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Geomicrobiology of Caves: A Review

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In this article, we provide a review of geomicrobiological interactions in caves, which are nutrient-limited environments containing a variety of redox interfaces. Interactions of cave microorganisms and mineral environments lead to the dissolution of, or precipitation on, host rock and speleothems (secondary mineral formations). Metabolic processes of sulfur-, iron-, and manganese-oxidizing bacteria can generate considerable acidity, dissolving cave walls and formations. Examples of possible microbially influenced corrosion include corrosion residues (e.g., Lechuguilla and Spider caves, New Mexico, USA), moonmilk from a number of caves (e.g., Spider Cave, New Mexico, and caves in the Italian Alps), and sulfuric acid speleogenesis and cave enlargement (e.g., Movile Cave, Romania, and Cueva de Villa Luz, Mexico). Precipitation processes in caves, as in surface environments, occur through active or passive processes. In caves, microbially induced mineralization is documented in the formation of carbonates, moonmilk, silicates, clays, iron and manganese oxides, sulfur, and saltpeter at scales ranging from the microscopic to landscape biokarst. Suggestions for future research are given to encourage a move from descriptive, qualitative studies to more experimental studies.

Keywords biokarst, biomineralization, caves, iron and manganese oxides, microorganisms, moonmilk, saltpeter, sulfur

Introduction

Microorganisms have been shown to be important active and passive promoters of redox reactions that influence geological formations (Ehrlich 1996). Studies of microorganisms in caves have been predominantly descriptive with only a few experimental studies reported, but the past decade has produced extensive research into microbial interactions with minerals within cave environments (see papers in Northup, Reysenbach, and Pace 1997; Sasowsky

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and Palmer 1994). We review these efforts in the context of dissolution and precipitation reactions involving carbonates, moonmilk, silicates, clays, iron, manganese, sulfur, saltpeter, and the formation of biokarst and phytokarst. An overview of caves and their speleothems (secondary mineral formations) is provided. Following this overview of the subject, the rest of this special issue brings together new results in the field of cave geomicrobiology.

Types of Caves

Spaces below the Earth's surface range in size from microfissures to hundreds of kilometers in length and, theoretically, most have no natural human-accessible entrances (Curl 1966). A "cave" is defined as any natural space below the surface that extends beyond the twilight zone, and that is accessible to humans (Gillieson 1996; Hill and Forti 1997). Caves can be classified in several ways, particularly by the type of rock and method of formation (Palmer 1991). The most common types of caves are those formed in limestone and other calcareous rocks, and as lava tubes in basaltic rock. Other types of caves are usually limited in extent, and include those in gypsum, granite, talus, quartzite, ice, and sandstone.

There are three primary mechanisms for the formation of caves. Classical limestone caves such as Mammoth Cave, Kentucky, and Altamira Cave, Spain, are formed as water passes through the soil zone, absorbing CO_2 and forming a dilute solution of carbonic acid. As the acidic water reaches the water table, it stays in contact with the limestone and dissolves more calcium carbonate (Gillieson 1996). As the water reaches the cave, carbon dioxide degasses into the cave air, which allows the formation of carbonate speleothems such as stalactites and stalagmites. Sulfuric acid-driven speleogenesis creates some limestone caves when hydrogen sulfide rises along fissures until it encounters the oxygenated zone. There, sulfuric acid forms that dissolves the limestone (Hill 1987, 1990, 1995; Jagnow, Hill, Davis, Duchene, Cunningham, Northup, and Queen 2000). Examples of sulfuric acid caves include Carlsbad Cavern and Lechuguilla Cave, New Mexico, and Movile Cave, Romania. Lava tube caves are formed by flowing lava, as can be seen on the Kilauea volcano, Hawaii. As molten lava flows out of a volcano, the surface lava cools more quickly and solidifies. When the eruption stops, the rapidly flowing lava may drain, leaving an empty tubular conduit behind.

Speleothems and Minerals

Cave Minerals of the World (Hill and Forti 1997) reviews the many types of speleothems and minerals found in caves. Spelothems are secondary mineral deposits formed by a physico-chemical reaction from a primary mineral in a cave (Moore 1952). Hill and Forti recognized 38 "official" speleothem types, with numerous subtypes and varieties, and describe over 250 different minerals found in caves. A particular speleothem can be composed of any of a number of different minerals or unconsolidated materials.

Microbial Ecology of Caves

Caves are considered to be extreme environments for life, and are often severely resource-limited due to the absence of light that precludes primary production of organic material by plants. Physical parameters, however, tend to be relatively mild, predictable, and constant. Entering a cave, one goes through a series of zones, beginning with an entrance zone that is strongly impacted by surface conditions. Deeper into a cave is the twilight zone, where limited light penetrates and surface conditions are ameliorated by cave conditions. In the deep cave, there is an absence of light, temperature that is at or near the MAST (Mean Annual Surface Temperature) for the region, and high humidity.

Many studies have described microorganisms occurring in both terrestrial sediments and aquatic cave environments around the world. One of the earliest publications was by Høeg (1946), who studied microbes on the walls of Norwegian caves. Caumartin (1963) provided an early review of microorganisms from caves. Other reviews include Vandel (1965), Dyson and James (1973), Dickson and Kirk (1976), Jones and Motyka (1987), and Rutherford and Huang (1994).

Many microbes identified from deep caves are identical to surface forms, opportunistic and active only under favorable growth conditions (Dickson and Kirk 1976; James 1994; Jones and Motyka 1987). Most are nonresidents transported into caves by water, air, sediment, and animals. However, these enrichment-based and cultural studies have focused on typical heterotrophic microbes known from surface studies; such techniques have been shown to grow less than 1% of microbes present in an environment (Amann, Ludwig, and Schleifer 1995; Winogradsky 1949). Culture-independent, molecular phylogenetic techniques have since shown that many novel organisms can be found in caves (Angert, Northup, Reysenbach, Peek, Goebel, and Pace 1998; Vlasceanu, Sarbu, Engel, and Kinkle 2000). James (1994) offered the caveat that biogenic and inorganic processes may be difficult or impossible to differentiate. Furthermore, contributions of chemolithotrophic microorganisms using iron, sulfur, and manganese have traditionally been considered to be of limited importance (Barr 1968; Caumartin 1963; Jones and Motyka 1987), but recent studies (Hose, Palmer, Palmer, Northup, Boston, and Duchene 2000; Sarbu, Kane, and Kinkle 1996) provide counterexamples.

Redox Environments in Caves

Prior to the 1990s, much of the work on microorganisms in caves concentrated on descriptions of organisms, and documented, to a limited extent, the relationship of organisms to redox environments. Caves have been viewed as highly oxidized environments with little in the way of reduced compounds to support the growth of microorganisms; however, recent studies have shown that microorganisms in caves exploit a variety of environments with redox gradients (see papers in Sasowsky and Palmer 1994). Microorganisms living at the interface between the host rock and cave passages can utilize reduced compounds in the host rock. Limestones and basalts are often rich in reduced sulfur, iron, and manganese.

