
Mussel power from methane

Biologists James Childress and Charles Fisher had just about given up on ever finding a sea animal that, with the help of bacteria, is nourished by methane. Plenty of biological communities that derive energy from hydrogen sulfide have been found at hydrothermal vents and other seafloor sites where the sun refuses to go (SN:1/12/80,p.28). But methane, which is known to provide both energy and carbon for some kinds of bacteria, didn't seem to be the kind of fuel that animals and bacteria had learned to use symbiotically.

So when Childress and Fisher, both at the University of California at Santa Barbara, were invited last year by researchers at Texas A&M University in College Station to examine clams and tube worms suspected of living on methane at oil and natural-gas seeps off the Louisiana coast (SN:10/12/85,p.231), they didn't have high hopes. And as expected, their studies showed that the clams and worms at the seeps were fueled by hydrogen sulfide, not methane. But when Childress's group studied one of the clams' and worms' neighbors — an as-yet-unnamed species of mussel — they hit pay dirt.

In the Sept. 19 SCIENCE, the California and Texas groups report that the mussels have intracellular bacteria in their gills that enable them to be powered almost entirely by methane. "A number of people have suggested this," says Childress. "But this is the first time anyone has demonstrated symbiosis with methane being consumed."

Another group of researchers, led by Colleen M. Cavanaugh at Harvard University, has found evidence pointing to the use of methane in bacteria living in the gills of another mussel species, this one found at deep-sea cold seeps off Florida's west coast (SN:12/15/84,p.374). Their paper is being reviewed by NATURE.

Cavanaugh cautions that both her work and Childress's, while highly suggestive of methane symbiosis, do not yet actually demonstrate it. If, in future work, the researchers are able to show that the bacteria are in fact using the methane as a nutrient and are exporting energy and carbon to the mussels, then methane can join hydrogen sulfide as the only compounds known to be involved in symbiosis in deep-sea environments. These findings, says Cavanaugh, "will lead to exploration of other habitats where this type of symbiosis may exist."

In oxidizing methane to carbon dioxide and formaldehyde, methane bacteria obtain energy. The formation of formaldehyde also leads to the production of carbon compounds that become incorporated in the bacteria. Presumably, the

bacteria produce glucose or some sort of carbon compound that is transferred to the mussels, possibly supplying most of the animals' nutritional needs for energy and carbon.

Childress's group found that both whole mussels and pieces of their gills consume methane at high rates, and that as their methane consumption increases, so do the production of carbon dioxide and the consumption of oxygen. The researchers also found that the ratio of stable carbon isotopes in mussel tissue reflects that in natural-gas methane. Using radioactive carbon-14, they were able to show that the carbon in the methane molecules being consumed was the same carbon in the carbon dioxide being released. Electron micrographs of the gill bacteria contain stacked membranes that are found in methane oxidizers (as well as in some other kinds of bacteria). What's more, since February the group has been keeping mussels alive just by bubbling air and methane natural gas through their tanks.

Cavanaugh's group took another tack in its studies of the Florida mussels. These researchers hunted for and found enzymatic activity known to occur only in methane oxidizers. "We've shown that the enzyme materials are only in the gills, where the bacteria are, and not in the mantle or foot tissues, where there are no bacteria," Cavanaugh notes. Micrographs of the bacteria in these gills also revealed internal membranes that resemble those of methane oxidizers.

After scientists discovered hydrogen sulfide bacteria in vent animals, they began to look for other kinds of bacteria that were supplying animals with energy by oxidizing compounds such as hydrogen, ammonia and methane. According to Cavanaugh, the idea of methane symbiosis was first suggested by British researchers in 1981. In a SCIENCE paper published earlier this year, LaVerne Kulm and Erwin Suess at Oregon State University in Corvallis and their colleagues suggested that they had found the first evidence of methane symbiosis in clams that live in the subduction zone off the Oregon coast (SN:12/15/84,p.374). But Childress thinks that their evidence — carbon isotope studies of four clams — is weak, partly because his group has found that there can be a tremendous variation in carbon isotope readings among clams. Moreover, he says, these same clam species, found in other locations, have developed a symbiotic relationship with sulfide-reducing bacteria, and it would be unlikely from an evolutionary standpoint that the clams also would have become adapted to methane bacteria. "I'm sure we'll show eventually that those animals don't use methane," he adds. According to Kulm, no mussels have been seen at the Oregon site.

