sulfuric-acid speleogenesis, including massive sulfur deposits (Spirakis and Cunningham, 1992). Workers have documented scores of mineralogical and geomorphic features that characterize sulfuric-acid speleogenesis (Davis et al., 1990; Palmer, 1991; DuChene, 1997; Polyak et al., 1998; Polyak and Provencio, 1998; Palmer and Palmer, 2000) in its 180+ km of surveyed passage (Gulden, 2004).

Two remarkable, actively forming caves with very high inputs of sulfide gases first explored in the 1980s provided the beginnings of evidence that bacteria may play an important role in sulfuric-acid speleogenetic caves via sulfide oxidation contributing to the overall acidity of cave walls and furthering the carbonate dissolution process and conversion to gypsum. Other evidence includes elemental sulfur granule production common in anaerobic and usually photosynthetic bacteria living in the dark (Hose et al., 2000a). Movile Cave, Romania, was opened and explored in 1986, and a robust, chemoautotrophic ecosystem of 48 identified species (34 endemic) living independently of direct solar energy (i.e., nonphotosynthetically) was identified (Sarbu et al., 1996). Moisture on gypsum-coated walls in this cave had a pH of 3.5–4.0. The following year, 1987, American caver Jim Pizarowicz discovered Cueva de Villa Luz in Tabasco, Mexico, and recognized a robust ecosystem living in a highly acidic environment. Although well known to the local Zoque indigenous populations and subject of fish biology investigation in the 1960s (Gordon and Rosen, 1962), this cave had not garnered the attention of the wider scientific community until Pizarowicz’s rediscovery. Distinctive, biological stalactite-like deposits, dubbed “snottites,” drip sulfuric acid with a pH of 0–3.5 (Pizarowicz, 1992; Hose and Pizarowicz, 1999; Hose et al., 2000a). Strongly acidic drips and hydrogen sulfide-charged condensation on the walls of these two caves provided compelling evidence of an important role for sulfuric-acid speleogenesis in some caves.

The 1990s saw a sharp increase in geomicrobiological investigations. Besides Cueva de Villa Luz and Movile, other caves with significant sulfidic inputs that have been studied include Cesspool Cave (Engel et al., 2001), Parker/Sulphur River Passage in Parker Cave (Angert et al., 1998), Frasassi Cave (Vlasceanu et al., 1997, 2000; Lyon et al., 2004), and extensive work in Kane Cave (e.g., Engel et al., 2001, 2004).

While the evidence of conspicuous microbial production of biofilms and other deposits are abundant in these caves, the direct relationship in any quantitative way to speleogenesis is unclear, and the matter has been debated vis-à-vis Movile and

TABLE 1. CAVES IDENTIFIED AS PARTIALLY OR FULLY FORMED BY HYPOGENIC, SULFIDE-RICH WATERS

Cave/region name	Country/State (region)	Active sulfidic community	Length/depth of largest cave (m)	Dominantly air- (A), onwater-filled (W), or mixed (M)	Temperature (°C)	Other notes	References
Frasassi Caves	Italy	Yes	>20,000/~160 (Grotta del Fiume—Grotta Grande del Vento Cave System)	A	13.0–13.5	~100 caves in area	Sarbu et al. (2000); Galdenzi and Maruoka (2003)
Cueva de Villa Luz	Mexico/Tabasco	Yes	1987/23	A w/subterranean springs and following stream	28	10 km from Tertiary andesite flow, 50 km from active volcano, and <60 km from petroleum basin	Hose and Pisarowicz (1999); Hose et al. (2000a)
El Sistema Zacatón	Mexico/ San Luis Potosí	Yes	?/329 (El Zacatón)	W	30–32	3 sulfidic, bio-rich cenotes; sulfidic conditions associated with Tertiary volcanic flows; bottom of El Zacatón is ~100 m msl	Gary et al. (2003)
Movile Cave	Romania/ Dobrogea	Yes	~300/~26	M	19.0–20.9	31 endemic species; demonstrated isolation from surface	Sarbu et al. (1996); Sarbu and Lascu (1997); Movile Cave Project (2004)
Cesspool Cave	USA/Virginia	Yes	20/<10	A w/subterranean spring and following stream	12–13	Developed in Quaternary travertine;	Hubbard et al. (1986, 1990); Engel et al. (2001)
Frasassi Caves	Italy	Yes	>20,000/~160 (Grotta del Fiume—Grotta Grande del Vento Cave System)	A	13.0–13.5	~100 caves in area	Sarbu et al. (2000); Galdenzi and Maruoka (2003)
Pozzo Merro	Italy	Yes	?/392	W	?	World's deepest pit; proximal to Pleistocene igneous rocks	Gary et al. (2003)
Lower Kane Cave and Hellsport Cave	USA/Wyoming	Yes	337 (Lower Kane Cave)	A w/subterranean springs and following streams	21–22	130 km east of Yellowstone geothermal field; within petroleum basin	Egemeier (1973, 1981); Hose and Richards (2000); Engel et al. (2003)
Upper Kane and Salamander Caves	USA/Wyoming	No	310 (Upper Kane Cave)	A	21	130 km east of Yellowstone geothermal field; within petroleum basin	Egemeier (1973), 1981; Hose and Richards (2000)
Carlsbad Cavern, Lechuguilla Cave, and other caves of the Guadalupe Mountains	USA/New Mexico	No	181,421/489 (Lechuguilla Cave)	A	13–20	Speleogenesis dated back to 12 Ma; immediately adjacent to petroleum basin and extensive evaporite deposits	Egemeier (1987); Hill (1987); Hose et al. (2000b); Gulden (2004)