Entry of flowing, dripping, and seeping water can bring energy into caves. For example, two springs only 2 m apart in Cueva de Villa Luz, Mexico, have drastically different water chemistries (Hose and Pisarowicz 1999; Hose et al. 2000). Anchialine caves are aquatic limestone caves with an inland opening to the surface and subterranean connections to marine waters characterized by a saline layer beneath less dense fresh water. A boundary layer sharply separates the two waters with gradients of dissolved oxygen, salinity, light, and temperature. Anchaline caves support high densities of microbes and other organisms that stratify by gradient (Wilson and Morris 1994).

The Edwards Aquifer of Texas provides an excellent example of subterranean redox boundaries (Schindel, Worthington, Alexander, and Veni 2000) with a freshwater/saline water interface with redox interfaces of sulfates, chlorides, H_2S, and bacteria. The deep artesian zone of the aquifer has developed an interesting ecology including two blind catfish species that probably feed on bacteria along the redox interface. Fungi growing in shallower artesian wells in the aquifer were presumed to utilize the energy from hydrocarbons at an oxygen boundary interface (Kuehn and Koehn 1988). These and many other examples (see this issue) demonstrate the rich potential for study of geomicrobiological interactions at redox boundaries in caves.

Dissolution and Precipitation Processes

In caves, a variety of precipitation and dissolution processes results in the deposition of carbonate speleothems, silicates, iron and manganese oxides, sulfur compounds, and nitrates, and the breakdown of limestone walls. Precipitation processes can be passive where microbial cells act as nucleation sites, or active where bacterially produced enzymes control mineralization. Microbially influenced corrosion or dissolution of mineral surfaces can occur through mechanical attack, secretion of exoenzymes, organic and mineral acids (e.g., sulfuric acid), and a variety of other mechanisms (reviewed in Sand 1997). Of particular interest in cave dissolution processes are reactions involving iron-, sulfur-, and manganese-oxidizing bacteria. These microbially mediated reactions can generate considerable acidity that can dissolve cave walls or speleothems. Laboratory investigations (Engel et al. 2001, this issue) demonstrate the ability of bacteria isolated from caves to dissolve calcium carbonate.

Sulfuric Acid Dissolution

Sulfuric acid-driven speleogenesis, where sulfuric acid causes the dissolution of limestone and results in the precipitation of gypsum, has been implicated in the formation of numerous caves (Davis 1980; Egemeier 1981; Galdenzi 1990; Galdenzi and Menichetti 1995; Galdenzi, Menichetti, and Forti 1997; Hill 1987, 1990, 1995; Korshunov and Semikolennyh 1994; Principi 1931). The involvement of bacteria in sulfuric acid-driven speleogenesis is under investigation. Egemeier (1973, 1981) suggested that the hydrogen sulfide present in the Kane Caves, Wyoming, is biogenic. Molecular phylogenetic studies of acidic biofilms in Cueva de Villa Luz, Mexico (Figure 1) and Frasassi Gorge, Italy, caves demonstrate the presence of *Thiobacillus* spp. (Hose et al. 2000; Vlasceanu et al. 2000). *Thiobacillus* bacteria gain energy from the oxidation of sulfur or sulfide to sulfuric acid and can contribute to dissolution of carbonate bedrock in these caves.

Corrosion Residues

Microbially influenced corrosion of limestone walls can be observed in Lechuguilla and Spider caves, Carlsbad Caverns National Park, New Mexico, in the "corrosion residues," which are fluffy, multicolored coatings composed of iron and manganese oxides and clays (Cunningham, Northup, Pollastro, Wright, and Larock 1995; Figure 2). Bulk chemistry and XRD studies of corrosion residues have demonstrated that these are not simply dissolution products but are highly enriched in certain elements such as Fe and Mn, possibly by microbial processes (Dotson, Schelble, Spilde, Crossey, and Northup 1999). Utilizing molecular phylogenetic techniques and enrichment cultures targeting iron and manganese oxidizers, Northup et al. (2000) have shown support for microbially influenced corrosion of limestone wall rock by iron- and manganese-oxidizing bacteria.

Corrosion Moonmilk

Moonmilk is usually considered to be a depositional product, as discussed in more detail later; however, it can also form by corrosional processes (Hill and Forti 1997). Caumartin and Renault (1958) and Caumartin (1963) suggested that moonmilk could be the product of microbial metabolism, which could biochemically corrode underlying bedrock, flowstone, and speleothems that would disintegrate when wet (Sweeting 1973). Gradzíński, Szulc, and Smyk (1997) concluded that moonmilk deposits from several caves in southern Poland were the result of either microbially mediated precipitation of autochthonous carbonates or microbial degradation of the host rock.

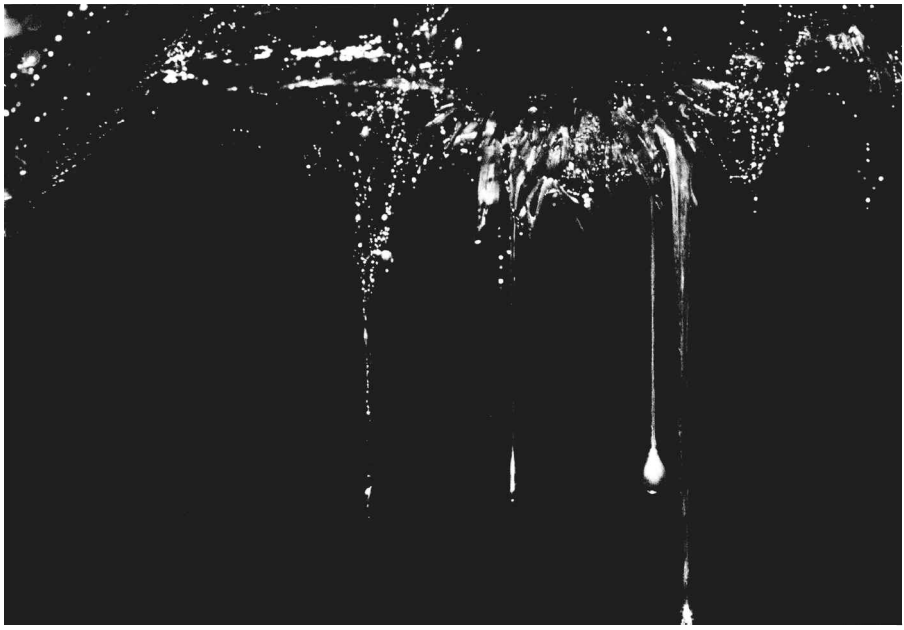

FIGURE 1 Acid biofilms ("snottites") in Cueva de Villa Luz, Tabasco, Mexico. Photo by Kenneth Ingham.