One of the problems with past studies at the Oregon, Louisiana and Florida

sites is that the initial explorations of the area were primarily geological; because the scientists did not expect to find oases for sea life, they were not prepared to do any in-depth biological studies. During the *Alvin*'s original dives off Oregon in 1984, for example, no live biological samples were collected. Researchers should learn more about the biology and geology next summer, when *Alvin* is scheduled to make 25 dives at the subduction zone off Oregon.

Similarly, no live specimens were taken from the Florida seeps. In October, Cavanaugh and others will collect mussels, tube worms and clams there using *Alvin*. At the Louisiana seeps, all the specimens have come from trawling. The Texas A&M group is planning to take its first look at those seeps at the end of this month with the *Johnson Sea Link*, according to Childress, who has requested time on the submersible next year to do a very extensive biological workup of the seeps.

Childress says his group is also planning to look for methane symbiosis at other locations, such as off the California coast. "My prediction," he says, "is that wherever you have methane-rich petroleum seeps or hydrocarbon seeps at depths of more than a few hundred meters, you have the potential for having these kinds of animals there."

— S. Weisburd

Upside-down clouds spark debate

By stretching an electrified wire across a 2-kilometer-wide canyon, two atmospheric scientists may have shed light on the mechanism that electrifies thunderclouds. However, others who study clouds are reluctant to call the results of the experiment a flash of insight.

Charles B. Moore and Bernard Vonnegut report in the Sept. 26 SCIENCE that by releasing small amounts of negative charge into the atmosphere, they were able to influence the electrical structure of developing thunderclouds, sometimes reversing the polarity of the cloud or turning it "upside down." This publication of results from their 1984 and 1985 experiments supports earlier announcements of their findings (SN:12/22&29/84, p. 396).

Normal thunderclouds look approximately like an electric dipole, with a negatively charged layer stationed beneath the upper, positively charged portion of the cloud. Cloud-to-ground lightning discharges this negative layer by lowering negative charges to the ground in a violent series of sparks or "strokes." Why the negative charge so regularly appears on the bottom of the cloud is a question that viable cloud-electrification theories must answer.

The more accepted theories stress the microphysics of precipitation within the cloud, says Vonnegut, of the State University of New York at Albany. According to these theories, variables such as the temperature and size of colliding rain, ice and hail particles determine the polarity of the thundercloud.

For 30 years, Moore and Vonnegut have advocated a second class of theories, so-called "induction" or "feedback" mechanisms. These theories hold that the kind of charge — usually positive — present in the atmosphere as a cloud develops will influence the cloud's polarity. They decided to test this idea by reversing the polarity of the air near Langmuir Lab in Socorro, N.M. With a voltage of -120 kilovolts on their wire, they branded the air with a negative charge.

Moore and Vonnegut report that when clouds grew from the negatively charged air, they were abnormally polarized — a large positive charge center was observed in the lower part of the cloud. An observing plane flying through the cloud also reported the presence of a negative charge center in the top of the cloud, again a reverse of the normal situation.

Positive charge regions are often observed in the lower part of an active thundercloud, but these regions are small and "are transient phenomena that exist only after lightning," says Moore, of the New Mexico Institute of Mining and Technology in Socorro. "Whatever we're seeing is very different."

That a small amount of negative charge released into the atmosphere could produce such a large anomalous positive charge in the cloud confirms, in Moore's opinion, that feedback may be involved in the electrification process, and that the polarity of the growing thundercloud is determined by whether the ambient charge is positive or negative.

Other researchers are reluctant to draw any conclusions without more evidence. Tom Marshall of the University of Mississippi in Oxford sent an instrumented balloon at Langmuir through one of Moore and Vonnegut's test clouds and says the cloud displayed a normal polarity. He measured a three-tiered charge structure in the cloud — positive top, negative middle and positive bottom. "The electric field profile [electric field as a function of altitude] looks very much like electric field profiles for other clouds," he says. "It's common to have a [small] lower positive charge."

If the cloud is of normal polarity, says Earle Williams of MIT in Cambridge, Mass., it suggests "that there's some microphysical mechanism going on to produce that positive charge."

