Deep Life in the Slow, Slow Lane

Microbial life may seem infinitely adaptable and durable, but microbiologists and geologists probing the most voluminous part of the biosphere—the deep subsurface—are finding it slow going

Life is a hardy sort. In recent years, microbes have been reported in the most unlikely nooks and crannies of the planet living under incredible conditions: in mine drainage with a pH of 1; 1500 meters down in ancient lavas, living off the rock itself; in ice-encased brines; 3.2 kilometers deep in sweltering South African gold mines; in cloud droplets; and, albeit in suspended animation, preserved for more than 250 million years in ancient brine pockets in subterranean salt.

Microbes seem to be thriving wherever there is liquid water at temperatures below the reigning lethal limit of 113°C. Indeed, an estimate by microbiologist William Whitman and colleagues at the University of Georgia in Athens has almost all the planet's microbes out of sight beneath their familiar haunts of soil and sea floor. Geologist William Fyfe of the University of Western Ontario in London has said that "probably the top few kilometers of the entire basaltic ocean crust is alive with … microbes." Going farther, Thomas Gold of Cornell University has proposed a "deep hot biosphere" that extends down everywhere into the crust as much as 6 kilometers. That's an extreme view, but the recognition of pervasive, all-enduring life has sparked visions of alien life just waiting to be found beneath the inhospitable surface of Mars.

Researchers exploring the deep subsurface here on Earth—continental crust, marine sediments, and ocean crust—are not so optimistic, however. They are finding deep life, but it mostly seems to be living indirectly off the energy of sunlight rather than using local, less tempting sources of energy, such as the rock itself. Even when feeding off organic matter that trickles down from plant life at the surface, deep microbes are usually starved into apparent dormancy; when cut off from photosynthetic fuel supplies, they can simply disappear.

"We're not finding a prolific amount of life" even when temperatures would allow it, says hydrogeologist Tullis Onstott of Princeton University, who leads a group studying microbial life in the rock of deep South African gold mines. What deep life they are finding is living "very, very slowly." Microbiologist Stephen Giovannoni of Oregon State University (OSU) in Corvallis finds the same leisurely lifestyle in the ocean crust. "One often gets the impression that the deep subsurface is just a Garden of Eden for microorganisms," he says. "I don't see from our research that is true." To pin down where and how life can exist, much less thrive, researchers of the deep Earth will have to manipulate microbial communities in situ and take up new molecular genetics tools, not to mention bolster waning funding.

Living off the rock?

The latest cautionary note for deep-life enthusiasts comes, ironically enough, from the recent discovery of a microbial community apparently living quite independently of the surface beneath hot springs in Idaho, the way any life on Mars would presumably be living. In their research, hydrogeologist Francis Chapelle of the U.S. Geological Survey in Columbia, South Carolina, and his colleagues took advantage of a landowner's personal geothermal energy setup. To heat everything from his house to his dog's house, the owner had dug wells tapping Lidy Hot Springs. The hydrogeologists used the wells to sample uncontaminated waters as they rose from more than 200 meters down in a 6-million-year-old volcanic ash bed. In these 60°C waters, they found relatively high levels of hydrogen gas and a community of microorganisms well suited to living off the hydrogen.

Down and dirty. Detecting sparse life in rock kilometers down in muggy, less-than-sterile South African gold mines takes some doing.

According to several different types of DNA analyses, more than 95% of the hot-spring microbes are Archaea, the domain of microbial life distinct from bacteria that inhabits some of the most extreme environments on Earth. Further gene analysis turned up gene patterns in 95% of these Archaea that were closely related to those of known methanogens, which derive their energy from the reaction of hydrogen with carbon dioxide to produce methane. Not that they were "thriving" on the nanomolar concentrations of hydrogen. About 100,000 cells per milliliter came up in the springs, compared with 10 to 1000 times that abundance of microorganisms in surface waters.

The catch for astrobiologists is the apparent source of the hydrogen. The normally scarce gas is presumably produced through some sort of interaction between water and rock. That's how microbiologist Todd Stevens and geochemist James McKinley of Pacific Northwest National Laboratory in Richland, Washington, explained the hydrogen in water 1500 meters down in the Columbia River basalt lavas of eastern Washington (*Science*, 20 October 1995, p. 377). Iron in the basalt seemed to be chemically reducing the hydrogen of water to hydrogen gas. Thus, according to this scheme for deep hydrogen production, the life-sustaining gas would be available anywhere that water and basalt come together. This hypothesis has implications for life on Mars: The Red Planet is mostly basalt.

