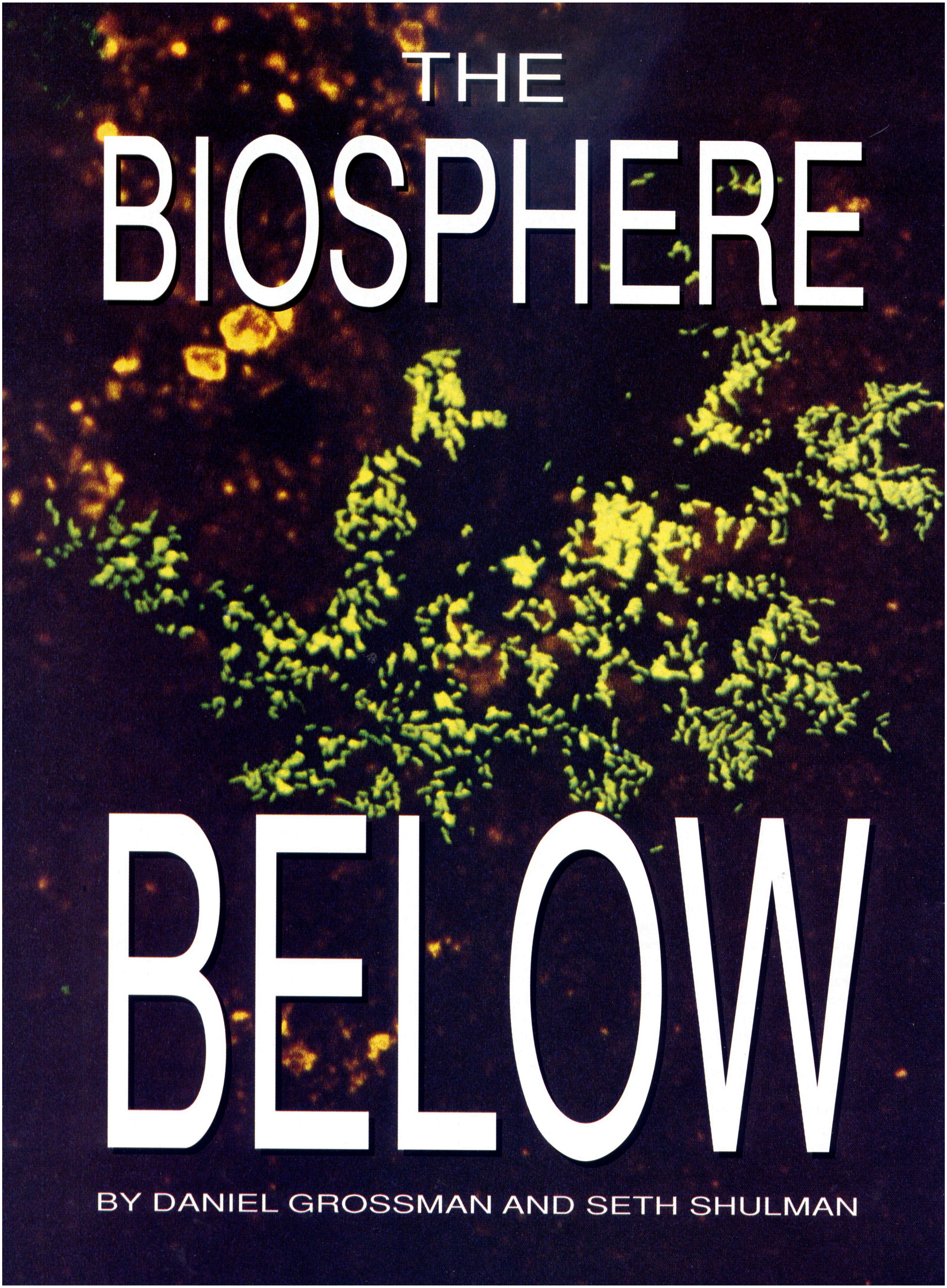


THE BIOSPHERE BELOW

BY DANIEL GROSSMAN AND SETH SHULMAN

More than a mile below Earth's surface, tiny creatures thrive in searing heat and crushing pressure. Scientists think these microorganisms might teach us about the origins and evolution of early life.