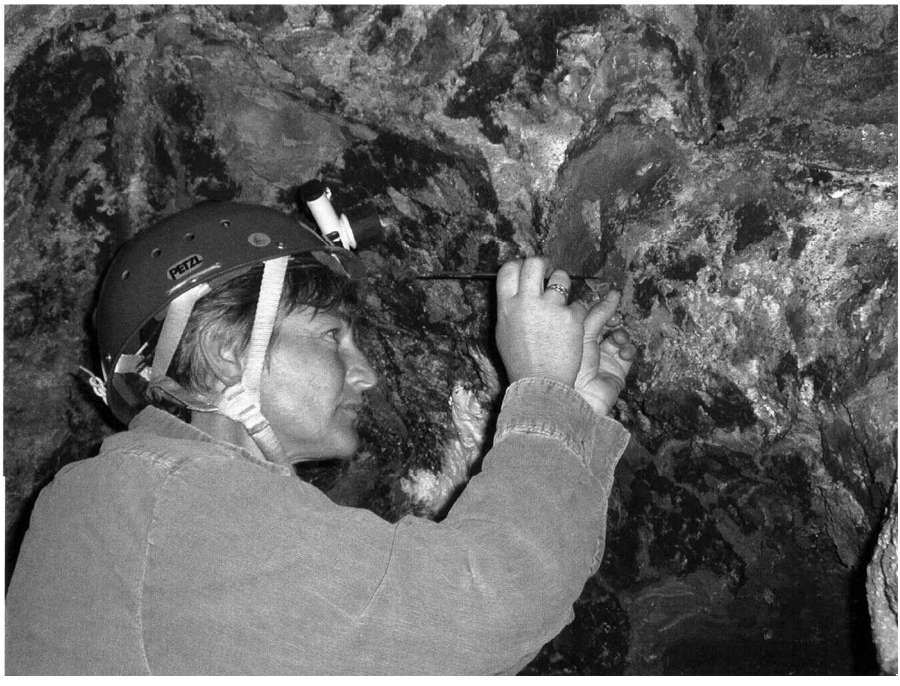

FIGURE 2 Anna-Louise Reysenbach sampling corrosion residues in Spider Cave, Carlsbad Caverns National Park, New Mexico, USA. Note the corroded and highly variable coloration of the walls. Photo by Kenneth Ingham.

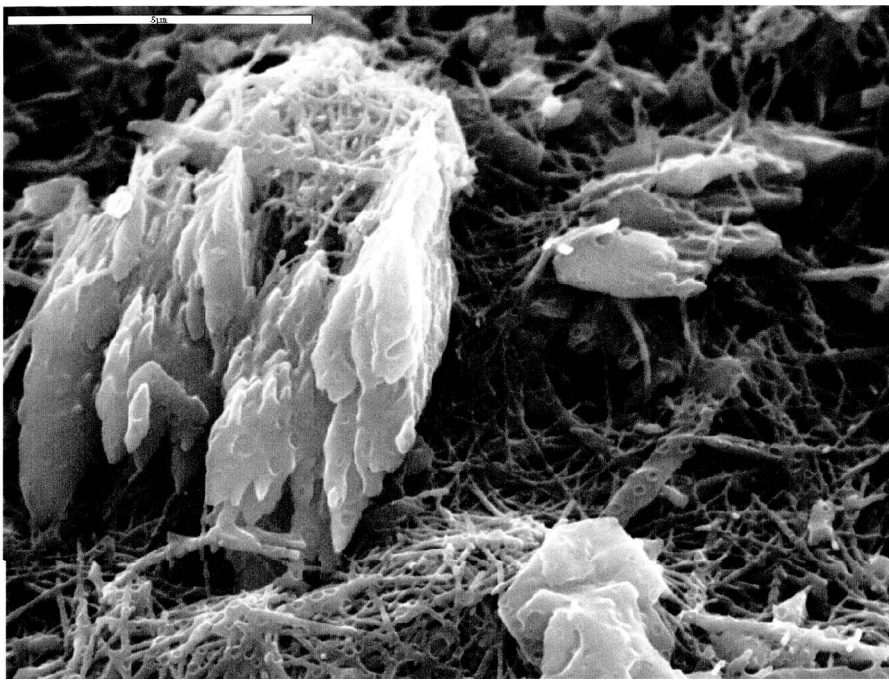

FIGURE 3 Scanning electron micrograph of moonmilk from Spider Cave, Carlsbad Caverns National Park, New Mexico, USA. Scale bar is 5 μm in length. Photomicrograph by Michael Spilde and Penelope Boston.

Microbial activity can cause degradation of speleothem structures, but whether that degradation can result in moonmilk has not yet been established. Most scanning electron microscopy (SEM) pictures of the microcrystals that make up moonmilk do not provide evidence of the weathering we would expect to see if microbial action routinely resulted in moonmilk production (Figure 3). Experimentation could establish corrosion as a mechanism for moonmilk formation.

Precipitation Processes

Various metals precipitate onto bacterial cell surfaces through biologically controlled mineralization (direct or enzymatic) or biologically induced mineralization (indirect or passive) (e.g., see Konhauser 1997, 1998; Lowenstam and Weiner 1989). In passive mineralization, dissolved metals sorb to amphoteric functional groups (carboxyl, phosphoryl, and amino constituents) on negatively charged cell wall surfaces, sheaths, or capsules. Bound metals (such as iron) provide sites for chemical interactions, reduce activation energy barriers, and serve as nucleation sites for crystal growth. Additionally, metabolic activity of microorganisms can change the pH of the surrounding environment leading to precipitation of various minerals. Several examples of these processes occur in caves and will be discussed next.

Summary. Experimental studies are shedding light on the ability of sulfur-oxidizing bacteria to contribute to speleogenesis and enlargement of existing caves, and the ability of iron- and manganese-oxidizing bacteria to corrode limestone walls, but future studies must establish the extent to which these bacteria contribute to these dissolution processes. In caves, biologically controlled and biologically induced mineralization are observed in the

formation of carbonates (including moonmilk), silicates, clays, iron and manganese oxides, sulfur, and nitrates (saltpeter).

Carbonates

Calcium carbonate speleothems predominate in most caves, and microbial studies have been done on stalactites, stalagmites, helictites, moonmilk, pool fingers, and cave pearls. An early study by Danielli and Edington (1983) demonstrated the ability of microorganisms isolated from cave deposits to precipitate calcium carbonate. More recent studies have attempted to identify the factors that control the contribution of microorganisms to carbonate precipitation (LeMétayer-Levrel, Castanier, Loubière, and Perthuisot 1997). Riding (2000) has provided an excellent review of "microbial carbonates," which included some cave deposits, and Jones (2000) described his extensive and detailed investigations of microbial karst sediments in the Cayman Islands, which span more than a decade in time.

Fungi, algae, and bacteria have all been implicated in the precipitation of carbonate dripstone in caves (Cox, James, Osborne, and Leggett, 1989a; Cox, James, Legget, and Osborne 1989b; Cox, Salih, James, and Allaway 1995; Danielli and Edington 1983; Went 1969). Microorganisms have been found fossilized within carbonate speleothems (e.g., Jones and Kahle 1995; Jones and Motyka 1987; Melim et al. 2001, this issue; Polyak and Cokendolpher 1992, this issue). However, because many of these studies were primarily observational in nature, the extent of microbial interactions in the production of carbonate speleothems is uncertain. Extensive documentation of microbial precipitation of calcium carbonate exists in the noncave, carbonate/travertine literature (e.g., Ehrlich 1996), but investigators have not established whether cave carbonate/travertine material has a similar origin.