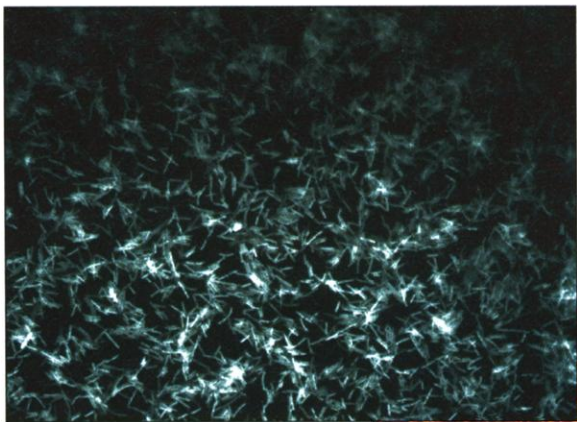
But this pervasive hydrogen source has since been called into question. Microbiologist Robert Anderson of the University of Massachusetts, Amherst, Chapelle, and Derek Lovley of UMass, a co-author on the Lidy Hot Springs paper, found that an unnaturally low pH is required to produce any hydrogen from water and basalt, and even then production quickly peters out. And microbiologist Norman Fry of the Central Public Health Laboratory in London and colleagues found that only 3% of Columbia River basalt microorganisms were hydrogen-consuming methanogens, according to their gene sequences; the rest make a living in diverse ways. That "looked very much like a microbial community you might see in a sediment," says Lovley, "in which organic matter is fueling metabolism." In that case, the bugs would be living off dissolved organic matter carried down from the surface or from fossil soils layered among the lava flows.

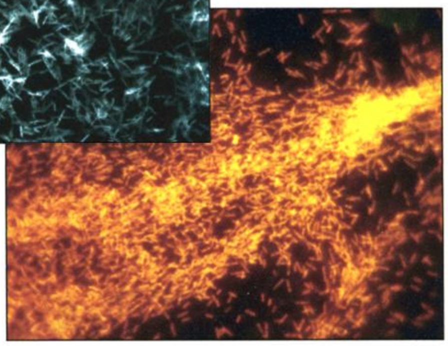
The Lidy Hot Springs organisms are prob-

CREDIT: DUANE MOSER/PNNL

ably not running their hydrogen economy on fuel generated by ubiquitous water-rock reactions, says Chapelle. Instead, they're most likely subsisting on hydrogen produced when the active faults that crisscross the area crack and crush rock. Fracturing creates active sites on mineral surfaces that can extract hydrogen from water to form hydrogen gas. Without the daily shifting of faults to produce fresh rock surfaces, he says, hydrogen would likely be truly scarce. He has screened more than a dozen other hot-spring sites around the Snake River Plain of Idaho and found that they appear to support carbon-based, not hydrogen-based, microbial communities. "I don't think hydrogen-based communities are going to be all that common," he says. "Carbon-based will be much, much more common." That should be discouraging for Mars explorationists. The Red Planet hasn't seen photosynthesizing life for billions of years, if it ever did.

Hope for a sizable continental biosphere independent of the surface may rest with another source of hydrogen: the natural radioactivity of certain crustal rocks. The radioactive elements such as uranium that decay and heat Earth's interior tend to be concentrated in the crust's most silica-rich rocks, such as granite. Mars doesn't have any detectable granite, but that's just what underlies Sweden, where microbiologist Karsten Pedersen of Göteborg University works in a subterranean laboratory tunneled as deep as 400 meters under 2-billion-year-old granite.





Rock lovers. Some microbes from hundreds of meters deep in Swedish granite may be living off hydrogen split off water by rock radioactivity.

Pedersen finds "lots of microorganisms" in water samples taken from fractures in the deep granite: 10,000 to 100,000 cells per milliliter, about "what you can pick up in clean surface water." He also finds lots of hydrogen, micromolar concentrations rather than nanomolar. And some of those microorganisms are hydrogen-consuming methanogens. What he doesn't know is how fast they are living. Life in deep granite is "probably quite slow," Pedersen assumes. Researchers are typically able to culture only 0.1% of the microorganisms they see in samples from any part of the deep subsurface. And even under optimal conditions, that small fraction takes weeks or months to grow into the density of cells generated overnight in vigorous lab cultures.

Taking it slow deep down

Just how slow and sparse deep life may get can be seen in the South African gold mines that Onstott and 30-some collaborators have been studying. In yet-to-be-published work, they find a density of microorganisms comparable to Pedersen's at similar depths. And at their greatest depth, 3.2 kilometers, they have found cells whose DNA marks them as one of the "hyperthermophile" archaeans known from sea-floor hot springs. But on descending from the shallowest depths, biomass decreases rapidly, in places to undetectable levels. The dearth of detectable life at greater depth puzzles Onstott. Life's currently known upper temperature limit of 113°C isn't approached until far below the mines. And there's plenty of fuel for living. Hydrogen concentrations soar to a million times what's typical of shallow aquifers. Most likely, radioactivity is generating hydrogen that accumulates in the water during the millions of years since the water trickled down from the surface to "puddle" in the deep rock.