At dawn on a gray morning, a mud-spattered crew works swiftly to thread two large, vertical pipes together. Above them, the steel derrick of a drilling rig towers at the base of a sparsely wooded slope. Deep below their feet, a pipe tipped with knobby, steel cutting heads dangles nearly a mile into the earth. Despite boots, heavy gloves and padded coveralls, the workers nimbly set in place a new casing to add to the growing auger.

These roughnecks have performed these maneuvers scores of times before in pursuit of deep deposits of oil and natural gas. But here, at a remote site called Parachute Creek in western Colorado's Piceance Basin, the object of the quest is different. This early-morning drilling crew is part of an effort to plumb Earth's depths for new forms of life.

Daniel Grossman

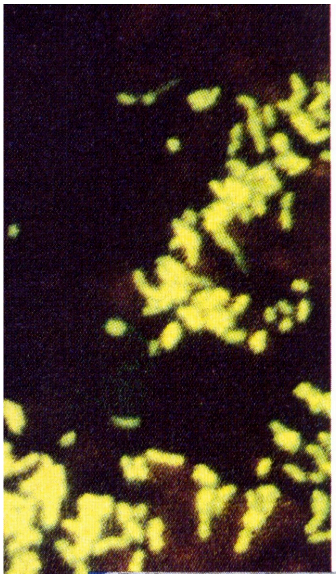
Roughnecks do the hardest and dirtiest tasks on a drilling rig. Here they attach more pipes in order to drill deeper into western Colorado's Piceance Basin in search of buried bacteria.

Over the past several years, this project has shown that the subterranean world supports living microorganisms, some of which thrive nearly two miles below Earth's surface. Evidence is mounting that this hidden biosphere is virtually teeming with life, flourishing in the cracks and pores of deep rock. So far, researchers have brought up more than 8,000 separate samples of microbes from deep underground. And they predict that future studies will uncover many more. In fact, underground life might make up a significant fraction of the total mass of life on our planet, says geologist T.C. Onstott of Princeton University, who helped oversee the Parachute Creek drilling operation.

However plentiful they might be, the subterranean microbes are only beginning to yield their secrets of survival to researchers. Still unknown is how the organisms manage to tolerate conditions that by traditional standards are nutrient-poor, lacking not only sunlight and oxygen but often water as well. (Some actually appear to eat toxic hydrocarbons such as toluene, xylene and trichloroethylene.) They somehow withstand near-boiling temperatures that would kill other creatures. And they live between the grains of solid rock.

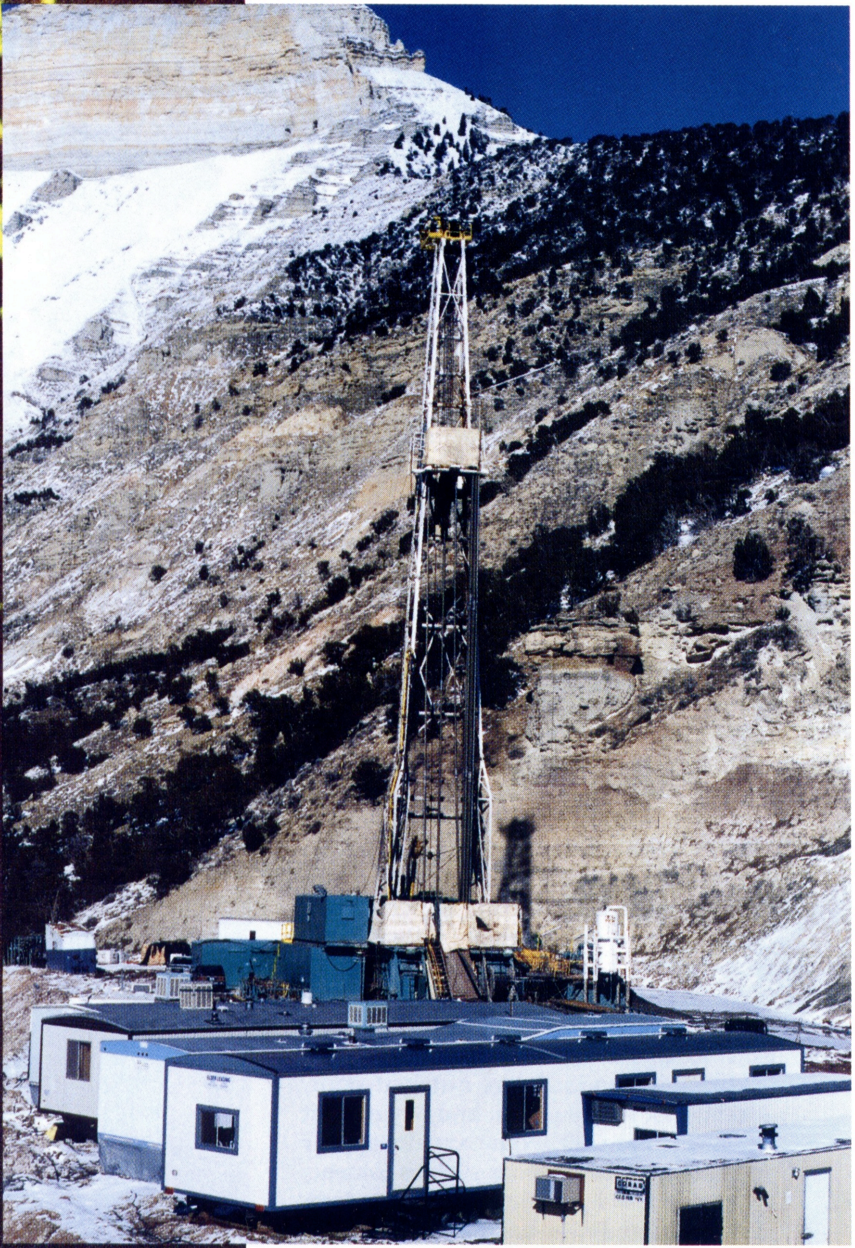
Equally mystifying is how the microbes wound up underground in the first place. They may be the evolutionary