Bacteria and fungi can precipitate calcium carbonate extracellularly through a variety of processes that include photosynthesis, ammonification, denitrification, sulfate reduction, and anaerobic sulfide oxidation (Castanier, Le Métayer-Levrel, and Perthuisot 1999; Ehrlich 1996; Riding 2000; Simkiss and Wilbur 1989). These processes increase the concentration of HCO_3^-, leading to increased alkalinity and causing calcium carbonate precipitation. An initial step in the precipitation of carbonate involves the adsorption of Ca^{2+} and Mg^{2+} to negatively charged cell surfaces. Once initial carbonate precipitates on the cell surface, the cell acts as a nucleation site—a critical stage in mineral precipitation. Subsequent $CaCO_3$ precipitation may be purely inorganic. Riding (2000) noted that microbial production of extracellular polymeric substances (EPS), which trap sediments, is often critical to the creation of microbial carbonates.

Castanier et al. (1999) proposed biologically controlled, or active precipitation of, $CaCO_3$ where carbonate particles are produced by ionic exchanges through the cell membrane of heterotrophic bacteria in an environment enriched in organic matter. An excellent study by Chafetz and Buczynski (1992) demonstrated that lab cultures inoculated from tidal-flat microbial mats from tidal flats produced the same carbonate precipitates observed in microbial mats in nature.

Fungal hyphae may act as nuclei for crystallization and as sites for attachment of crystals. Went (1969) showed fungal hyphae coated with calcium carbonate crystals extending down into the drop of water at the end of active stalactites. Algae and cyanobacteria precipitate calcium carbonate by changing the microclimate as a result of fixing carbon dioxide. They may subsequently trap and bind the particles to carbonate speleothems in the entrance and twilight zones of caves (Contos et al. 2001, this issue; Cox et al. 1989a,b, 1995; James, Patsalides, and Cox 1994; Jones and Motyka 1987). Heterotrophic bacteria have also been implicated in the precipitation of calcium carbonate in caves (Danielli and Edington 1983).

Terrestrial oncoids, laminated, microbially formed constructions, from sinkholes in dolostones from Grand Cayman and Cayman Brac, showed a diverse microbial component (Jones 1991). The oncoids grew when calcifying filaments and spores trapped and bound detritus within the associated mucus. Jones (1991) provided criteria for differentiating abiotic from biotic coatings on grains. Terrestrial oncoids resemble cave pearls, a speleothem that may have a microbial association. Gradzíński (1999) found a strong microbial component to irregular, but not regular, cave pearls.

LeMétayer-Levrel et al. (1997) investigated microorganisms in helictites from Clamouse Cave, France, and showed that bacteria appeared to act as nucleation sites for piled calcite rhombohedra. Such carbonate production in Clamouse was controlled by the type of bacteria present, abiotic factors such as temperature and salinity, the nature and amount of nutrients available, and time. Laboratory experiments allowed calculation of a "carbonatogenic yield," the ratio of organic matter input to calcium carbonate output (mass), to show that bacterial heterotrophic carbonatogenesis may play a major role in limestone deposition.

Although not documented in caves, nannobacteria—purported to be microbial cells 50–200 nanometers in diameter—have been proposed by Folk (1993, 1999) to exert an active role in carbonate deposition. However, significant controversy exists over whether these "nannobacteria" are cells, biomarkers, or inorganic precipitates. Southam and Donald (1999) cautioned that SEM cannot be used to differentiate between geochemical and geomicrobiological precipitates. Future studies may address the issue of nannobacteria in cave deposits.

Carbonates—Moonmilk

Moonmilk, also known as "Mondmilch" and by a wide variety of other names (Bernasconi 1981; Reinbacher 1994), describes a range of microcrystalline mineral aggregates with a physical appearance ranging from a very soft paste to cottage cheese that dry to the consistency of talcum powder. Moonmilk has been used since ancient times for its putative medicinal value (Shaw 1997).

Moonmilk is usually composed of calcite or aragonite in limestone caves, and of hydromagnesite in dolomite caves, but it can also be composed of a wide range of carbonate and noncarbonate minerals (Hill and Forti 1997). Formation is not limited to a defined location within a cave or to a specific chemical reaction, but to physicochemical conditions. Given the variability of minerals that form moonmilk, it is not surprising that several mechanisms, biotic and abiotic, have been proposed for its formation, one or more of which may be involved in the deposition of moonmilk in a particular cave. Høeg (1946) reported microorganisms in moonmilk and suggested that microbial activity was the cause of moonmilk deposition, an idea later supported by Davies and Moore (1957). Bernasconi (1976) provided an early review of these mechanisms. More recent reviews by Hill and Forti (1997) and Northup et al. (1997) include the effects of freezing, disintegration of bedrock or speleothems, and microorganisms on the precipitation of moonmilk.

The evidence that microbes may play a role in formation of moonmilk is largely circumstantial and based on presence. A wide range of microbes, particularly bacteria and streptomycetes, but also fungi, algae, and protozoa, can be cultured from moonmilk, often in very high densities (Northup et al. 2000). Putative cells and an organic matrix can frequently be seen with SEM or in thin sections, but not in all cases (Figure 3). There is no known benefit of calcium carbonate precipitation in bacterial metabolism, although detoxification of calcium has been suggested (Simkiss 1986).

An extensive survey of moonmilk deposits from high-altitude caves in the Italian Alps has shown no evidence of microbial involvement in calcite precipitation, although the majority of samples were from fossil deposits (Borsato, Frisia, Jones, and van der Borg

2000). A review of factors contributing to the formation of ancient and modern moonmilk deposits includes elevation and temperature, along with surface cover of soils and conifer forests, and with low discharge rates of seepage water and high humidity.

Gradzíński et al. (1997) proposed stages in the progressive formation of moonmilk where cells and an organic matrix first provide a structural framework; then, active bacterial cells are calcified and the extracellular organic matrix fills the remaining space with calcite. The growth of hydrogen-oxidizing (knallgas) bacteria facilitates calcite precipitation by creating an alkaline microenvironment.

Danielli and Edington (1983) reported one of the few experimental studies that investigated the role of microbes in the formation of moonmilk. Their study built on the earlier work of Williams (1959), who isolated from moonmilk a Gram-negative rod that precipitated calcium. Danielli and Edington (1983) isolated a wide range of colony types (the majority of them Gram-negative cells) from moonmilk collected in three caves in Wales, where calcite precipitation was a common characteristic of these isolates. These authors suggested that cells were using the organic salt anion for energy and dumping the calcium as a waste product. When the calcium exceeded the solubility threshold, precipitation resulted. Calcium carbonate encrusted cells served as a nucleation site for crystal formation.

A similar study by Cañaveras et al. (1999) investigated the possible role of microbes, predominantly streptomycetes, in the formation of hydromagnesite moonmilk from Altamira Cave, Spain. Cañaveras et al. (1999) noted that acicular crystals of aragonite are frequently interpreted as biogenic in origin based on discussions by Jones and Kahle (1993) and Verrecchia and Verrecchia (1994). Cañaveras et al. (1999) concluded that microbes could be involved in hydromagnesite moonmilk formation but were unable to rule out a possible inorganic origin.