Villa Luz Caves (Sarbu and Lascu, 1997; Vlasceanu et al., 1997; Palmer and Palmer, 1998; Hose et al., 2000a). A past presence of sulfur microbial communities is more subtly evident in relict caves in the Guadalupe Mountains (Cunningham et al., 1995; Northup et al., 2000, 2003; Hose et al., 2000b; Boston et al., 2001; Spilde et al., 2005). Here, large elemental-sulfur deposits and speleothems with distinctive biological-appearing morphologies (Fig. 2) suggest a role for microbiology in the early history of such caves. Several other caves with much lower levels of incoming hydrogen sulfide are providing major additional insights about microbial interactions with sulfur cave habitats and their possible role in speleogenesis and subsequent cave development (e.g., Engel et al., 2004), including those at elevated hot-spring temperatures (Barton and Luiszer, 2005). Engel and co-workers (2004) provided a plausible mechanism for geochemical influence of microbial mats on the cave speleogenesis in Kane Cave, Wyoming. This area of cave geomicrobiology is still immature, with only a handful of investigators involved, but hopefully the community will expand significantly over the coming years.

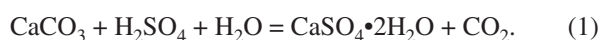
SULFUR MINERALOGY IN SULFUR CAVES

Striking sulfur minerals and elemental-sulfur deposits in caves can be a diagnostic signature of sulfuric-acid speleogenesis, whether their origins are abiotic or biologically mediated. Sulfur is a highly reactive species that produces elaborate suites of secondary sulfur minerals and actively enters into specialized metabolism of many microorganisms that use various forms of sulfur compounds as energy sources (for a review, see Overmann and van Gernerden, 2000). Some organisms are producers of sulfur minerals as apparent byproducts of their metabolism (e.g., Kelly et al., 1997). As Hill and Forti (1997) pointed out, elemental sulfur is the only native element that forms as a primary cave mineral, not derived from secondary chemical reactions with other (primary) minerals. In Cueva de Villa Luz, for example, deposits of native sulfur are present on the walls and ceilings around sulfidic springs (Fig. 1), apparently resulting from direct deposition by oxidation of H₂S gas emitted from the

springs. The abiotic oxidation of H₂S to elemental sulfur occurs quickly on a scale of minutes to hours (White, 1994). Elemental sulfur can also be produced by the anaerobic reduction of sulfates, mainly gypsum, by sulfate-reducing bacteria and by organisms such as the green and purple sulfur bacteria that produce elemental sulfur within their bodies as a byproduct of chemosynthetic metabolism (Ward et al., 1992). Intracellular sulfur bodies in great abundance have been observed in a number of sulfur bacterial strains isolated from the Yellow Roses area of Cueva de Villa Luz (Boston, personal observations).

Elemental sulfur in caves is relatively rare but has been reported in widely distributed cave systems in Austria, Iceland, Italy, Mexico, Russia, Turkmenistan, Uzbekistan, and the United States (Hill and Forti, 1997). Prominent deposits of sulfur have been described in Cueva de Villa Luz in Mexico (Hose and Piasrowicz, 1999) and caves of the Guadalupe Mountains of New Mexico, particularly Lechuguilla Cave and, to a lesser extent, Carlsbad Cavern (Spirakis and Cunningham, 1992; Cunningham et al., 1994).