However, Marshall adds that he's "very confused" by the magnitude of the charge. "I don't think the cloud was upside down, but on the other hand I don't think it was a normal cloud either."

— R. Monastersky

Reasons for boning up on manganese

Manganese may be an important factor not only in preventing osteoporosis but also in regulating the production and release of insulin and in fighting the cell and tissue damage caused by a number of environmental insults, according to research presented at the recent American Chemical Society meeting in Anaheim, Calif. Moreover, many individuals may be receiving insufficient levels of this essential trace metal. One reason, new data show, is that the body cannot absorb it from many of the richest dietary sources.

Biologist Paul Saltman of the University of California-San Diego (UCSD) in La Jolla became interested in the importance of dietary manganese six years ago after meeting the physician attending basketball-superstar Bill Walton. At the time, Saltman notes, Walton was constantly plagued by broken bones. Saltman recalls looking at the X-rays of an ankle of Walton's that was not healing, and realizing, to his shock, that "this guy has osteoporosis or an osteoporosis-type disease."

Examining Walton's blood, Saltman turned up no manganese, half the normal levels of zinc and copper, and lots of calcium. He says the calcium levels suggested Walton "was not synthesizing bone well." Other trace metal levels suggested a "remarkable" imbalance. Walton started taking mineral supplements, dropped his macrobiotic diet and "within six weeks was back playing [basketball]," Saltman says.

The first major clues to the cause of Walton's condition came in a one-year experiment by Saltman and his co-workers, in which rats on a low-manganese diet developed porous bones.

Bone tissue is constantly breaking down and being reformed. So in another test the UCSD team measured the activity of cells responsible for the breaking down and laying down of bone tissue. Manganese-deficient rats broke down half as much bone as rats on a minerally balanced diet. Cells responsible for laying down new bone were inhibited even more, he says, resulting in bone loss and increased porosity.

Most recently, working with rheumatologist J. Yves Reginster at the University of Liège in Belgium, Saltman and UCSD co-worker Linda Strause compared blood and bone samples from 14 Belgian women with "hard-core osteoporosis" to those from age-matched women without osteoporosis. Of the 25 factors they looked at, Saltman says the only statistically significant difference was in manganese. And it was striking. Blood levels of manganese in the osteoporotic women were a quarter of

those in the other group.

Sheri Zidenberg-Cherr and her colleagues at the University of California at Davis have found reduced activity in one enzyme — manganese superoxide dismutase — in manganese-deficient rats. This enzyme is important in the body's defense against free-radical damage from ozone and other environmentally polluting oxidants (SN:3/31/84,p.197), explains co-worker Carl Keen. "We can link a reduction in the activity of this enzyme," he adds, "with increased tissue-lipid peroxidation — thought to be one of the major types of tissue pathology linked with a host of environmental insults."

By manipulating dietary manganese levels in rats, the Davis team has been able to block the production or release of insulin from the pancreas, producing a condition that resembles diabetes. This suggests, Keen says, "that pancreatic manganese concentrations may be one of the regulatory steps in insulin's synthesis or release."

Human studies at the University of Texas in Austin suggest that the National Research Council's recommendation of 2.5 to 5 milligrams of manganese daily is too low. Jeanne Freeland-Graves has found that at least 3.8 mg must be consumed daily to prevent the body from depleting its stores of the metal.

Ironically, the richest stores of manganese — in wheat bran, tea and spinach, for example — are unavailable to the body, according to studies by Constance Kies at the University of Nebraska in Lincoln. Factors like phytate and fiber in bran, tannins in tea and oxalic acid in spinach inhibit the absorption of many minerals, including manganese. Though manganese is present in far smaller quantities in meats, milk and eggs, Kies says its higher bioavailability in them can make them an important source.

Kies's studies also show that mineral supplements of iron, magnesium and calcium can inhibit manganese uptake. The calcium finding is particularly worrisome, she says, because U.S. women — who in general receive "far less manganese than even the NRC recommends" — are being encouraged to offset their risk of developing osteoporosis with calcium pills. In fact, Kies says, because of their effect on manganese absorption, it is possible "that taking these calcium supplements might make the osteoporotic situation worse." However, Kies's data indicate that when the source of calcium is milk, "there is no statistically significant decrease in manganese absorption." — J. Raloff

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