Summary. Biotic and abiotic hypotheses for the formation of moonmilk do not need to be mutually exclusive. Given the variety of mineral types involved and the range of physicochemical conditions, microbes are clearly involved in the formation of moonmilk by dissolution or by serving as nucleation sites in some cases, but they may play a minor or negligible role in other cases. Microcosm studies would be very useful in understanding the role of various factors in formation of moonmilk.

Scanning electron microscopy has been an important tool in the study of cave microbial carbonates, and enrichment culture studies have demonstrated the ability of bacteria from caves to precipitate calcium carbonate. Additional studies that combine these techniques and add a molecular phylogenetic component are needed to establish the extent of microbial involvement in carbonate precipitation and to identify the organisms involved.

Silicates

Silicate speleothems are not as abundant as carbonate or sulfate speleothems and occur in any of three groups: (1) framework-structure, including silica minerals such as quartz; (2) sheet-structure, primarily as clay minerals; and (3) ore silicate minerals that tend to be rare and localized (Hill and Forti 1997).

The most common nonclay silicate mineral in caves is opal, an amorphous precipitate commonly found in lava tubes or interlayered with calcite in limestone caves (Hill and Forti 1997). A variety of microbial forms have been observed associated with opal in caves (Kunicka-Goldfinger 1982; Urbani 1976, 1977, 1996a,b). They have also been observed as opal–sulfur coralloids, flowstone, conulites, and crusts in Santa Ninfa Cave, Sicily (Forti 1989, 1994; Forti and Rossi 1987). Silicaceous algal diatoms (*Meolosira*) are concentrated in layers and covering silica coralloids in Togawa Sakaidanipdo Cave, Japan (Kashima 1986; Kashima, Ogawa, and Hong 1989). These coralloids occur in the twilight zone, are

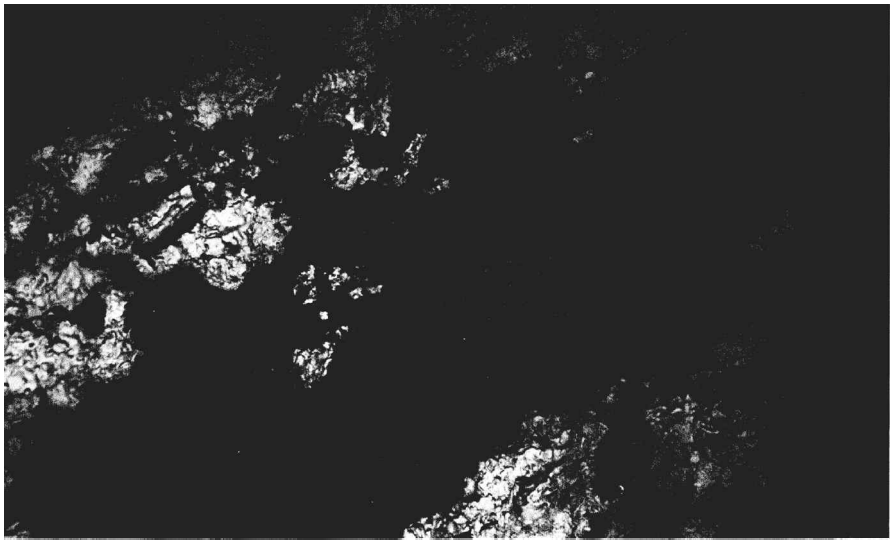

FIGURE 4 Vermiculations in Snot Heaven, Cueva de Villa Luz, Tabasco, Mexico. Photo by Kenneth Ingham.

frequently oriented toward the cave entrance, and often have more filaments than samples from deeper into the cave (Kashima 1986; Kashima et al. 1989; Urbani 1996a).

Quartz can occur in caves as spar crystals or as a dense variety known as chalcedony and usually has a high-temperature origin (Hill and Forti 1997). Microbes are known to cause the dissolution of quartz (Hiebert and Bennett 1992). For example, Feldmann, Neher, Jung, and Graf (1997) observed basidiomycete fungal hyphae that had bored into quartz crystals. The hyphae were rimmed with iron encrustations that may have formed from reactions of Ca-oxalate with Fe^{3+}.

A wide range of clay minerals are found in caves, but most clay minerals are actually detrital and washed into caves and thus are not true cave minerals as defined by Hill and Forti (1997). Clays occur in caves as part of "soils," corrosion residues, and wall vermiculations (Figure 4). Clay is sometimes rich in iron, can contain anaerobic pockets, and can retain and neutralize metabolic waste products, all factors that may enhance microbial colonization. Konhauser and Urrutia (1999) showed that microbial surfaces initiated biomineralization of metals at low concentrations followed by autocatalytic formation of iron and aluminum silicate clays in natural and experimental studies.

Vermiculations are thin, irregular, discontinuous deposits of mud and clays ranging in size from 1 mm to 1 cm or more in any dimension, often surrounded by a light-colored halo (Parenzan 1961). Vermiculations can form from a variety of mechanisms, including biological, on smooth, dense, moist surfaces in a cave (Hill and Forti 1997). Anelli and Graniti (1967) suggested that the halo surrounding vermiculations is caused by acids and other organic substances secreted by fungi. Urbani (1996b) described vermiculations composed of clay, gypsum, and green algae. Vermiculations in Cueva de Villa Luz, Mexico, contain a diverse population of microorganisms and macroorganisms, described as "biovermiculations" by Hose et al. (2000).

Corrosion residues in Lechuguilla and Spider caves, New Mexico, are rich in clays. Northup et al. (2000) demonstrated that the makeup of these clays changes substantially from the host rock outward to corrosion residues. Silica is substantially reduced in corrosion residues as compared with host rock. The extent to which the microbial community present is responsible for this transformation is under investigation.

Summary. The involvement of microbes in the formation and weathering of silicate speleothems is an area open to study. Opal and quartz are widely distributed in caves; descriptive and experimental studies are needed. Clays have been cultured for microorganisms, but no studies have conclusively shown a role for microorganisms in formation or alteration of clays in caves or formation of vermiculations.

Iron Oxides

In caves, iron oxides and hydroxides are most often observed as coatings or crusts and as powder in clastic cave fills, but they also exist as typical speleothems such as stalactites. Several descriptive studies have established the association of bacteria with iron deposits in caves, but experimental evidence for an active microbial role in the formation of iron deposits in caves is still lacking.

Amorphous hydrated iron oxide is the initial form of iron precipitated in caves. Crystalline forms such as goethite or hematite then result from the recrystallization of this iron oxide. Goethite (FeOOH) is commonly found in cave sediments, but it may also form boxwork, flowstone, or helictites. Hematite (Fe_2O_3) can form as crystal inclusions in other minerals or in other cave occurrences, usually under high temperatures (Hill and Forti 1997). Sources of reduced iron for iron-oxidizing bacteria include limestone (Melim 1991), infiltrating waters, and dissolved ferrous iron in cave pools.