These bacteria (stained green) were found in rock cores taken from a deep hole near Hanford, Washington. The bacteria were living in sedimentary rock without sunlight, oxygen and traditional food sources for thousands of years at least. Some scientists believe such bacteria may be descendants of microorganisms from the age of the dinosaurs.



heirs of microbes that started out on the surface but then were buried in place by geological processes. It's also possible that the ancestors of today's underground microbes transported themselves downward, migrating into Earth's crust from the planet's surface millions of years ago.

The most startling possibility is that the microbes are extremely ancient living fossils — little-changed descendants of some of the first forms of life. A few scientists have proposed that life itself originated deep underground and then migrated upward when the surface environment became more hospitable. The microbes being recovered today, in fact, may be close cousins to primordial bacteria that stayed behind, says Thomas Gold, a retired geochemist from Cornell University in Ithaca, New York. Until now, most scientists have believed that life began on the surface, perhaps in pools of water rich in amino acids and other compounds. If studies of these underground microbes can prove Gold's theory, scientists will have to rewrite the first chapter of life on Earth.



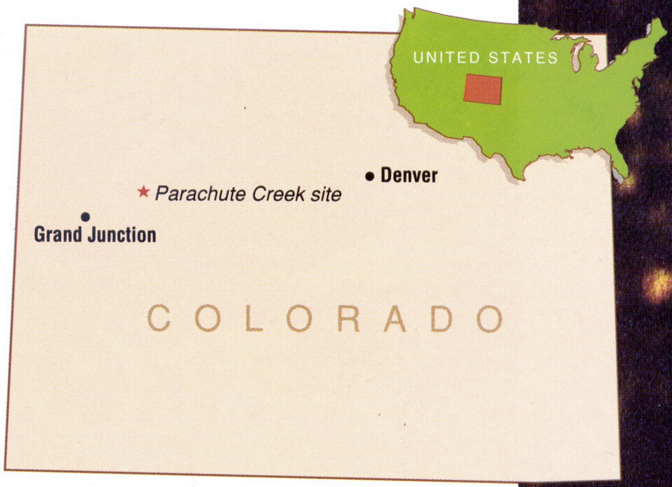
Daniel Grossman

A drilling rig in western Colorado marks the site of an experiment looking for life deep underground. In the trailers below the tower, scientists prepare core samples to be analyzed for traces of microbial life.