"It appears there's far more energy there than is being utilized by microorganisms," says Onstott. He guesses that although there's plenty of fuel available, there's nothing left to burn it with. The deep mine water is devoid of oxygen, and other oxidizing agents, such as ferric iron, may not be exposed on mineral surfaces accessible to microorganisms, says Onstott. "Are they really doing anything down there?," he wonders. "They could be dormant cells with sequenceable DNA that we're not able to resuscitate."

Researchers have been examining another major compartment of the deep biosphere—ocean sediments—to gauge just how slow life can go this side of outright death. All ocean sediments are buried with varying amounts of organic matter that microorganisms might feed on, but oxygen that can be used to burn that fuel runs out a few centimeters beneath the sea floor. Then the dissolved sulfate of seawater takes over from oxygen as the sulfate diffuses downward through the sediment. The balance between the downward diffusion of sulfate and its consumption by microorganisms, as evidenced in its changing concentration with depth in the sediment, is a measure of how fast life is living beneath the sea floor—at least, life that uses sulfate as an oxidizing agent.

Oceanographers Steven D'Hondt, Scott Rutherford, and Arthur Spivack of the University of Rhode Island (URI), Narragansett Bay, drew on sulfate profiles measured in Ocean Drilling Program (ODP) sediment cores from around the world in order to estimate deep life's pace (*Science*, 30 November 2001, p. 1820, and 15 March, p. 2067). Assuming that all the cells counted in deep-sea cores are alive, each on average is metabolizing sulfate at a rate 1/100,000 that of the least active microbes in near-shore sediments, the URI group reported. "Either these things are taking very few breaths very rarely, or they're generally inactive," says D'Hondt.

Oceanographer John Parkes of the University of Bristol, U.K., who has counted cells found as deep as 850 meters in 14-million-year-old sediment, thinks most of the cells he sees are alive if not "well." For one, the URI group did not account for any oxidizing agents except sulfate, he notes; other agents, such as iron in sediment minerals, could be contributing as well. And life may have a way of shutting itself down for millions of years, he says, holding itself together molecularly but not growing. Then it might bounce back briefly and divide relatively frequently, say, once every decade, before putting itself to "sleep" once again.

D'Hondt is now seeing some support for Parkes's view. D'Hondt and 26 colleagues returned at the end of March from 2 months of coring sediments off Peru from ODP's *JOIDES Resolution*. They found clear evidence that manganese and iron were being used as oxidizing agents in energy production, not just sulfate. Shipboard geochemists also found signs that oxygen and nitrate, powerful oxidants, were diffusing up from the underlying crust to drive microbial activity in the bottom few tens of meters of sediment. Even so, a glance at the initial geochemical results suggests that deep-sediment metabolic activity beyond the near shore is still low in the extreme, says D'Hondt.

Coming in from the cold

The third realm of the deep biosphere, the ocean crust that covers two-thirds of the planet, is if anything more mysterious than

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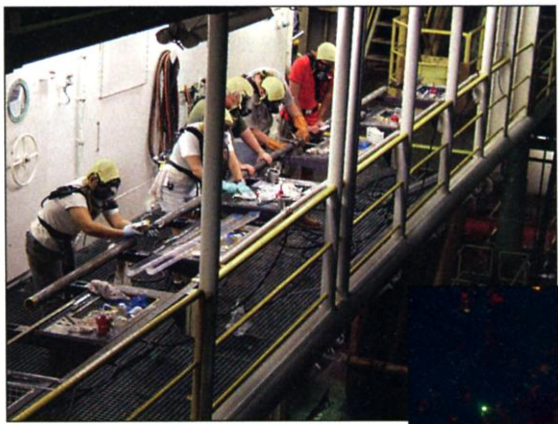

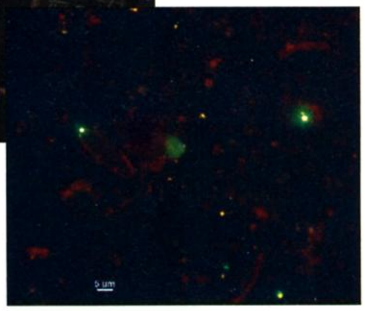

Smelly work. Processing ocean sediment cores onboard *JOIDES Resolution* can require respirators for the mud's hydrogen sulfide, but it can yield slow-living microbes (right, green dots) from 30 meters deep.

the continental crust or the overlying sediments. Microorganisms are down there, but "we don't know what they're doing or what they're using for an energy source," says geochemist Jeffrey Alt of the University of Michigan, Ann Arbor.