Oxidized iron precipitates onto a wide range of bacterial cell surfaces or sheaths either through enzymatic or passive mineralization (Konhauser 1997, 1998; Lowenstam and Weiner 1989). Microbial iron mineral formation has been documented in the formation of ferric hydroxide (e.g., ferrihydrite), iron oxides, magnetite, iron sulfates and sulfides, iron silicates, iron phosphates, and carbonates (siderite, $FeCO_3$) in a wide variety of aqueous and some terrestrial habitats (reviewed in Konhauser 1997, 1998). Generally, abiotic formation of iron oxides occurs above pH 6, whereas biologically produced iron oxides form at low pH. However, biological iron oxidation can also occur at circumneutral pH (Emerson and Moyer 1997).

Circumstantial evidence for iron biomineralization in caves comes largely from observational studies (Caldwell and Caldwell 1980; Caumartin 1963; Crabtree 1962; Dyson and James, 1981; James, 1994; Klimchouk 1994; Luiszer, 1992; Maltsev 1997). In one of the more detailed studies of possible biogenic structures, Jones and Motyka (1987) found spherical bodies with high concentrations of iron or manganese in stalactites in Grand Cayman Island, British West Indies. The most unusual iron oxide deposits found in caves are stalactites from Lechuguilla Cave, New Mexico, that resemble the "rusticles" found on the hull of the Titanic. These formations consist of iron oxides coating organic filaments that may be fossilized *Clonothrix* or other iron-oxidizing bacteria (Davis, Palmer, and Palmer 1990; Provencio and Polyak 2001, this issue).

Peck (1986), in one of a few experimental studies, reported on the presence of *Gallionella ferruginea* and *Leptothrix* sp. in enrichment cultures inoculated with mud from cave pools and sumps and from moist Fe and Mn wall crusts and formations in Level Crevice Cave near Dubuque, Iowa. In these experiments, *Gallionella ferruginea* showed iron hydroxide precipitations and *Leptothrix* sp. showed iron-impregnated sheaths. Sterile control cultures showed no iron precipitation. This study demonstrated the presence of iron-oxidizers in cave sediments; however, the presence of microbes in the absence of quantification and demonstration of metabolic activity does not necessarily establish activity in situ.

Summary. Studies of the geomicrobiology of iron in caves must continue to move beyond the documentation of the presence of iron-oxidizing bacteria. The study by Beard, Johnson, Cox, Sun, Nealson, and Aquilar (1999) on the biogenic fractionation of iron

isotopes has the potential of providing a new technique for investigating the role of iron bacteria in caves. Combining mineralogical and biological techniques in the correlation of the location and activity of microorganisms to changes in mineral composition will also assist us in determining the extent of microbial involvement in the formation of iron minerals (Spilde, Crossey, Dotson, Schelble, Northup, Barns, and Dahm 2000).

Manganese Oxides

Manganese compounds in caves are found as soft deposits that may occur in clastic deposits (Cílek and Fábry 1989); as coatings on walls or speleothems (Figure 5) (Gascoine 1982; Hill 1982; Kashima 1983; Moore and Sullivan 1978, 1997; Rogers and Williams 1982); or as consolidated crusts (Hill 1982; Jones 1992; Moore 1981; Peck 1986). The most common manganese mineral found in caves is birnessite (Hill and Forti 1997). In addition, poorly crystalline manganese oxides and hydroxides (pyrolusite, romanechite, todorokite, rhodochrosite) also occur in caves (Onac, Pedersen, and Tysseland 1997a; Onac, Tysseland, Bengeaue, and Hofenpradli 1997b).

Abiotic oxidation of Mn(II) is controlled by pH and redox reactions between Mn(II) and Mn(III, IV). Biotic oxidation of manganese can occur by two mechanisms: indirectly or directly. Indirect oxidation results from the release of oxidants, acids, or bases into the environment surrounding the microbial cell, and leads to a change in redox conditions in the surrounding microenvironment (reviewed in Tebo, Ghiorse, van Waasbergen, Siering, and Caspi 1997). Direct oxidation may occur through the binding of Mn(II) to negatively charged substances on the bacterial cell surface, or through the action of Mn(II)-binding proteins which are both intra- and extracellular (Ghiorse 1984). Mn oxidation is accomplished

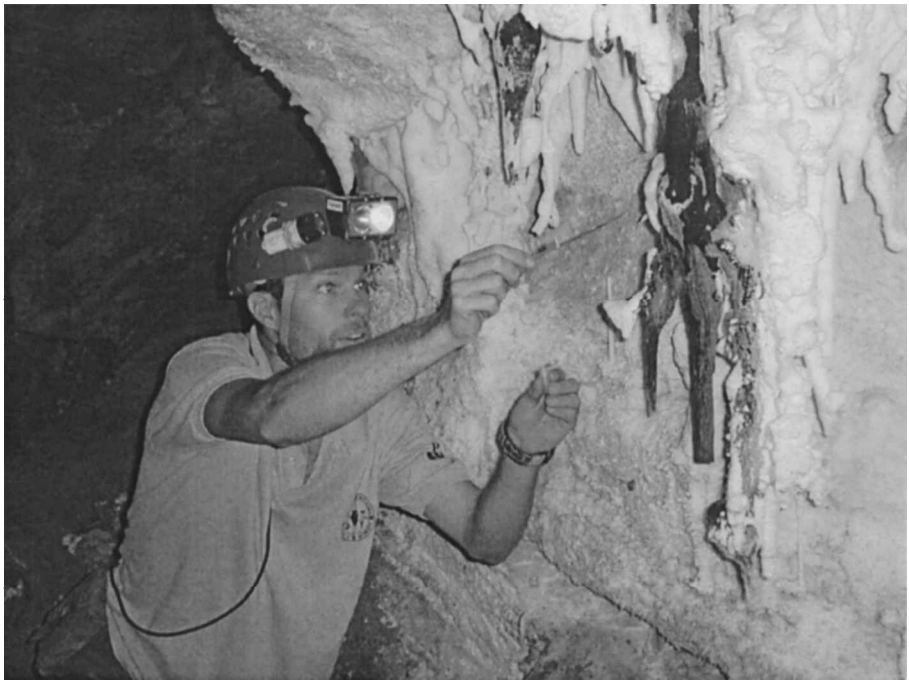

FIGURE 5 Stan Allison sampling manganese oxide coatings that cover white stalactites in Spider Cave, Carlsbad Caverns National Park, New Mexico, USA. Photo by Kenneth Ingham.

by a phylogenetically diverse set of bacteria that fall within the alpha, gamma, and beta subdivisions of the Proteobacteria and within the Gram-positive bacteria. Several fungi are also known to oxidize Mn(II). Microbial oxidation of Mn is affected by oxygen, temperature, pH, light, and metal concentration in the environment. Biological oxidation of Mn(II) can speed up the rate of oxidation by up to five orders of magnitude.