Back in the 1960s, Frank Wobber, the geologist who heads the U.S. Department of Energy's Subsurface Science Program, was tantalized by the initial findings of oil-company geologists that microorganisms were present in some oil reservoirs. But many scientists dismissed the notion that life could exist thousands of feet underground. With the exception of plankton, which absorb sunlight at moderate depths in the oceans, no bacteria or other microorganisms were thought to survive below the depths reached by the roots of plants. "Many biologists back then had a healthy skepticism about the prospect of subsurface microbes at great depth," Wobber recalls. "They told me I was wasting my time."

Then, in 1977, an oceanographic team using the submersible *Alvin* discovered animals living in total blackness at hot springs on the seafloor. Cut off from solar energy, these animals are part of a food chain based on bacteria that survive on chemical energy that they extract from sulfur compounds spewing from underwater vents. Despite this discovery, scientists remained skeptical that microbes could survive in deep rock.

Today, however, few would contend that Wobber is wasting his time. Since it began in the mid-1980s, Wobber's $15-million-per-year research program has amassed an impressive collection of subsurface microbes from U.S. sites that has piqued the interest of many different groups. "I never could have predicted how fast and how far things would change," Wobber says.



EARTH: Phil Kirchmeier

Scientists from the U.S. Department of Energy came to the Parachute Creek region of western Colorado in search of microorganisms living more than a mile below Earth's surface.

The National Cancer Institute and pharmaceutical companies are clamoring to investigate the organisms, which may produce substances that could be used in new drugs from antibiotics to treatments for cancer and AIDS. Petrochemical companies and firms specializing in environmental cleanup are testing the newly discovered microbes for the ability to break down oil spills and toxic contaminants. Even the National Aeronautics and Space Administration is interested, because the discovery of the subterranean microbes suggests that similar forms of life could possibly exist within the crusts of planets with hostile surface environments.

Many of Wobber's colleagues were skeptical of his findings at first because they suspected the microbes were actually surface organisms that contaminated the cores accidentally during drilling. These scientists have come around because Wobber and other researchers studying subterranean life have adopted exacting procedures to make sure that this is not the case.

To be successful in bringing up untainted rock, the research team must combine some of the brawny tools of the rock quarry with the painstaking procedures of an operating room. For instance, during the recent drilling at Parachute Creek, the team worked quickly in a makeshift laboratory to isolate the newly extracted cores from possible contamination by surface microbes and by atmospheric oxygen. Frederick Colwell, a microbiologist from Idaho National Engineering Laboratory in Idaho Falls, loaded the heavy plugs of rock into what looked like a plastic tent on top of a table. The tent was filled with the inert gas argon, Colwell explained, because oxygen in the air might kill the subterranean microbes.

Three assistants reached into sleeves fitted with gloves sealed to the tent's clear walls. With a hydraulic press, the workers cut squat chunks like thick slices of a round bread loaf from the core. With sterile hammers and chisels, they trimmed off the outer crust of each slice, which may have been tainted by surface bacteria during drilling. Then they broke the interior rock, which held the microbes they sought, into crouton-sized pieces. Finally they packed the stones in sterile plastic bags and cardboard boxes to be shipped by overnight mail to researchers across the country.

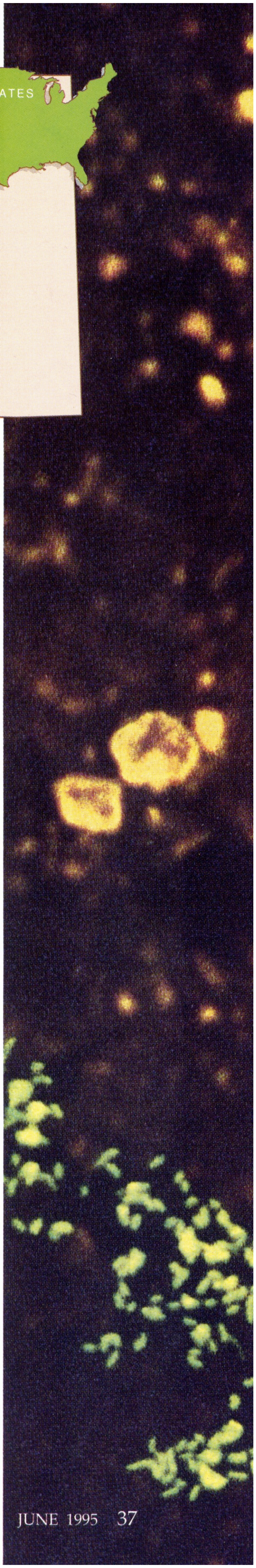
To know for sure that the cores are free of impurities, the researchers add easy-to-identify but inert contaminants such as fluorocarbons and microscopic latex spheres during the drilling process. These tracers are designed to mimic the movement of bacteria present in the drilling muds. If the contaminants bathing the exterior of a core have not penetrated to the interior, where the sought-after bacteria live, there is good reason to assume that microbes in the muds have not been able to contaminate the interior either. If, on the other hand, researchers find tracer contaminants in the interior of the core recovered from underground, they can't use it for biological studies.

