

A Study for Behavior and Products of Cave Microorganisms

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1. Introduction

The plant life of caves is made up of species that can live in total darkness. If any plant in a cave contains chlorophyll, the chlorophyll obviously cannot be energized by light rays from the sun. With a few exceptions noted below, none of the plants in the perpetually dark zone contains chlorophyll. The chief plant life there consists of bacteria, including actinomycetes, and fungi.

These chemoautotrophs may play an important role in the food chains of some caves. After synthesizing food from inorganic material, they serve as food for such small animals as protozoans, which in turn feed larger animals. Thus, the chemotrophs theoretically can serve as the basic food supply for the life of the entire cave.

Heterotrophic bacteria and fungi break down waste material deposited by cave-dwelling animals as well as organic material brought in by flowing water or visiting animals. In so doing they perform two functions. First, they act as scavengers ; second, they release

chemical compounds for further use as nutrient material for other organisms. Heterotrophic bacteria cannot exist in areas devoid of organic material, but autotrophic bacterial can. Life can therefore go on in a cave that is sealed from the surface, provided its flora includes a few autotrophic microorganisms.

2. Characteristics of the Microflora

Since a cave is normally connected by an entrance to the surface, microorganisms found in the dark zone are similar to species found in the surface soil. The spores by means of which they propagate are so small that they can readily be carried deep into the cave by percolating soil water, as well as by currents of air, by streams of water flowing into the cave, or by animals. When they have settled in a suitable environment, the spores germinate and develop into the mature forms of the species.

Demonstrations in English caves have shown that the mere passage of explorers into and out of a cave can cause extreme contamination by certain typee of bacteria that had not previously existed in the cave.

Sterile petri dishes placed in a virgin cave area and then cultured have proved to be free from bacteria of certain outside species, but

when cultured after a party of ten or twelve people had passed through this same area, they contained many of these bacteria.

Among the autotrophic microorganisms commonly found in caves are the chemoautotrophic iron bacteria. This is not surprising, since most caves contain everything that these bacteria need in order to live, including an abundance of moisture and of iron compounds, and a sufficient quantity of certain indispensable trace elements. Caumartin has shown how these resources might be utilized by the iron bacterium *Perabacterium spelei*.

This species is anaerobic - that is, it requires no free oxygen - and it can fix nitrogen obtained from the air. It derives the carbon it needs from iron carbonate in the walls of the cave. Decomposition of the iron carbonate supplies the energy required for the bacterium's metabolism. This process liberates ferrous ions that are oxidized to produce the ferric mineral goethite[FeO(OH)], which is the brown pigment of cave silt.

Energy is also released by the slow process of transformation from one clay mineral to another in the sediment which partly fills caves. Muscovite, an insoluble mineral derived from beds of shale associated with the limestone, is the principal original constituent of the clay-sized fraction of cave sediment.

This muscovite formed in the shale when it was under great pressure in the Earth. As a consequence, muscovite is unstable in the

low-pressure cave environment, and it slowly changes there into calcium-montmorillonite, another clay mineral. We suggest that this and similar transformations of clay minerals may be important energy sources for cave chemoautotrophs.

The peculiar odor, suggesting damp earth or moldiness, so characteristic of some caves, is produced by cave actinomycetes, which are moldlike filamentous bacteria. Some actinomycetes synthesize carotene, a common pigment of certain cave-dwelling insects. The brownish color often found in droplets of water coating cave walls is also caused by this pigment.

Microorganisms are themselves digested and utilized by cave insects as a source of amino acids and other materials required for growth. Some microorganisms live in the intestines of cave insects. Here they aid digestion by secreting enzymes that help to convert food to a form that can be absorbed.

Cave microorganisms therefore play an important role in enabling cave animals to survive and grow on what would otherwise be an inadequate diet, and to do this in an environment entirely without sunlight.

3. Saltpeter

A material that owes its origin in part to microorganisms is cave saltpeter, which was extensively mined to make gunpowder during the War of 1812 and the Civil War. This highly soluble material, consisting mainly of nitrocalcite $[\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}]$, impregnates silt in many caves. The saltpeter-bearing silt, or “petre dirt,” was processed for the wars by leaching it with water and then boiling the liquor with wood ashes to convert it to niter $[\text{KNO}_3]$.

The bacterium *Nitrobacter agilis*, responsible for the final stage of forming the nitrate of saltpeter from nitrogenous organic material, is common in saltpeter caves. A puzzling aspect of the problem, however, is that some of the caves richest in saltpeter contain no obvious accumulations of such organic material as bat guano.

A clue to the puzzle is that during the War of 1812 the principal source of saltpeter other than caves was the soil around houses and under their floors. Before the days of modern plumbing, it was in this soil that nitrogen compounds from human waste accumulated. This points to an hypothesis, originated in the first edition of this book, that the ultimate source of the raw material for saltpeter in caves may be the urine of the cave rat, *Neotoma magister*.

These animals leave a trail of urine droplets and musk in the dark zones of caves, which helps them to find their way from the surface

back to their nests. In some dry western caves, where cave rats have been crossing an area of limestone for thousands of years, thick accumulations of dehydrated urine called *amberat* have formed. On silt, however, or in wet caves, bacterial action may break down the urea and convert it to cave saltpeter.

4. Manganese Minerals

Microorganisms also probably play a part in the origin of the black manganese deposits commonly present in caves. The manganese minerals form sootlike layers that cover the walls of certain passages or coat cobbles in cave streams.