Several studies have proposed microbial participation in the formation of cave manganese deposits (Broughton 1971; Cílek and Fábry 1989; Crabtree 1962; Gradzíński, Banaś, and Uchman 1995; Hill 1982; Jones 1992; Laverty and Crabtree 1978; Moore and Sullivan 1978; Northup et al. 2000; Onac et al. 1997a,b; Peck 1986; White 1976). For example, Moore (1981) found manganese-oxidizing bacteria such as *Leptothrix* in a stream in Matts Black Cave, West Virginia, and attributed the formation of birnessite in this cave to the precipitation of manganese around sheaths of bacteria. The presence of rods, sheets, strands, and smooth spheroid morphologies in fossil remains of manganese precipitates in stalactites, karst breccia, and root calcrete crusts in Grand Caymen caves led Jones (1992) to conclude that many of these manganese precipitates were biogenic. Recently, Spilde, Brearley, and Papike (2001) have shown todorokite precipitation in microbial cultures isolated from todorokite-rich material in Lechuguilla Cave, New Mexico.

Irregularly shaped crusts of manganese flowstone (2–20-mm thick) are found in Jaskinia Czarna Cave (Tatra Mountains, Poland). Filaments and globular bodies are interpreted as bacterial or fungal cells that participated in the formation of the flowstones as evidenced by their three-dimensional morphology and the amorphic character that is more common in biogenic manganese oxides. The high Mn/Fe ratio of 72.1:1 in the crusts was attributed by Gradzíński et al. (1995) to biologically mediated precipitation.

Summary. Most of these studies use the presence of bacterial shapes in the oxides and the recovery of species of bacteria known to oxidize manganese from cave waters as limited evidence that these microorganisms may precipitate manganese oxide minerals in caves. Isolation of putative manganese-oxidizing bacteria from cave deposits, followed by production of manganese oxides in enrichment cultures and not in sterile controls (e.g. Peck 1986), is needed to establish that the organisms found in the deposits actually produce manganese oxides. In addition, microcosm studies in situ could establish rates of manganese oxide production in caves. As we learn more about the mechanisms involved in microbial oxidation of manganese, it may be possible to analyze manganese oxide deposits for the presence of enzymes and proteins involved in manganese oxidation.

Sulfur Compounds

Geomicrobiological studies of the sulfur cycle in caves have recently documented sulfur oxidation in caves and cenotes (Brigmon, Bitton, Zam, Martin, and O'Brien 1994a; Brigmon, Martin, Morris, Bitton, and Zam 1994b; Hill 1987, 1990, 1995; Hill and Forti 1997; Marcella, Heydari, Stoessell, and Schoonen 1994; Martin and Brigmon 1994; Wilson and Morris 1994) and several caves with extensive sulfur bacterial communities have been investigated (Angert et al. 1998; Hose et al. 2000; Sarbu, Galdenzi, Menichetti, and Gentile 2000; Vlasceanu et al. 2000). Most of these studies have been qualitative in nature where the presence of sulfur bacteria was identified by cultivation or culture-independent molecular phylogeny.

To establish a biogenic origin for sulfur in caves, investigators have searched for gypsum and sulfur deposits that are enriched in the light isotope of sulfur (Hill 1987, 1990, 1996; Hose et al. 2000; Mylroie, Carew, Bottrell, and Balcerzak 1994; Pisarowicz 1994). ^{34}S values of Cueva de Villa Luz sulfur are −11.7‰ (hydrogen sulfide), −23.7‰ (native sulfur), and

−23.4‰ (gypsum), which are supportive of a biogenic origin for the last two compounds (Hose et al. 2000). Sulfur isotopes of gypsum and native sulfur in Carlsbad Cavern and Lechuguilla Cave, New Mexico, are within the same range as the Cueva de Villa Luz (Hill 1996).

Sulfate generated by sulfur/sulfide-oxidizing bacteria can be used as an electron-acceptor by sulfate-reducers. This reaction produces bicarbonate that can complex with calcium, resulting in the precipitation of calcite (e.g., as in Weebubbie Cave, Nullarbor, Australia) (Contos et al. 2001, this issue; James and Rogers 1994). Dolomite formation from sulfate reduction has been observed in the Madison aquifer, South Dakota, by Palmer and Palmer (1994).

A pioneering study by Sarbu et al. (1996) of Movile Cave, Romania, showed that this ecosystem is based on sulfide/sulfur oxidation reactions. Movile Cave contains thick microbial mats of fungi and bacteria including sulfide-oxidizing species of *Thiobacillus* and *Beggiatoa*, and sulfate-reducers such as *Desulfovibrio* found in partially flooded galleries of the cave (Sarbu, Kinkle, Vlasceanu, Kane, and Popa 1994; Sarbu et al. 1996; Vlasceanu, Popa, and Kinkle 1997). These mats contain deposits of elemental sulfur and support a unique and diverse biota (Sarbu and Kane 1995; Sarbu and Popa 1992; Sarbu et al. 1996). These microbial communities receive no organic input from the surface, and the ecosystem appears to be entirely subterranean, relying exclusively on energy produced within the system. Other ecosystems that are based in part on energy from sulfide/sulfur reactions include caves of Frasassi Gorge, Italy (Vlasceanu et al. 2000) and the submarine caves of Cape Palinuro, Italy (Mattison, Abbiati, Dando, Fitzsimons, Pratt, Southward, and Southward 1998; Southward et al. 1996).

Sulfur-based microbial communities also exist in Cueva de Villa Luz, Mexico (Hose et al. 2000) and Sulphur River passage, Parker Cave, Kentucky (Angert et al. 1998; Olson and Thompson 1988; Thompson and Olson 1988). Molecular phylogenetic analysis of the whole filamentous bacterial community in Sulphur River has revealed the presence of bacteria whose closest relatives are sulfide-oxidizing bacteria capable of precipitating elemental sulfur as well as producing sulfuric acid (Angert et al. 1998).

Summary. The discovery of several caves around the world that contain microbial communities utilizing sulfur compounds has greatly expanded opportunities to study the sulfur cycle within karst. Studies to date have concentrated on identification of bacteria present that can utilize sulfur compounds and have documented the presence of isotopically light sulfur compounds of probable biogenic origin. A few studies have elucidated the role these and other chemolithotrophic bacteria play in the ecosystem of caves. Additional work is needed and we must continue to employ culture-independent molecular phylogenetic studies of microbial communities in cave sulfur environments to identify microbial community members.

Nitrates

Nitrocalcite (calcium nitrate) is the "saltpeter" commonly found in dry cave sediments. In the United States, saltpeter earth was mined for the niter component of gunpowder (Shaw 1997). A unique feature of saltpeter mining is that the saltpeter earth will regenerate itself if it is placed in contact with cave walls and the floor. Experimental work done by Olson and Krapac (1995, 1997) suggests 10 or more years may be needed.

Many studies support the role of nitrifying bacteria in the origin of saltpeter earth (Faust 1949, 1967, 1968; Hill 1981a,b; Hill, Eller, Fliermans, and Hauer 1983). Fliermans and Schmidt (1977) isolated *Nitrobacter* from saltpeter deposits in Mammoth

Cave, reporting bacterial densities more than a 100 times higher than in surface soils with different species predominating. Stable isotope analysis shows that saltpeter is selectively enriched in the lighter isotope of nitrogen (Jameson, Boyer, and Alexander 1994), as expected from microbial activity.