Sulfates are the second most important class of cave minerals, and gypsum is the third most common cave mineral after calcite and aragonite (Hill and Forti, 1997). Widespread cave deposits of gypsum and occurrences of elemental sulfur suggest a link to H₂S-rich fluids or gases (Davis, 1980; Egemeier, 1981, 1987; Hill, 1987). The chemical oxidation of H₂S-rich water by oxygenated waters or biologically by bacterial oxidation in both the vadose and phreatic zones forms sulfuric acid that aggressively attacks limestone bedrock. Thus, the most abundant gypsum deposits in limestone caves result from sulfuric-acid speleogenesis, where limestone reacts with sulfuric acid to produce gypsum by the reaction:



In addition to gypsum, sulfuric-acid speleogenetic caves frequently host a large variety of unusual sulfates and other rare minerals (Table 2). Complexes such as svanbergite [SrAl₃(PO₄)(SO₄)(OH)₆] and tyuyamunite [Ca(UO₂)₂V₂O₈·5-8(H₂O)] are secondary minerals resulting from dissolution of trace elements from the

TABLE 2. IMPORTANT SULFUR MINERALS IN ACID SPELEOGENETIC CAVES

Mineral	Formula	Origin
Sulfur	S	H ₂ S oxidation, biogenic sulfate reduction
Alunite	KAl ₃ (SO ₄) ₂ (OH) ₆	Acid alteration of clays
Anhydrite	CaSO ₄	Precipitation from saline solution, dehydration of gypsum
Barite	BaSO ₄	Precipitation from aqueous solution
Celestite	SrSO ₄	Precipitation from aqueous solution
Epsomite	MgSO ₄ · 7H ₂ O	Evaporation
Gypsum	CaSO ₄ · 2H ₂ O	Sulfur oxidation, acid alteration of limestone, evaporation, precipitation from aqueous solution
Hexahydrate	MgSO ₄ · 6H ₂ O	Dehydration of epsomite
Jarosite	K ₂ Fe ₆ (SO ₄) ₄ (OH) ₁₂	Oxidation of Fe-sulfides
Kieserite	MgSO ₄ · H ₂ O	Dehydration of epsomite
Melanterite	FeSO ₄ · 7H ₂ O	Oxidation of Fe-sulfides
Mirabalite	NaSO ₄ · 10H ₂ O	Evaporation
Potassium alum	KAl(SO ₄) ₂ · 12H ₂ O	Evaporation
Thenardite	NaSO ₄	Dehydration of mirabalite

bedrock and the subsequent concentration in clays at the bedrock-acid reaction boundary (Hill and Forti, 1997). Other minerals, such as alunite, natroalunite, and endellite (hydrated halloysite), are the result of acid alteration of insoluble clays present in the bedrock (Polyak and Guven, 1996) and are good marker minerals of sulfuric-acid speleogenesis.

The extent to which geologically significant deposits of sulfur and sulfur minerals are attributable to microbial action in all sulfurous environments is controversial (Machel, 1992; Spirakis and Cunningham, 1992; Ehrlich, 1996). However, production of minerals (elemental sulfur and gypsum) by living cultures in the laboratory strengthens the evidence (Boston et al., 2001; Hose et al., 2000a; Spilde et al., 2005), as does the observation that most H₂S in Kane Cave appears to be consumed by subaqueous sulfur-oxidizing organisms, i.e., those living under the water surface rather than in the air-filled portion of the cave (Engel et al., 2004). Additional preliminary evidence is provided by isotopic values of elemental sulfur and gypsum in Cueva de Villa Luz that match well the $\delta^{34}\text{S}$ values obtained from gypsum and sulfur in Lechuguilla Cave (Pisarowicz, 1994; Hose et al., 2000a), implying a similar origin. In Barton and Luiszer (2005), the authors presented further evidence that both sulfate reduction and sulfide oxidation are coincident processes, each of which may be contributing to speleogenesis, even in the absence of free oxygen.

MICROBIOLOGICAL SYSTEMS IN SULFUR CAVES

Most cave ecosystems are very low in nutrients and are based on detritus (Culver, 1985; Simon et al., 2003). However, in the geologically controlled sulfuric-acid speleogenetic caves, the input of energy-rich hydrogen sulfide gas greatly boosts biological productivity via chemolithoautotrophy (Hose et al., 2000a; Sarbu et al., 2000). With respect to abundant biological productivity, cave sulfur systems share this trait in common with other types of sulfur systems, including deep-sea hydrothermal vents (Ruby et al., 1981; Wirsen et al., 1993), surface sulfur springs (Brock, 1978; Frund and Cohen, 1992; Canfield and DesMarais, 1993; Teske et al., 1998; Reysenbach and Cady, 2001; Elshahed et al., 2003), solfataras (Huber et al., 1996), and highly acidic pyrite-oxidizing subsurface communities below ground under the Río Tinto in Spain (González-Toril et al., 2003; Stoker et al., 2004). Comparison of these chemically related but otherwise highly different systems in terms of fundamental biogeochemical cycling, biodiversity, and biomineralization could be a fruitful focus of future research. Such comparisons could help us to determine whether the organisms involved are closely related or whether aspects of the chemistries resemble each other but are produced by different microbial communities specific to each habitat.