With a few exceptions, including several occasions when the drill-site team spiked cores with tracer contaminants just to keep their unwitting colleagues on their toes, no tracers have shown up in any recent core. So the microbes found in the samples appear to be bonafide members of subterranean communities.

"It's the job of scientists to be skeptical," says project scientist David Boone, a microbiologist at the Oregon Graduate Institute of Science and Technology in Portland. "But we involved in this research — virtually all of us — have become confident that these samples represent organisms that have been recovered uncontaminated."

Only a tiny fraction of the subterranean life brought up so far has been fully studied. But already researchers have discovered several entirely new species and even new genera of microbes.

Subterranean microbes can be crudely classified into two types, heterotrophs and lithotrophs, says Jim Fredrickson, a



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Frederick Colwell places core samples inside a plastic tent to prevent them from being contaminated by surface microbes or by oxygen that might kill any subterranean microbes.

microbiologist from Pacific Northwest Laboratory in Richland, Washington. Heterotrophs make their living by eating the remains of organic material buried in sedimentary rock, such as partially decayed plant material. Some of these heterotrophs appear to be close relatives of microorganisms found on Earth's surface.

But unlike life on the surface, many of the underground microbes need no oxygen to live. And their motion can also be limited. Some of them seem to move very little; others probably move around by flowing with groundwater seeping through porous rock deposits. Heterotrophs grow very slowly: Their cells may take hundreds of years to divide, whereas some surface microbes divide in just days or hours. Frederickson says the density of heterotrophs is surprisingly high, considering the scarcity of organic material wedged in rock fissures more than a mile below Earth's surface.

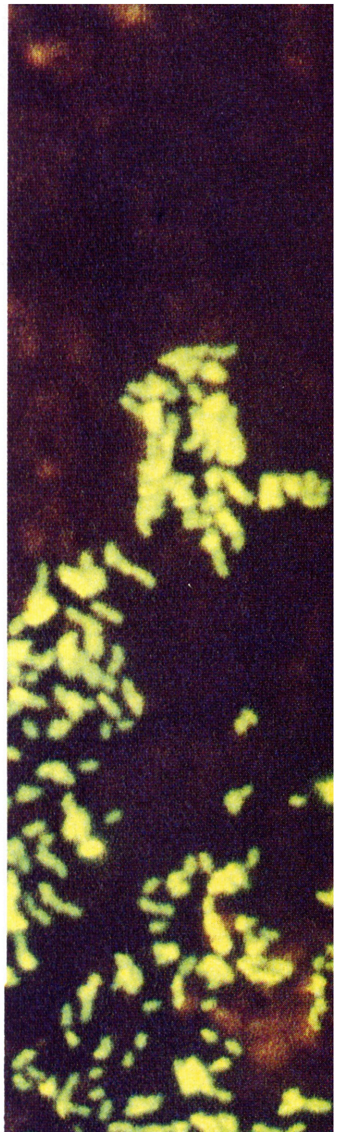
Perhaps even more intriguing, Fredrickson says, are lithotrophs. These could be the most common subterranean denizens. And research suggests that these microbes may well be unlike any life on the surface. They live off geochemical energy, eating hydrogen present in deep rock formations. So far very little is known about them. Scientists aren't even able to pinpoint the origin of the hydrogen they break down for energy. Some geologists contend that it percolates up from high-temperature geochemical reactions in the mantle. Others believe that it could be the product of a relatively low-temperature chemical reaction that occurs when groundwater comes into contact with certain common rocks, such as basalt.

As some researchers study how the microbes survive, others are trying to learn why microbes are present underground at all. Did they migrate down from the surface? Are they ancestors of organisms that once lived on the surface but were buried in place and somehow adapted to life underground? Or did they originate underground to begin with?