When deep-diving oceanographers stumbled on a riot of life at sea-floor hot springs dotting the ridge crests in 1979, microbes were obviously a crucial part of it. More apparent evidence of vibrant deep microbial activity came in 1991, when oceanographers observed clouds of "fluffy white stuff" billowing from the East Pacific Rise following a volcanic eruption. After researchers found more flocs laden with thermophilic microorganisms pouring from recently disturbed hot springs, they concluded that "there's a biosphere below the sea floor," says oceanographer John Delaney of the University of Washington, Seattle.

Just how massive this ocean crustal biosphere might be remains unclear. Microbes are obviously active along the crest of the midocean ridge system, which stretches for 60,000 kilometers through the global ocean. For example, something seems to be nibbling on the glass that makes up about 5% of ocean crustal rock; samples of the glass brought up by deep drilling are scarred with pits filled with DNA-containing material (*Science*, 2 May 1997, p. 703). But the "snowblower events" of white floc billowing from ridge crests may not reflect a deep aquifer system "humming with life," as one oceanographer put it. On closer inspection, microbiologist Craig Taylor of Woods Hole Oceanographic Institution in Massachusetts found that the white stuff is not so much bacteria as sulfur filaments produced by bacteria consuming the hydrogen sulfide in hot spring waters. And the mats are probably the product of a brief "bloom" of bacteria feeding on a surge of hydrogen sulfide released by a quake or eruption rather than the release of a huge bacterial mass that's always feeding beneath the surface.

Microbes are making some sort of living at the ridge crest, where seawater heated to hundreds of degrees by underlying magma extracts the most chemicals from the fractured rock. But the crust cools as it spreads away from the ridge crest, and as the temperature drops, deep microbial life diminishes too. Ninety kilometers east of the Juan de Fuca Ridge, water rises through an ODP drill hole that penetrates 3.5-million-year-old crust. The water is 60°C, and microbiologists James Cowen of the University of Hawaii, Manoa, and Giovannoni of OSU find a concentration of cells less than that in seawater. A common misconception holds that "the deep-sea subsurface is packed with cells," says Giovannoni. "It's not; it's a fairly low biomass."

In even older, colder crust, life seems to have quit, or nearly so. The diffusion of oxygen and nitrate up from 40-million-year-old crust into the lowermost sediment, as found during the most recent ODP cruise, means that "whatever [biological] activity is going on in the crust isn't enough to strip out the nitrate and oxygen," says D'Hondt. "There's not much activity in old, cold crust."

To pin down how much life the bulk of the deep biosphere harbors and how much living it is doing, researchers will have to sharpen their tools of exploration. Oceanographers will have to figure out how to retrieve uncontaminated samples of ocean crustal life, as colleagues have done for marine sediments and continental crust. Then a means of gauging the pace of deep life must be developed. Culturing microorganisms in place will soon be attempted in deep mines, but molecular techniques for measuring gene expression may prove useful as well.

Deep-life researchers will also have to look for new sources of funding. Radioactive waste disposal in Sweden will keep work on deep granites going, and ocean drillers have made sedimentary and crustal life a focus of their next 10-year international drilling program (*Science*, 13 November 1998, p. 1251). But elsewhere attention is shifting toward shallow ground where microbes might help clean up pollutants. Life beneath the surface of Mars may get more attention than Earth's vast if thinly spread store of deep life.

–RICHARD A. KERR

NEWS

Geobiologists: As Diverse As the Bugs They Study

Derek Lovley and Kenneth Nealson have alternately sparred with each other and spurred each other on as leaders of the field they helped create

In the mid-1980s, Derek Lovley and Kenneth Nealson achieved something most scientists can only dream about: They put their stamp indelibly on a nascent scientific discipline. The two researchers independently announced the discovery of microbes that live off metals. The claims were surprising, even heretical, but the near-simultaneous findings "opened up a new field of study," particularly in the United States, says Yuri Gorby, a microbiologist at Pacific Northwest National Laboratory (PNNL) in Richland, Washington. It turns out that these tiny metal-processing organisms have played a pivotal role in the vast sweep of geological history.

Since those early discoveries, Lovley's and Nealson's careers have followed similar trajectories. Today, the two men are considered intellectual leaders. Lovley chairs the microbiology department at the University of Massachusetts (UMass), Amherst; Nealson is now a distinguished geobiology professor at the University of Southern California (USC) in Los Angeles, and he works part-time in the astrobiology program at the Jet Propulsion Laboratory (JPL) in neighboring Pasadena. "There are a lot of parallels in what they have contributed and similarities in what they have worked on," says PNNL microbiologist Jim Fredrickson. Yet the two are opposites in personality and outlook, and according to numerous colleagues, their scientific and professional relationship is often characterized as one of intense rivalry.

Each has championed his own findings and the importance of the particular bacteria he first worked on 20 years ago. As a result, Lovley and Nealson have sometimes clashed at meetings and in print. "For a long time, the two weren't talking to each other," says