Deep-aquifer anaerobic sulfate-reducing bacteria reside at depths well in excess of 3 km below the surface (Olson et al., 1981; Daumas et al., 1986; Chapelle and Lovley, 1990; Fredrickson and Onstott, 1996). They live in systems that

appear to be largely closed with respect to at least geologically short-term exchange of materials to or from the surface. Transport in these systems is dominated by fracture and porosity characteristics and the resulting hydrology. Although similar in some respects to microbial communities inhabiting deep subsurface aquifer and rock masses (Phelps et al., 1989; Chapelle and Lovley, 1990; Fliermans and Hazen, 1991; Pedersen, 1993; Amy and Haldeman 1997), cave microorganisms are subject to a different, typically larger suite of environmental conditions and selective pressures (Moser et al., 2001). These include potential airborne transport of organisms from one area of a cave to another, fluid and particle transport via large conduits, and the delivery of gaseous or aerosolized compounds to the site of growth, which would occur much more slowly in a solid-rock-matrix environment.

Bacterial slimes, films, and mats dominate in many sulfur environments and perhaps serve protective functions in active sulfuric-acid speleogenetic caves against the aggressive chemical environment by allowing organisms to maintain local conditions of redox, dissolved oxygen, pH, and other parameters within their preferred limits. Although these biofilms occur in both water- and air-filled portions of caves, in many respects the moist layer of water on subaerial sulfur masses, gypsum blocks, and cave walls produces mats analogous to the subaqueous biofilms investigated by Costerton et al. (1981, 1987). Biofilms allow organisms to maintain discrete environments around themselves, including a niche for significant anaerobic populations living beneath the outer oxygen-utilizing biota (Costerton et al., 1987).

IDENTITY, BIODIVERSITY, AND ANTIQUITY OF THE SULFUR LIFESTYLE

Studies of sulfuric-acid speleogenetic caves from various areas of the world have investigated sulfur-oxidizing and sulfate-reducing microbial communities. Within the sulfur oxidizers, two main groups have been identified in Cueva de Villa Luz and other caves that may be playing a direct role in wall-rock dissolution: "thiobacilli" and members of the *Epsilonproteobacteria*. The thiobacilli, gram-negative, rod-shaped bacteria, have been identified in Parker Cave's Sulphur River in Kentucky (Angert et al., 1998), Cesspool Cave in Virginia (Engel et al., 2001), Movile Cave in Romania (Vlasceanu et al., 1997), Cueva de Villa Luz in Mexico (Hose et al., 2000a), and the Frasassi Gorge caves in Italy (Vlasceanu et al., 2000). *Epsilonproteobacteria*, which usually occur in sulfuric-acid speleogenetic caves as filamentous, uncharacterized environmental isolates, have been found in Lower Kane Cave in Wyoming (Engel et al., 2003), Parker Cave's Sulphur River in Kentucky (Angert et al., 1998), Cesspool Cave in Virginia (Engel et al., 2001), and Cueva de Villa Luz in Mexico (Northup et al., 2005). Engel et al. (2003) noted that all *Epsilonproteobacteria* isolated to date either oxidize sulfur compounds with oxygen, nitrate, various sulfur species, or metals as electron acceptors, supporting the sulfur-

cycling role of these organisms. Sulfate-reducing bacteria reside in several of the black mud habitats in Cueva de Villa Luz (Hose et al., 2000a). Luxuriant dense mats dominated by purple and green sulfur bacteria (e.g., *Chromatium* spp. and others, and several members of the Chlorobiaceae) line the subterranean spring passages in Villa Luz (Boston et al., 1999). They appear to be surviving in the total darkness by metabolizing hydrogen sulfide and/or sulfur. Sulfate-reducing bacteria have been recovered from most of the microbial mat, slime, and mud deposits within Cueva de Villa Luz, even when dissolved oxygen is high in the overall vicinity (Hose et al., 2000a). Common sulfur organisms also in the streams of many of these caves and include genera like *Beggiatoa* and *Thiothrix*. For our purposes, they are less interesting, since we have not yet seen evidence that they significantly affect the geochemistry of wall-rock dissolution nor contribute significantly to secondary mineral precipitation.

In 2000, Ruldolf Amann (2000, p. 1) wrote of the “large microbiota incognita... present in common habitats.” Caves represent an uncommon habitat of largely unknown microbial biodiversity and possess the potential to have an even greater microbiota incognita. Using molecular biology techniques that do not require the growing of microbes, researchers have been working to disclose the nature of these novel microorganisms. They have discovered new major groups in the environment, including new kingdoms of microorganisms (DeLong and Pace, 2001). Applying this methodology to cave communities has led to the discovery of many novel organisms, such as mesophilic Archaea in Lechuguilla Cave (Northup et al., 2003) and *Epsilon-proteobacteria* in sulfuric-acid speleogenetic caves, such as Lower Kane Cave (Engel et al., 2003), Parker Cave (Angert et al., 1998), and Cueva de Villa Luz (Northup et al., 2005). Because of the extreme and unusual nature of many sulfuric-acid speleogenetic caves, the potential exists to greatly expand our knowledge of this microbiota incognita.