To help answer these questions, geologists working on the project are studying rocks from different depths. Princeton's Onstott explains that the Parachute Creek site has an ideal geologic history for addressing this question.

During the Cretaceous Period, which lasted from about 145 to 65 million years ago, a great body of water known as the Cretaceous Seaway stretched all the way from the Arctic Ocean to the Gulf of Mexico, says Onstott. Today's Parachute Creek site once sat near the western coast of this seaway, where a river flowed out into a broad delta. Rich organic silt and fine sand were carried in the river's currents and settled out in the placid flows of the delta, building up a sequence of sedimentary layers. Toward the end of the Cretaceous, mountain ranges began to rise up near the delta, separating the seaway from the present-day Piceance Basin. A new sequence of layers then began to pile up on top of the silt and sand. Crushed by the material above, the delta deposits compacted into shale and sandstone. Today geologists call these rocks the Mesaverde Formation.

Roughly 40 million years ago, buried under a 6,000-foot cap of rock (1,830 meters), the Mesaverde Formation was subjected to searing temperatures as high as 284 degrees Fahrenheit (140 Celsius). Scientists think the heat should have completely destroyed any life in the rock, Onstott says. Then, between 5 million and 10 million years ago, the entire region was uplifted, creating today's Colorado Plateau and cooling the Mesaverde to a



temperature that could permit life again. The DOE scientists believe that the Mesaverde may have been sterile until that time. So Onstott and his colleagues hope to use cores from this isolated formation to gauge the speed at which microbes move through deep rock.

This is crucial information because it will help tell scientists why microbes are present at such depths. The core at Parachute Creek penetrated a layer of Mesaverde sandstone that is sandwiched between layers of dense shale. If the researchers find organisms living in the sandstone, they will know that they got there since the rock was sterilized millions of years ago. This would mean that the microbes migrated down from the surface fairly quickly in geological time, squeezing through fractures in the shale, and it would suggest that rapid transport of the microbes from the surface is one likely explanation for their presence deep in Earth.

On the other hand, if the scientists don't find microbes in the Mesaverde sandstone, this would mean that the microbes have not been able to move downward too far since the rock was sterilized. In other words, microbes do not migrate very fast. This would support the idea that microbes found underground are actually very ancient, the ancestors of surface organisms that were buried in place as sediments accumulated on top of them. Of course, it would also leave open the possibility that the microbes originated underground.

Biologists are also working on the question of how the microbes got underground. To get some answers, they are using recent genetic techniques to examine the relationships between subterranean organisms and microbes that live above ground.

This work is already forcing scientists to reconsider their ideas about the well-studied *Bacillus* genus of bacteria. *Bacilli* have always been defined as rod-shaped microbes that flourish in the presence of oxygen. But Boone says he has discovered a genetic relationship between surface *Bacilli* and a microbe from below that he has named *Bacillus infernus*, or "*Bacillus* from hell," that is actually poisoned by oxygen.

Boone says the genetic makeup of this microbe indicates that it belongs to the same genus as the surface *Bacilli* despite living without oxygen, a substance that was thought to be a requirement for all *Bacilli.* Could *Bacillus infernus* be a living fossil, a distant relative of surface-dwelling *Bacilli* bacteria that moved underground and then evolved to survive without oxygen? Boone says it is too early to say for sure. But biologists are hard at work trying to determine the relationships between such underground organisms as *Bacillus infernus* and surface dwellers.

To study the bacterial family tree, microbiologist Sandra Nierzwicki-Bauer of Rensselaer Polytechnic Institute in Troy, New York, is comparing the genetic makeup of related microbes taken from different depths underground. If she finds that the deepest microbes share the most similarities in genetic makeup and the ones near the surface are quite different from each other, then she may be able to conclude that the ancestors of both groups started underground and sent out migrants that moved toward the surface, evolving along the way. In fact, this is what preliminary results from some of the

