Methylmercury: Bacterial Degradation in Lake Sediments

Abstract. *During the first 50 days of a long-term period of incubation of lake sediments with inorganic mercury (Hg^{2+}), low concentrations of methylmercury were observed to build up. Upon continued incubation there was a rapid decrease in amount of methylmercury in the system and a concomitant evolution of volatile inorganic mercury (Hg^0). Transfer of the mixed culture to growth media containing methylmercury resulted in the degradation of methylmercury and the volatilization of Hg^0. Four bacterial isolates were obtained from the mixed culture which, in pure culture, rapidly degraded methylmercury to methane and Hg^0. The presence of methane in head space gases was confirmed by flame-ionization gas chromatography, and the presence of Hg^0 in head space gases was confirmed by mass spectrometry.*

It is generally believed that bacteria produce the methylmercury found in lake sediments through the methylation of inorganic mercury (Hg^{2+}). Two types of methylation are possible: microbial (enzymatic) and chemical (nonenzymatic methylation of Hg^{2+} by methylcobalamin). Methylation presumed to be enzymatic has been reported in rotting fish, in sediments (*1*), and in a pure culture of *Clostridium cochlearium* isolated from soil (*2*). It has been demonstrated that under labratory conditions methylcobalamin produced by methanogenic bacteria could bring about the methylation of Hg^{2+} (*3*). Whether the methylation of Hg^{2+} actually occurs in the aquatic environment by the same mechanisms as those studied in the laboratory has yet to be conclusively demonstrated. Although it is generally accepted that methylation occurs in the aquatic environment, most workers have been unable to find even trace amounts of methylmercury in a wide variety of naturally occurring sediments or in water. In this report we offer evidence that the failure to find methylmercury in sediments is due, at least in part, to the presence of bacteria capable of degrading methylmercury.

Long-term incubation studies were carried out on the biomethylation of Hg^{2+} in sediments taken from the delta area of the St. Clair River, Michigan. We used various combinations of growth media and gaseous atmospheres in an effort to identify organisms or groups of organisms capable of methylating Hg^{2+}. One flask was included containing a sediment : water mixture (50 : 50 by volume) [150 g (wet weight) of sediment plus 100 ml of water] to which was added 2 mg of Hg^{2+}. The mercury, added as $[^{203}Hg]HgCl_2$, had a radioactivity of 2.7×10^7 disintegrations per minute. The flask was incubated under aerobic conditions without shaking for 146 days. The flask was flushed continuously with a stream of sterile, water-saturated air at a flow rate of approximately 20 cm^3/min. A control containing heat-sterilized sediment (121°C for 30 minutes) was maintained under the same conditions. A trap was attached to each flask to collect any volatile mercury species in the effluent flushing gas. It was found that a trapping solution consisting of 100 g of KBr and 15 g of $HgBr_2$ dissolved in 1 liter of water quantitatively traps dimethylmercury (DMM) and volatile elemental mercury (Hg^0) (*4*).

Figure 1 indicates that, during the first 50 days of incubation, the concentration of methylmercury in the sediment increased from zero to 0.31 μg/g (wet weight), as determined by electron-capture gas chromatography. This increase was followed by a period of rapid decrease in the amount of methylmercury, and there was a concomitant rapid increase in the amount of Hg^0 volatilized to the trap. There were no organomercurials found in the trap contents, an indication that the methylmercury produced had been degraded and that the volatile radioactive species was trapped as mercury vapor (*5*).

In an effort to determine if the disappearance of methylmercury was due to microbial degradation, 1 ml of the