Microbes are clearly involved in the generation of calcium nitrate by chemolithotrophy (Focht and Verstraete 1977). Through nitrification, *Nitrosomonas* bacteria convert ammonium ions (or ammonia) to nitrite:

$$2NH_3 + 3O_2 \rightarrow \ 2NO_2^- + 2H^+ + 2H_2O$$

Nitrite is then converted to nitrate by *Nitrobacter*:

$$2NO_2^- + O_2 \rightarrow \ 2NO_3^-$$

These processes are widespread in soils, usually associated with decomposition of organic compounds.

One question that still remains relates to the original source of the nitrogen as well as the source of nitrogen for regeneration of saltpeter earth (Hill 1992, Lewis 1992, Moore 1994). Hess (1900) first proposed a seeping groundwater hypothesis where bacterial decomposition of organic matter above the cave released nitrate ions that were transported into the cave where evaporation of water in dry passages would result in a buildup of nitrate in the saltpeter earth. Pace (1971) and Hill (1981a,b) proposed modified seeping groundwater mechanisms, with ammonia or ammonium ions carried in from surface soils. Hill (1981a,b) demonstrated that drill cores up to 30 cm in depth into wall limestone contained very low levels of nitrate, whereas protected areas of limestone in caves had high concentrations of nitrate (a few ppm vs. hundreds to thousands of ppm). Potential sources of nitrogen in caves include bat guano (Hill 1987); ammonium–urea from amberat (cave rat feces and urine) (Moore and Sullivan 1978); bacterial nitrogen fixation (Faust 1967; Lewis 1992); fertilizers and sewage; volcanic rocks; and forest litter (Hess 1900; Hill 1981a,b; Moore 1994). Hill (1981a,b) argues that highly organic surface soil is the primary source for nitrogen compounds entering the cave. Nitrogen content in surface soils varies with climate, vegetation type, topography, soil type and porosity, and microbial activity (Bartholomew and Clark 1965; Hill 1981a,b).

Summary. Microbial activity, specifically the coupled autotrophic reactions of nitrification, is central to the deposition of saltpeter in cave sediments. The nitrogen source may be from deposits of bat guano or amberat, but in most caves it is probably from decomposing organic matter leaching into seeping groundwater entering the cave by capillary action and gravity with evaporation concentrating nitrogen compounds into saltpeter passages (Hill 1981a,b). The form of nitrogen entering the cave may be variable, depending on the source.

Biokarst and Phytokarst: Landforms Shaped by Microorganisms

Karst is defined as large areas of limestone with distinctive surficial and underground geomorphological features such as karren (a minor form of karst due to solution) and caves. Biokarst is the result of a dominance of organic influences on specific process-form relationships with geomorphology on a landscape scale (Viles 1984). Within karst, biokarst is often recognized as small-scale features of localized importance. These features were earlier considered to form primarily from hydrological mechanisms, but biotic action, soil, and vegetation, as influenced by climate, lithology, and human impacts, can modify

limestone. Biokarst represents a larger scale phenomenon of erosion and consolidation of caves and karst.

Viles (1984) reviewed the major forms of biological modifications of karst, including bioerosion (Neuman 1966) and phytokarst (Folk, Roberts, and Moore 1973; Jones 1989). Organisms involved ranged from bacteria and cyanobacteria up to trees and animals. Folk et al. (1973) studied spongy phytokarst pinnacles on Hell, Grand Cayman Island, later attributed by Viles and Spencer (1986) to a suite of weathering processes as well as phytokarstic algal boring and erosion.

Biokarst effects in caves usually involve stalactites, moonmilk, and sinter crusts. Bull and Laverty (1982) described phytokarst pinnacles in cave entrances in Sarawak, Malaysia, that were oriented to light and formed as erosional structures from boring and solution by red algal and cyanobacterial growth. They characterized four distinct forms of "directed phytokarst" with formation controlled by both structure and aspect. Lithological control was specifically excluded.

Cox et al. (1989a,b) reported speleothems exhibiting a characteristic morphology that resembled crustaceans from two caves in New South Wales, Australia. Locally described as "craybacks" or "lobsters," the structures are up to 4 m long by 3 m high. Environmental factors, including location within the twilight zone of these sea caves, provided strong control on formation. The authors describe these speleothems as "cyanobacterial subaerial stromatolites." Formation is both biologically controlled and biologically induced, with calcite precipitation and aeolian sediment trapping important in the deposition of these speleothems.

Summary. Studies of biokarst involving caves are still in the very early descriptive stages. Viles (1984) urged more work in three critical areas; (1) more observation and quantitative description; (2) additional work on mechanisms, both biotic and abiotic, including determination of rates of formation; and (3) establishment of process-form links where the biotic contribution is the dominant force in development of a particular biokarst feature.

Conclusions

Friedman and Sanders (1978) noted that "Purely inorganic chemical reactions can take place only where simple organisms are totally absent. At the surface of the earth, environments devoid of such organisms are uncommon." That same observation is true for environments below the surface. Studies of dissolution and precipitation of carbonates, moonmilk, silicates, clays, iron and manganese oxides, sulfur, and saltpeter in caves span only a few decades. A variety of organisms with biogenic potential have been discovered and some fascinating systems and environments have been described from caves. These studies provide insights into biomineralization in general, and in the formation of speleothems in particular.

Future Directions

Caves should be used as experimental study systems for geomicrobiology, not because they are strange, but because they are simple and often locally abundant, allowing for replicate studies (Culver 1982; Frey 1963). This review illustrates that studies of cave geomicrobiology are largely still qualitative in nature. Although qualitative studies are still needed and serve as a critical base of information, we encourage more quantitative, experimental studies in the future. To determine microbial contribution to mineral formation in caves, we need to draw on the wealth of rigorous research done on surface systems. Barton and Pace (2001, this issue) and Jones (2001, this issue) offer critical guidelines concerning what constitutes hard evidence for biogenicity of cave deposits. Because culture-based

techniques allow us to grow less than 1% of the microorganisms present using standard culture techniques (Amann et al. 1995; Winogradsky 1949), we should expand the use of molecular phylogenetic techniques to study the makeup of microbial communities in caves. An excellent example of this is the recent study by Vlasceanu et al. (2000) where molecular phylogeny was used to establish the role of microorganisms in cave enlargement. Stable isotope techniques can provide information on microbial contribution to mineral formation (Hose et al. 2000) and ecosystem bioenergetics (Sarbu et al. 1996). Finally, experiments with microorganisms from cave environments will allow cave geomicrobiology studies to establish the role that microorganisms play in the dissolution and precipitation processes that go on in caves.

This article reviews work on microbial activities in caves that can be applied to the recognition of biomarkers for subsurface life on other planets (Boston, Ivanov, and McKay 1992; Boston 2000; Boston et al. 2001; Cunningham et al. 1995; McKay, Ivanov, and Boston 1994). Allen et al. (2000) stated that although no single feature can be taken as conclusive evidence for life, a consortium of biomarkers could provide strong circumstantial evidence of life. Searching for biomarkers in caves will also allow us to understand the contribution of microbes to biokarst and speleogenesis.

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