The widely distributed sulfur-based microbial systems mentioned herein have led to much speculation about the degree of relatedness of organisms in these environments and their possible relationship to early organisms. Sulfur metabolism (particularly at depth) has been playing a controversial role in thinking about the very origins of life since the early 1990s (Drobner et al., 1990; Wächtershäuser, 1990, 1991, 2000). These investigators hypothesized pyrite oxidation as the earliest type of metabolism. Subsequently, further plausibility has been afforded to this hypothesis by investigators at the Carnegie Institution who have synthesized $\text{CH}_3\text{-CO-COOH}$ (pyruvic acid), an essential compound in Wächtershäuser’s pre-biotic scheme, from CO with iron sulfide (pyrite) at 250 °C and pressure simulating a 7 km depth within rock (Cody et al., 2000). A complementary hypothesis, possibly salient to the origin of some subsurface microorganisms, is the notion that surface sterilization of any very early life form may have occurred during Earth’s early biological history due to the Early Heavy Bombardment Period, in which leftover material from the formation of the solar system pelted the planet (e.g., Sleep et al., 1989;

Sleep and Zahnle, 1998). It has been hypothesized that only subsurface microbiota would have survived this evolutionary bottleneck and been available to repopulate the surface. If this scenario is to be believed, then all life on Earth may be the distant descendants of subsurface sulfur, hydrogen, and other microorganisms in the subsurface.

SULFUR CAVE SYSTEMS AS MARS ASTROBIOLOGY ANALOGS

It is known that Mars has volcanic lava-tube caves (Boston, 2003), but the question arises whether there are other speleogenetic mechanisms that may have operated on that planet (Grin et al., 1998, 1999; Boston, 2003). Orbital missions to date have not directly detected hard-to-image features like solutional caves. While Mars is very different from Earth, possible speleogenetic mechanisms can be postulated based on what we do know about the planet. Interest in possible Martian caves extends beyond the intrinsic geologic interest in speleogenesis for its own sake to the potential of caves as repositories of climate signals, geochemical data, unusual minerals, and, importantly, life or traces of past life (Boston et al., 1992, 2001; Grin et al., 1999, 1998; Boston, 1999, 2000; McKay et al., 1994).

Recent results from the spate of Mars missions that have flown successfully since the Pathfinder Mission in 1997 have added pieces to the puzzle. There is strong evidence of near-subsurface water ice (Boynton et al., 2002; Feldman et al., 2002; Mitrofanov et al., 2002). Based on calculations of geothermal heat flow on Mars (Solomon and Head, 1990), it has been inferred that there may be a liquid water zone beneath the surface (Boston et al., 1992). The Mars Exploration Rover (MER) Spirit that landed in Gusev Crater uncovered evidence that the crater may have been a shallow lake or small sea at one time (Squyres et al., 2004). Baker and colleagues (1991) first advanced this notion of shallow large water bodies in the 1990s. The MER missions of 2004–2005 have also confirmed abundant sulfur compounds in the Martian surface in fine-grained sediments and within tested rocks (Gellert et al., 2004). High sulfur abundance on the surface was documented by the Viking missions to Mars in the mid-1970s (Toulmin et al., 1977; Clark and Baird, 1979; Banin et al., 1992) and again by Mars Pathfinder (Rieder et al., 1997). Sulfur figured in early models of hypothetical sulfur-driven microbial communities that might be found on Mars (Clark, 1979). Sulfur-based food chains were an early inspiration of post-Viking thinking (Clark, 1979), and applying the notion of chemolithotrophic communities to subsurface Mars communities followed thereafter (Boston et al., 1992; Fisk and Giovannoni, 1999).

The latest, recently emerged, puzzle piece that could indicate a subsurface microbial presence concerns apparent detection of CH_4 and weaker evidence for NH_3 , both chemically reduced gases, in the Martian atmosphere (Formisano et al., 2004; Mumma et al., 2004; Krashnopol'sky et al., 2004; Wong et al., 2004). These investigators detected the methane both

from the orbital European Space Agency's Mars Express Mission and ground-based telescopic measurements. Such evidence of "leakiness" from the subsurface has been suggested as a potential indicator of reduced gases in the subsurface that could be energy sources for subterranean microbial biota (Levine et al., 1989; Boston et al., 1992). Whether the CH₄ detection is valid, and whether other chemical explanations prove to be correct, awaits further research.