In most limestone caves, as in Weber Cave, Iowa, these layers are composed of the mineral birnessite[(Na,Ca)Mn₇O₁₄ · 3H₂O]. In Jsaper Cave, South Dakota, however, where the ground water contains a higher than average amount of barium, the mineral is romanechite[BaMn₉O₁₆(OH)₄]. The deposits tend to be layered like stalactites. They consist of crystals so extremely minute that they are almost beyond the limit of resolution of the X-ray diffraction technique normally used for identifying such minerals.

5. Moonmilk

Laboratory cultures made from the water found on certain cave deposits reveal the presence of bacteria and other microorganisms that may play a part in the construction of certain calcareous mineral deposits and in the disintegration of the limestone wall rock.

Microorganisms have been shown, for example, to play a major role in the origin of a curious cave material known as *moonmilk*. This is a soft, white, claylike substance present on the walls of many caves. Its name comes from Switzerland, where in the 15th century it was called Monmilch (gnome's milk), because the people there believed that the caves were inhabited by gnomes. *Mon* (sometimes written *Moon*) meant gnome, but the word has become mistaken for *Mond*, which is German for moon.

When the mineral constituents of moonmilk are removed by dissolving it in a weak acid, an abundant organic residue remains, which consists chiefly of such bacteria as *Macromonas bipunctata*, along with actinomycetes and algae.

This microflora probably assists in breaking down the minerals of the wall rock and aids in their conversion to the solids contained in the moonmilk.

The larger mineral bodies in calcite moonmilk have a distinctive surface sculpture that can be seen under the scanning electron

microscope. The bodies consist of rods of calcite with an average size of 1×8 micrometers.

A diagonal grain is impressed on the surfaces of the rods, and parallel ridges commonly trend along the lengths of the rods superimposed on the diagonal grain. The combined effect produces bodies somewhat resembling ears of corn.

The diagonal grain is aligned with the crystal structure, as can be seen through an optical microscope with polarized light. Because the crystal structure of calcite normally parallels the long dimension of calcite crystals, the grains in calcite moonmilk were once erroneously identified and named as a separate new mineral, "lublinite".

More research is needed to determine the energy source of the microorganisms in moonmilk. The snow-white rather than brown color of most moonmilk suggests that oxidation of iron is not the source. We tentatively suggest that soluble organic compounds from the soil provide the energy for the microorganisms that control the growth of this strange substance.

6. Medical Use of Cave Actinomycetes

Cave actinomycetes have been the subject of much research as a possible source of antibiotics. Several expeditions into caves in Central America, South America, and the United States have been conducted to collect cave silt that contains actinomycetes and molds. From these, scientists hope to obtain new and powerful antibiotics.

In the 16th and 17th centuries, long before modern “miracle drugs” were dreamed of, physicians used dried moonmilk from European caves as a dressing for wounds. They did so primarily because this substance would stop bleeding and act as a dehydrating agent, but they also believed it had curative qualities.

Now that we know that moonmilk contains actinomycetes, and that some actinomycetes possess antibiotic properties, we see that the early use of this cave material in medicine may have had a valid scientific basis, even though the early physicians did not know what it was.

It is of passing interest to note here that speleologists testify that sometimes when they enter a cave while suffering from a cold, they find that after they have been underground for several hours their symptoms largely disappear.

A probable explanation for most of these cases is that deep inside caves the air is almost free from pollen, and the clean air would

alleviate symptoms caused by some allergies. It is possible, however, that in some cases the cold victim obtains relief by inhaling an unknown cave product that may someday be used medically in treating common colds.

7. Harmful Microorganisms

Not all species of cave microflora can be considered beneficial to people. As noted above, certain bacteria cause the breakdown of organic material - a welcome sanitary process when the material broken down is organic waste. But the same bacteria also disintegrate what might have been especially interesting remains.

Countless vertebrates, including humans, have been buried in caves, yet it is rare to find anything more than their bones, because the cave bacteria have usually caused complete decay of all other parts.

The only exceptions to this are in certain caves with extremely low humidity ; in these the microflora is nearly inactive, and in especially dry areas it is sometimes possible to find desiccated bodies. Thus, in recent years, a sequence of extinct marsupial species with skin and fur intact has been studied from the caves under the Nullarbor Desert of Australia. Also, in drier parts of caves in Mammoth Cave National Park, Kentucky, desiccated human bodies over 2,000 years old have

been founds.

8. Relation of Microorganisms to Cave Food Chains

Microorganisms are an extremely important constituent of the cave environment. They are involved in the development of cave deposits such as moonmilk, in the production of food for cave animals, and in the breakdown of organic material in the cave.

Ideally, a nearly closed ecologic system could exist in the dark zone of a cave, where the energy required for metabolism is derived from minerals in the wall rock and in sediment on the floor.

The basic food cycle in such a cave setting would depend on chemoautotrophic bacteria. These bacteria could serve as nutrient material for cave-dwelling animals, with no organic input from outside the cave.

Thus, the primary energy sources exploited by cave organisms are either minerals broken down by autotrophic bacteria, or surface-derived organic material utilized by heterotrophic bacteria. In the quantitatively more important latter case, the decomposer bacteria are eaten by protozoans which are eaten by such aquatic cave-dwelling animals as flatworms, isopods, and amphipods, which are eaten in turn by larger animals such as crayfish, salamanders, and

fish.

Finally, these aquatic forms release waste material that supports the heterotrophic bacteria that helped to initiate the chain, and the cycle is complete.