Hydrogen-Based Microbial Ecosystems in the Earth

Todd O. Stevens and James P. McKinley (*1*) report finding hydrogen gas (H_2) of possible geochemical origin, and they propose that this H_2 supports lithotrophic methanogenic bacteria that are physiologically active beneath the Columbia River plateau.

Methanogenic bacteria are ubiquitous in the biosphere's anaerobic habitats (for example, in soils and sediments), and the ability to use H_2 as an electron donor for carbon dioxide reduction to methane is almost universal among methanogens (*2*). In order for methanogens to be linked to photosynthesis, H_2 is usually produced by an anaerobic microbial food chain responsible for the decay of photosynthetically produced plant materials. But H_2 production is also commonly associated with geothermal activity. Furthermore, a variety of habitats where geothermal H_2 is emitted have been shown to support methanogenic bacteria (*2*, *3*). These previously described microorganisms do precisely what was postulated for the microbial community beneath the Columbia River plateau: They grow in anaerobic habitats at the expense of abiogenic H_2. Thus, as a strictly physiological phenomenon, the subject of Stevens and McKinley's report is not unique.

There are, however, three ecological aspects of the work that merit attention: (i) The proposed H_2 source for methanogenic life was neither biogenic (from an anaerobic food chain) nor geothermal; (ii) C isotopic ratios suggested that methanogenesis was occurring in situ, within the basaltic subsurface deposits; and (iii) lithotrophy (regardless of its aerobic or anaerobic basis) has not been previously reported in subsurface environments. Given the diversity of microbial biogeochemical reactions and efforts by scientists to describe them (*4*), it is important to place new discoveries within the scholastic context of microbial ecology.

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Stevens and McKinley (*1*) suggest that H_2 generated from rock weathering supports an autotrophic microbial community in deep anaerobic basaltic aquifers in the western United States. Data about microbiological populations, hydrogen concentrations, and carbon isotopes, as well as a laboratory experiment showing H_2 production from a basalt-water interaction, are provided as evidence for this conclusion. However, each of these lines of evidence has an alternative interpretation.

The strongest evidence for the proposed abiological H_2 production [figure 4 of the report (*1*)] demonstrates that H_2 accumulates when some basalts are incubated in buffered water, but these experiments were conducted in phosphate buffer at pH 6, whereas the pH of the ground waters at this site is alkaline (pH = 7.5 to 9.9). It seems likely that H_2 production could be favored by artificially lowering the pH, and so this experiment cannot be used to support the hypothesis (*1*).

Stevens and McKinley suggest that the isotopic signature of the inorganic C dissolved in the ground water also provides evidence for abiological H_2 production. The enrichment of ^{13}C in dissolved inorganic carbon in the methanogenic zones is probably the result of H_2-dependent methanogenesis. However, similar isotopic signals are observed in the methanogenic zone of organic-rich marine sediments in which the source of H_2 is organic matter fermentation (*2*). Thus, the isotopic data do not uniquely identify these aquifers as ecosystems driven by abiological H_2 production.

Furthermore, Stevens and McKinley state that the ground waters with high sulfate are "depleted in ^{13}C," which "suggests that sulfate-reducing bacteria oxidize biologically fixed carbon, which is relatively rich in ^{12}C." This statement describes an environment in which degradation of organic matter, rather than consumption of abiologically produced H_2, is fueling microbial metabolism.

High concentrations of H_2 dissolved in the ground water are stated by Stevens and McKinley to be "mostly three or more orders of magnitude above the range . . . that would be expected from microbial fermentation of organic matter." However, the reference cited (*3*) states that the H_2 concentrations in anaerobic sedimentary environments are independent of the rate of H_2 production from rates of organic matter fermentation. Thus, high H_2 concentrations are not evidence that H_2 is coming from an abiological source.

Stevens and McKinley found that microbial enumerations recovered higher numbers of H_2-consuming microorganisms than fermentative bacteria. However, it is well known that viable counts are unreliable indicators of true microbial numbers and that organic-rich media (such as that used to enumerate the fermentative bacteria) may be toxic to heterotrophic microorganisms living in organic-poor environments such as aquifers.

Finally, Stevens and McKinley state that "the igneous rocks in the study area contained little organic carbon," suggesting that this is an organic-poor environment. However, given the low rates of microbial metabolism in deep aquifers, a "little" organic C may go a long way.