To illustrate a plausibility of the argument linking sulfur cave ecosystems to Mars, Figure 4 shows a hypothetical Mars impact crater catastrophic speleogenetic mechanism incorporating some of the newest findings from various missions. Such conceptual models can help to guide thinking about potential future subsurface mission targets using orbital, aerial, or ground-based robotic searching strategies involving geophysical techniques like ground-penetrating radar, seismic methods, and others.

Some areas on Mars may possess an icy subsurface layer that acts as an impermeable barrier to upward percolation of reduced gases produced below (Fig. 4A). An impacting object striking the surface of Mars would inject significant energy at that site (Fig. 4B). The icy layer would probably melt out to a significant distance from the impact entry point. Also as a result of impact, extensive fracturing would occur in the parent rock. This stratum may be basalt or other volcanic rocks, but may also be evaporite basin material, notably gypsum and magnesium sulfates, indicated by MER rover data (Gellert et al., 2004). A type of catastrophic hydrothermal speleogenesis may then ensue due to the massive fracturing of the site and violent release of hot water and steam. Evidence for such massive floods has long been recognized on Mars (Baker and Milton, 1974) and has been tied to impact events. Although controversial, recent suggestions that even the shapes of the ice floes result from such a massive impact-related catastrophic flood have been put forward by the European Space Agency's Mars Express Team (Murray et al., 2005). Subsequent to impact, more familiar, Earth-like, secondary solutional processes could continue for some few to several tens of thousand years based on the amount of energy that was imparted on impact (Fig. 4C). Based on their observations of the Haughton Crater site in Devon Island in the Canadian Arctic, Cockell and colleagues estimated that such a transient geothermal site could have persisted for several thousand years on Mars (Cockell and Lee, 2002; Cockell et al., 2002). An impacting object that struck a gypsum or carbonate bed could produce a large amount of sulfide, SO₂, and other gases. D'Hondt et al. (1994) hypothesized that sulfuric acid resulted from the Cretaceous-Tertiary (K/T) impactor striking evaporite deposits on Earth. Finally, once the heat of impact is exhausted, the site can refreeze and return to a state similar to the pre-impact condition, with the important exception that caves have been formed beneath the crater (Fig. 4D). Such caves could provide new habitat opportunities for any subsurface fracture-inhabiting biota to invade. Additionally, caves could provide a much more accessible site than unbreached rock layers for robotic life detection

missions with or without drilling (Dubowsky et al., 2004). Detailed discussion of the relative merits and deficits of this hypothetical scenario is beyond the scope of this paper; however, it illustrates why some astrobiologists are taking such an interest in the formation of sulfuric-acid speleogenetic caves on Earth and their associated ecosystems.

THE FUTURE—THE BIG QUESTIONS AND THE LITTLE DETAILS

Study of sulfuric-acid speleogenetic caves and their microbial communities is in its infancy. A small set of extremely important questions awaits further work. The first of these is identification of the ultimate source of H₂S coming into a particular system. Derivation from petroleum basins, magmatic systems, or the biological degradation of sulfate-containing strata are all possibilities. Learning to distinguish between these options will require extensive analysis of stable-isotopic ratios, thorough understanding of the fundamental regional geology, dating techniques, and mineralogical and biological analyses.

Second on the list of important questions is the assessment of the extent of direct biological contribution in speleogenesis, specifically bedrock dissolution. Here again, stable-isotopic analyses are critical, along with laboratory and field attempts to quantify the rates at which microbial communities may help dissolution. Tracer studies, so critical in establishing rates of various *in situ* biological processes, are often difficult to accomplish in caves where impact of investigations must be kept to a minimum because of conservation considerations and restrictions in these fragile and commonly nonrenewable environments. Proxies for dissolution rates in nature derived by observation of dissolution rates observable in laboratory cultures can provide some insight but clearly do not provide direct quantification of what is going on in the caves. Further methodological developments on this front would be very valuable.

The third critical issue worthy of study is the determination of biogenicity of any minerals in these sulfur caves. Biogenicity can be either passive (that is, the result of preferential precipitation of minerals on biofilm and microbial cell bodies or filaments) or accomplished by active metabolic processing of materials (Lowenstam and Weiner, 1989; Konhauser, 1997, 1998). Both types of processes are equally interesting and tell us different things about the mineralogy and biology involved. Study in this area requires the successful growth of organisms in the laboratory and unequivocal production of the minerals in question by the captive microbes (Boston et al., 2001).

In summary, sulfur-driven microbial communities in caves constitute a type of ecosystem of relevance to many fields—speleogenesis, cave mineralogy and geochemistry, extremophile geomicrobiology, biomineralogy, and astrobiology. As awareness of the special nature of such systems grows, cave explorers will be alerted to their value, and more of these systems will undoubtedly be identified.