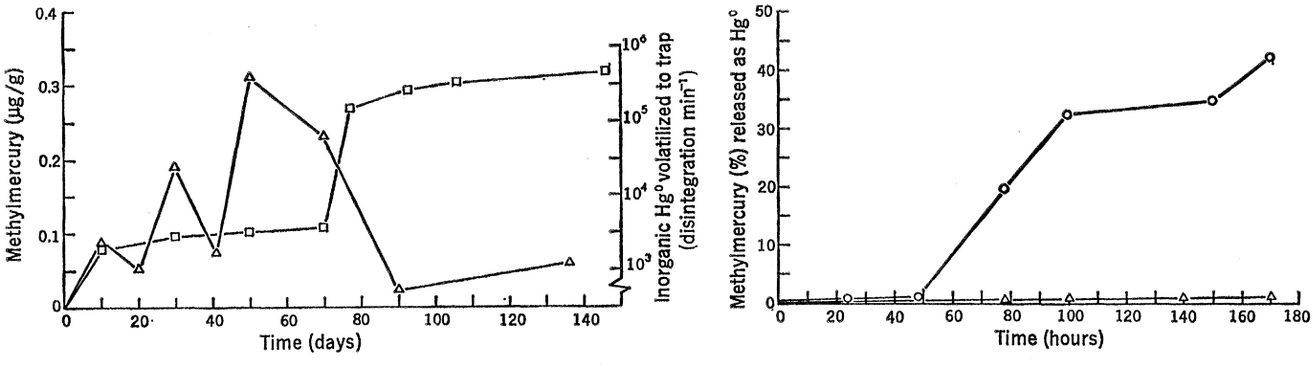

Fig. 1 (left). Plot of the time course of methylmercury production as a function of the volatilization of Hg^0 in sediment: □, disintegrations per minute of Hg^0 volatilized to the trap; △, micrograms of methylmercury per gram of sediment as determined by gas chromatography. At the indicated time intervals, 25 ml of the mixture were aseptically removed for analysis. The mixture was extracted with 35 ml of toluene after treatment with 5 ml of acidic KBr solution. Additional cleanup was accomplished by extraction of the toluene with 1.5 ml of buffered cysteine. The cysteine was treated with 0.6 ml of 3*M* KI and extracted with 2 ml of benzene. Methylmercury iodide in the benzene extracts was determined by means of a gas chromatograph (Microtek 2000 R) with an electron-capture (tritium) detector and a glass column (122 by 0.63 cm) packed with a mixture of 1.5 percent OV-17 and 1.7 percent QF-1 on Supelcoport (100 to 120 mesh). The operating conditions were as follows: column temperature, 125°C; inlet temperature, 180°C; detector temperature, 165°C; carrier flow, 70 ml per minute of nitrogen. A linear calibration curve was obtained for the 0- to 1.0-ng range. We monitored the volatilized mercury species by directly inserting the trap into the counting well of a gamma scintillation spectrometer (Packard 2001). Counting efficiencies were determined by use of a standard ^{203}Hg reference source. Corrections for background and decay were made for each reported value of the number of disintegrations per minute. Fig. 2 (right). Degradation of methylmercury by a mixed culture from sediment: ○, mixed inoculum from sediment; △, uninoculated control. The trap containing $HgBr_2$ and KBr was monitored for radioactivity and, at the termination of the experiment, was analyzed for organomercurials. The contents of the flask were checked for radioactivity and, at the termination of the experiment, were analyzed for residual methylmercury.

contents of the flask was transferred to 100 ml of tryptic soy broth (TSB) containing 25 μg of labeled methylmercury bromide, [^{203}Hg]CH_3HgBr (MMB), with a radioactivity of 1.71×10^6 disintegrations per minute. Figure 2 depicts the evolution of Hg^0 during the growth of the mixed culture as compared to that of an uninoculated control containing MMB. Analysis of the contents of the flask after 170 hours of incubation indicated the complete disappearance of methylmercury. (The methylmercury was completely recovered from the control flask.) The discrepancy arising from the fact that only 42 percent of the methylmercury added was recovered (as Hg^0) although all the methylmercury had disappeared from the flask contents may be explained in terms of the radioactivity present in the cell fraction (*6*, *7*).

Serial dilutions of the mixed culture from the TSB flask were plated out on tryptic soy agar, and representative colonies were selected from high-dilution plates. After the cultures had been purified by streaking, they were tested for methylmercury degradation by incubation in TSB containing 0.25 part per million (ppm) of labeled MMB (radioactivity, 1.03×10^6 disintegrations per minute). Four of these cultures degraded methylmercury, as shown in Fig. 3. Although these species have not been characterized, all were short, Gram-negative rods and appear to be *Pseudomonas* sp.