The discovery of active H_2-dependent methanogenesis in deep basaltic aquifers of the western United States lends further credence to the suggestion (*4*) that lithotrophic microbial ecosystems exist in the deep terrestrial subsurface of Earth and possibly other planets. However, there are as yet insufficient data to conclude that H_2 produced from abiological basalt weathering is the primary electron donor supporting the microbial community in these aquifers.

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Response: Madsen raises a point about the significance of the microbial communities that we reported within the Columbia River Basalt Group (CRB) (*1*). Certainly, we are not the first to propose that microorganisms can gain energy from oxidation of geochemically produced H_2. Some investigators have even proposed hydrogenotrophy-based ecosystems in the subsurface environment (*2*). To our knowledge, however, actual evidence for in situ hydrogenotrophic

primary production has not been reported. The studies cited by Madsen do not contain such evidence.

Some confusion may have arisen among readers because we drew an additional distinction in our discussion: We proposed that the microbial system within the CRB may be not only (chemo)lithoautotrophic, but also independent from photosynthesis, which is not quite the same thing. Sulfide- and methane-oxidizing communities at deep-sea hydrothermal vents are chemolithoautotrophic but depend on photosynthesis because they require O_2 as an electron acceptor. Many chemolithoautotrophic microorganisms can be found in anaerobic sediments, but the community as a whole—and ultimately, the lithoautotroph population—is usually dependent on degradation of organic matter.

All of the environments described in the references cited by Madsen are profoundly affected by photosynthetic processes. They contain photosynthetic mats, organic-rich sediments, or oxygenated water. It seems a reasonable hypothesis that geochemically produced H_2 may contribute electrons to microorganisms in some of these locations; however, it was not demonstrated. We suspect that chemolithoautotrophic primary production is widespread in the subsurface.

Lovley and Chapelle suggest that the evidence in our report may be insufficient to demonstrate a chemolithoautotrophy-based system. Any of our points of evidence alone is not sufficient, but we believe that, taken together, they provide a strong case. Because of the inherent difficulty in sampling the subsurface, indirect observations and simulations must be used. Our report is an initial study and not the final word on this system. We address each paragraph of the comment by Lovley and Chapelle, in order.

The pH of our experiment [figure 4 of our report (*1*)] is below that of the aquifers; however, H_2 production does occur in the in situ pH range and is typically 20 to 85% of that found at pH 6 in vitro (*3*). We agree that our study would have been strengthened by data from experiments at higher pH, although other variables also determine H_2 production (*3*). The experimental survey of H_2 production in different rocks was done at pH 6 because that was found, in initial experiments, to be the optimal pH for H_2 evolution.

Dissolved inorganic C (DIC) depleted in ^{12}C by autotrophic methanogenesis has been reported in organic matter-rich sediments, but not in close relation with dissolved inorganic C (DIC) depletion as we observed. If the methane formation had been associated with heterotrophic H_2 production, DIC would have been produced, not consumed. [The unit label in the axis label for figure 3b in our report (*1*) was incorrect: DIC was reported in mmol/L, not in mg/L.]

The high-sulfate zones were not detailed in our report because we as yet have no further information about them. The isotopic evidence supports autotrophy in the (predominant) low-sulfate aquifers. However, the ^{13}C-DIC depletion in some high-sulfate waters is so great as to suggest anaerobic methane oxidation—a phenomenon observed several times in the field, but not demonstrated in the laboratory. Alternatively, it could indicate a volcanic, rather than atmospheric, source of C (the source of the S is also unknown, but could be volcanic). The nature of processes occuring in the high-sulfate waters remains unknown.

The high H_2 concentrations show that it is present in sufficient quantities to provide an electron-donor for microbial metabolism, not necessarily that H_2 comes from an abiological source. However, we also demonstrate an abiological source (basalt weathering) that could explain this observation.

The cultural enumeration data are a contributing line of evidence that demonstrates that the microorganisms we postulate are in fact present in the ground waters. The observation that autotrophs outnumbered the heterotrophs (within the inherent limitations of the technique) does separate the CRB aquifers from sedimentary systems we have observed and from other reports (*4*). We have also demonstrated that basalt can serve as the sole electron donor for microbial growth and respiration in vitro (*1*, *3*).

There are clearly many avenues left to explore in understanding the CRB system. We believe that it may become an important model for microbial ecology and are pleased that our report has stimulated discussion about this topic.

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