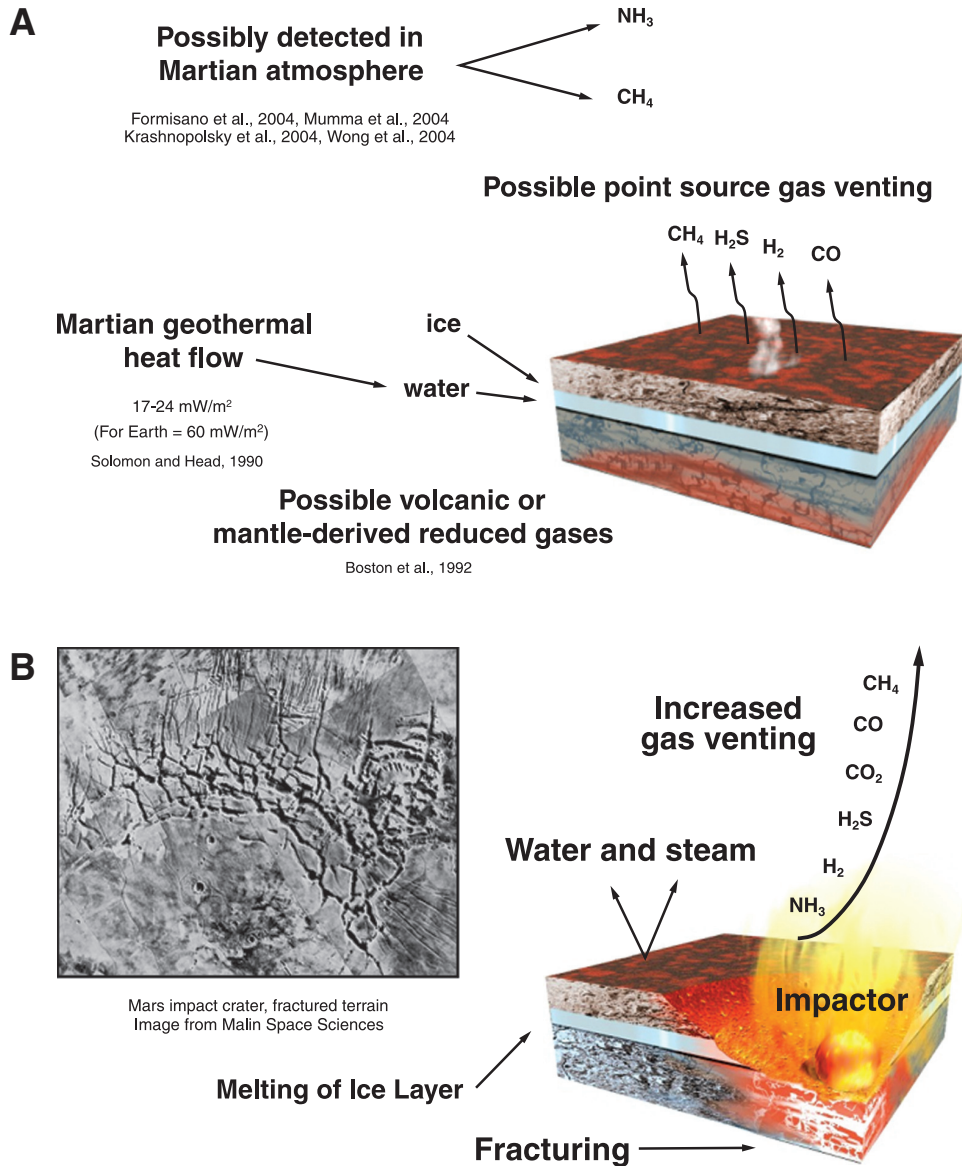


Figure 4 (on this and following page). Series of four panels presenting a hypothetical model of hypogenic-like cave formation that might occur on Mars following impact cratering into subsurface evaporite and ice-bearing strata. We have expanded the notion of impact crater-induced transient geothermal habitats introduced by Cockell and co-workers (Cockell and Lee, 2002; Cockell et al., 2002) to encompass the possibility of the creation of fractures and resulting cavities beneath such impact features. Graphic © 2004 by R.D. Frederick. Used with permission. (A) Depiction of possible background condition with subsurface ice layer and possible slight leakiness of reduced gases from geothermal or biological activity below. Reports of methane and ammonia have recently been made by European Space Agency (ESA) Mars Express investigators (Formisano et al., 2004; Wong et al., 2004) and ground-based astronomical teams (Krashnopol'sky et al., 2004; Mumma et al., 2004). (B) Illustration of an impacting body creating massive fracturing, near-instantaneous flash heating of subsurface ice to water and steam, and initial mechanical creation of subsurface macroporosity. The photographic insert shows an example of such fracturing at a Mars impact crater site. (C) Illustration of a geothermal system created by the transient heating from the energy of an impact. Possible chemistries dependent upon sedimentary composition of impact target area are listed. Sources for hydrogen sulfide include release of the gas