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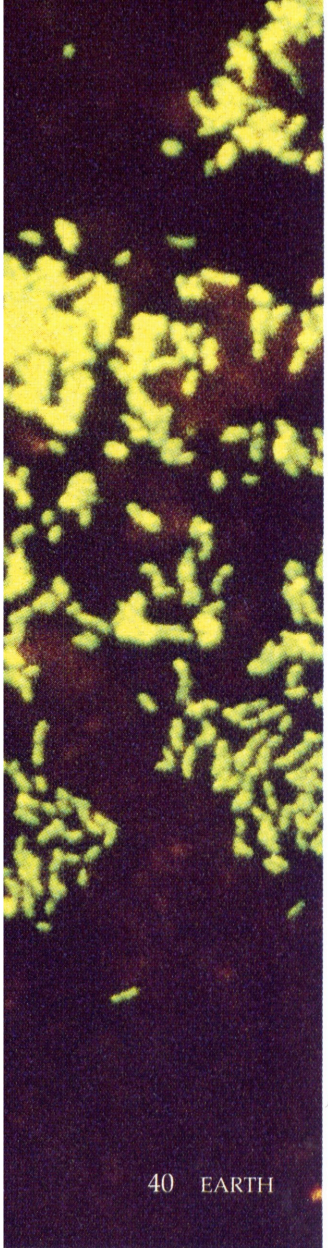
After the exterior of the core has been removed, technicians crack the sample into smaller pieces. The rocks are then shipped to scientists who will study them for signs of bacterial life.

Courtesy Henry Aldrich

The recently discovered bacterium Bacillus infernus, or "Bacillus from hell," was named after the depths at which it lives. Bacillus infernus has been found over a mile underground, far from the oxygen on which other strains of Bacillus depend.



boreholes across the United States have shown so far, she says.

But these results still don't prove that life itself originated deep below. "Trying to prove anything about events that took place 4 billion years ago is extremely difficult," says microbiologist Todd Stevens of Pacific Northwest Laboratory. "There just isn't that much evidence left."

Stevens, who has been studying microbes from deep within the volcanic rock of the Columbia River area in the Pacific Northwest, notes that Earth's surface was hostile to life for hundreds of millions of years during the planet's infancy. Geologists think that meteorites bombarding Earth may have created seas of molten magma covering the surface. Until about 3.8 billion years ago, these impacts may have repeatedly sterilized the surface and any life on it. By contrast, the environment underground, although hot, may have been relatively conducive to life. Stevens says that bacteria that originated aboveground might have survived in the crust while the surface was still hostile to life.

Stevens also says that deep underground is "a very likely place for the origin of life." Cornell's Thomas Gold proposed a few years ago that life may have originated underground and moved upward as the surface became more hospitable. He says these primordial microbes could have derived the energy they needed to survive from chemicals in Earth's interior — just as many of today's underground life forms do — and later evolved the ability to photosynthesize energy from sunlight when they reached the surface.

Several scientists from the Department of Energy drilling program say that Gold's theories can be considered little more than speculation. In support of his ideas, Gold points out that today's underground microbes are strikingly similar to the group of surface bacteria known as *Archaebacteria*. Members of this group include the most primitive forms of life found today, and they are widely considered to be close relatives of early life forms. *Archaebacteria* are found in places just as inhospitable as the underground microbes, places like hot springs, brine pools and oxygen-deficient muds. Many break down chemicals to obtain their energy. And many *Archaebacteria* are thermophilic, meaning they like heat. In fact, like subterranean microbes they thrive in temperatures as high as 176 to 194 F (80 to 90 C).

The similarities between underground microbes and the very ancient *Archaebacteria* are tantalizing evidence that both may be the direct descendants of primordial heat-loving organisms that lived beneath Earth's surface, Gold says. Are *Archaebacteria* surviving heirs of some of the first organisms to find their way to Earth's surface from life's birthplace deep underground? And could some of today's underground microbes in fact be living fossils of organisms that, for whatever reason, never left the nest? We may never know. But Gold says these are distinct possibilities.

As microbiologists continue to speculate about these fundamental questions, the search for life deep underground continues. In addition to the Department of Energy's program, researchers from the University of Bristol in England have reported finding microbes beneath the ocean floor. Scientists in Sweden have recovered bacteria from more than 12,000 feet down in granitic rock. Such international efforts are only beginning to decipher the riddle of subterranean life. Says Onstott, "There are so many questions still to answer." ⊕

Boston-based journalists Daniel Grossman and Seth Shulman wrote about how sea turtles navigate using Earth's magnetic field in the February 1995 issue of Earth.