The pseudomonads isolated appear to be quite similar to the species isolated from soil by Tonomura *et al.* (*7*) and reported to convert organomercurials to metallic mercury and an organic moiety. More recently, the enzyme system involved has been isolated (*8*) and characterized by Japanese workers (*9*).

The degradation of methylmercury was further verified by short-term incubation of cultures that degraded methylmercury with 25 μg of MMB in closed flasks containing 50 ml of TSB medium. The head space gases were analyzed for methane by flame-ionization gas chromatography with an OV-1 column. Methane was produced by the four cultures with concomitant volatilization of mercury, an indication that the methylmercury was degraded to methane and Hg^0. No methane was produced by cultures that did not degrade methylmercury incubated under the same conditions.

The presence of Hg^0 in the head space gas of one of the isolates (sample 1-2) was confirmed by mass spectrometry. Head space gases from an inoculated control without methylmercury and an uninoculated control containing 5 ppm of MMB did not contain Hg^0.

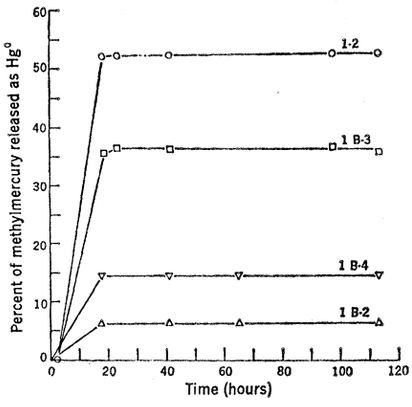

Fig. 3. Degradation of methylmercury by pure cultures isolated from sediment. Volatile mercury was monitored in a trap containing $HgBr_2$ and KBr attached to the flask. Curves are shown only for cultures that degraded methylmercury.

The results indicate that demethylators are present in mercury-containing sediments and could be responsible for the degradation of at least some of the methylmercury produced in sediments. Thus these organisms may serve a useful purpose in maintaining the environmental methylmercury concentrations at a minimum. However, data are not available in the literature on the prevalence of such organisms in the aquatic environment or the conditions which might favor their growth. We have isolated some 200 cultures from various sediments and environmental samples taken from areas where fish containing methylmercury have been found. The results of experiments, currently in progress, with these isolates will be presented elsewhere (*10*).

WILLIAM J. SPANGLER
JAMES L. SPIGARELLI
JOSEPH M. ROSE, HOPE M. MILLER
Midwest Research Institute,
Kansas City, Missouri 64110

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4. We confirmed the quantitative trapping of DMM and Hg^0 by preliminary experiments, using $HgCl_2$, CH_3HgBr, and Hg^0 (produced in aqueous solution by the chemical reduction of Hg^{2+}), all labeled with ^{203}Hg, and unlabeled DMM. Of the labeled mercury species tested, only Hg^0 and DMM were found in the $HgBr_2$/KBr trap. If DMM were formed from [^{203}Hg]-MMB by a microbial reaction in the incubation flask, the [^{203}Hg]DMM formed would volatilize to the trap and be converted to [^{203}Hg]-MMB in the trapping solution. The resulting radioactivity would be seen as a false positive test for Hg^0. To eliminate this possibility, all trap contents containing radioactivity were extracted and analyzed for methylmercury by electron-capture gas chromatography.
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Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectra of Human Serum Lipoproteins

Abstract. *Human serum lipoproteins have been studied by Fourier transform nuclear magnetic resonance of carbon-13 in natural abundance. Spectra of high-density, low-density, and very-low-density lipoproteins were recorded and partly assigned. The prominent features of these spectra reflect the qualitative and quantitative composition of the lipid moiety of these complexes. The results suggest that carbon-13 nuclear magnetic resonance will be a useful technique for studies of the structural and dynamic parameters of lipoproteins.*

The structure and metabolism of human serum lipoproteins have come under increasing scrutiny in recent years, reflecting accentuated interest in the topic of protein-lipid interactions generally and the recognition of the important physiological roles of lipoproteins specifically (*1*). The structural properties of serum lipoproteins have been probed by a variety of physical and chemical techniques; nevertheless, the structural organization of these