from below, where, in our scenario (Boston et al., 1992), it may already be slowly percolating up from magmatic sources or from heat-shock vaporization of gypsum and other sulfates recently shown to be present at the Mars Exploration Rover (MER) rover sites (Gellert et al., 2004). The Cretaceous-Tertiary (K/T) impactor, reputed to have created the Chicxulub crater near the Yucatan Peninsula, is thought to have created enormous quantities of SO_x gases by vaporization (Gupta et al., 2001); however, this took place in a free oxygen-dominated, 1 bar atmosphere. We believe the shock chemistry in the Martian case would be significantly less oxidized and produce less-oxidized forms of vaporous sulfur compounds, but this has not yet been modeled to our knowledge. Hypothetical solutional speleogenesis in impact fractures is illustrated. Insert shows airborne photograph of Houghton Crater impact site, a 23-m.y.-old crater on Devon Island in the High Arctic of Canada. Synthetic aperture radar is taken by the Intera STAR X-band radar system. Frame width is 36 km; crater is ~20 km wide. Image is courtesy of the Geological Survey of Canada. (D) Illustration of a return to background condition after impact-induced heating dissipates. Water that is still left in the system refreezes and reseals the system. The major change from pre-impact is the presence of caverns beneath the crater site.

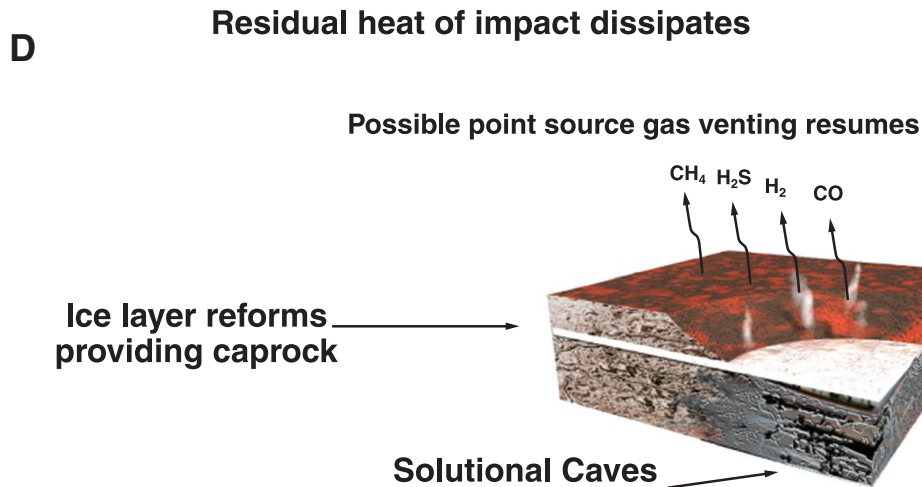
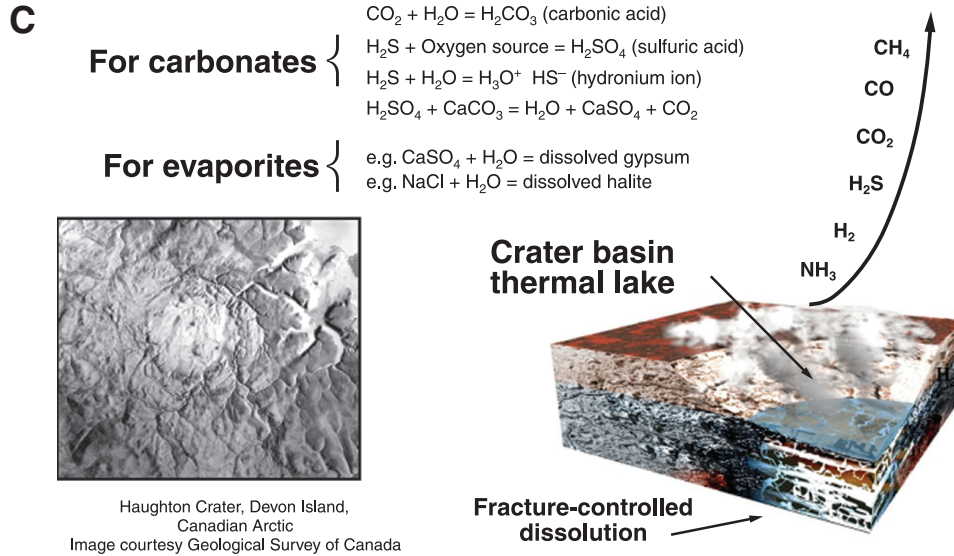


Figure 4 (continued).

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