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Effect of Salinity on Methylation of Mercury

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Monomethyl and dimethylmercury are potent neurotoxins subject to biomagnification in food webs (NATIONAL ACADEMY OF SCIENCES 1978). This fact was tragically demonstrated by the Minamata and Niigata poisoning incidents in Japan in which 168 persons who ate seafood from mercury polluted waters were poisoned, 52 fatally (KATZ 1972, D'ITRI and D'ITRI 1978). Shortly after these two incidents, work conducted in freshwater environments by WESTOO (1966) and JENSEN and JERNELOV (1969) demonstrated the microbial conversion of inorganic and phenylmercury compounds to mono- and dimethyl-mercury. It has been since widely assumed that microbial methylation played a significant role in the Japanese poisoning incidents, even though these were associated with estuarine waters and involved some methyl-mercury as well as inorganic mercury discharges, making the role of microbial methylation uncertain. Consideration of some fragmentary evidence from the literature, however, indicates that the rate and the significance of microbial methylation of mercury in freshwater and saltwater environments may not be the same. YAMADA and TONOMURA (1972a, 1972b and 1972c) showed that sulfide ions interfered with the methylation of mercuric ions by Clostridium cochlearium. Sulfate interfered with methylation of mercury in a mixed anaerobic culture of methylating and sulfate-reducing bacteria. Similar results were reported by TALMI and MESMER (1975). FAGERSTROM and JERNELOV (1971) reported very low rates of mercury methylation from HgS as compared to $HgCl_2$. Based on these results, one may speculate that in sulfate-rich and anaerobic marine and estuarine sediments mercury is less likely to be methylated than in low-sulfate freshwater sediments. The same anaerobic conditions that are conducive to mercury methylation will give rise, in sulfate-rich sediments, to H_2S that will tie up mercuric ions and make them less available for microbial methylation. In addition, sulfate may interfere with methylation of mercury through its effect on the redox potential. Reduction of sulfate occurs at redox potentials less negative ($\sim$ - 200 mV) than those required for reduction of CO_2 ($\sim$ - 400 mV). Consequently,

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significant methanogenesis does not occur until all available sulfate is reduced (LEXMOND et al. 1976). Sulfate being a major anion in seawater (TAIT and DESANTO 1972), estuarine sediments are generally rich in sulfate while most freshwater environments are not. Regardless of its exact mechanism, a demonstrated relationship between mercury methylation rates and water salinity would greatly influence our thinking about mercury pollution effects in marine versus freshwater environments. Since we were unable to locate published reports on this subject, we have undertaken to investigate the influence of salinity on the rate of mercury methylation in an estuarine sediment.

EXPERIMENTAL

Sample Preparation and Incubation. Estuarine sediment used in these studies was collected from under a typical Spartina alterniflora stand on the bank of the Cheesequake Creek, Cheesequake State Park, NJ. Collection was made at low tide, using a hand-operated core sampler. Core sections from 50 to 20 cm in depth were slurried with water from a freshwater pond in a 2:1 final sediment:water ratio, using a Serwall Omni Mixer under a nitrogen atmosphere. Without addition of salts, this slurry had a salinity reading of 0.1% as determined by a YSI Model 33 salinometer. Sea salts were added from concentrated stock solutions of Seven Seas Marine Mix (Utility Chemical Co., Paterson, NJ) in pond water. Final salinities of the slurries were adjusted, after equilibration, using the salinometer. Ten ppm (based on dry sediment) of mercury was added as $HgCl_2$. While stirring under a nitrogen stream in order to assure homogeneity but minimize oxygen exposure, 30 g amounts of the slurry were dispensed into tared 75 ml test tubes. The tubes were flushed with nitrogen, were tightly closed with Teflon-lined screw caps and were incubated in the dark at 28°C. Controls of two kinds were included: tubes without added mercury and tubes with mercury plus 0.2% NaN_3 to suppress microbial methylation. At appropriate time intervals, tubes were sacrificed for analysis. The contents of the tube to be sacrificed were homogenized using a vortex mixer. Half of the sample was used for analysis of methylmercury, the other half was dried overnight at 105°C to determine the actual dry weight of the sediment in the individual samples.

Analytical Procedures. Analysis of methylmercury (monomethyl and dimethylmercury combined) was performed according to LONGBOTTOM et al. (1973). In precisely weighed sediment samples ($\sim$ 15 g) all methylmercury was converted to CH_3HgBr and subsequently extracted into toluene. After clean-up by sodium thiosulfate treatment, CH_3Hg^+ was converted to the iodide form and was extracted into benzene. After drying of the extract by anhydrous Na_2SO_4, CH_3HgI was analyzed by gas chromatography using a Tracor Model MT-220 instrument with a ^{63}Ni electron capture detector and on-column injection of 5 µl samples. A 183 cm long

0.73 cm OD glass column packed with 10% Silar 10-C on 80/100 mesh Gas Chrom Q was used under the following operating conditions: 95% argon, 5% methane carrier at 90 ml/min, inlet 210°C, oven 200°C, detector 280°C. Peaks were quantitated by means of a disc integrator and appropriate methylmercuric iodide (Matheson, Coleman and Bell, Norwood, OH) standards. Detection limit was 0.01 µg mercury/g sediment. Recovery of methylmercury from spiked sediment samples was 95% ± 7%. All methylmercury values were recorded as µg Hg per gram of dry sediment.

RESULTS AND DISCUSSION

The rate and extent of microbial methylation of Hg^{++} ions in anaerobically incubated estuarine sediment samples (Fig. 1) was clearly in an inverse relationship to salinity. Methylation was most rapid at the lowest salinity (0.1%). In this case, 2.3% of the added mercuric ion was present as methylmercury after 15 days of incubation as compared to 0.05% at 3% salinity. An increase in salinity beyond 2% had comparatively little additional effect on methylation. There was a marked decline in methylmercury concentrations after 15-20 days of incubation. Measurements on mono- and dimethylmercury-spiked estuarine sediment samples, not reported here in detail, suggest that these declines were a consequence of microbial demethylation. Anaerobic demethylation of mercury is known to occur (SPANGLER et al. 1973a, 1973b; BILLEN et al. 1974). The pattern of initial increase and subsequent decline in methylmercury was consistent in our experiments, and very similar patterns were observed previously in sediment samples by OLSON and COOPER (1976). We interpret these patterns as consequences of microbial succession, either in response to the methylmercury build-up, or to some other changing environmental parameter. It is not clear at this stage, however, whether these patterns would occur in nature, or whether they are artifacts of sample preparation and incubation procedures.

It would be desirable to relate our laboratory experiments to reported field observations. OLSON and COOPER (1974) measured *in situ* mercury methylation activity in San Francisco Bay estuarine sediments. They noted methylation activity far below the levels reported by JENSEN and JERNELOV (1969), FAGERSTROM and JERNELOV (1971) and SPANGLER et al. (1973b) for freshwater sediments incubated under laboratory conditions. OLSON and COOPER (1976) also compared methylation of mercury in sediment samples collected from three locations in San Francisco Bay. The sediment collection points ranged from shallow, nearshore areas to deep water. Presumably, the nearshore sediment sample had a lower salinity than the deep water one. Mercury methylation activity was found to be highest in the nearshore and lowest in the deep water sediment sample. However, this lower activity was not attributed to salinity, nor was the actual salinity of the San

Francisco Bay sediments reported. While the methylation rates reported by OLSON and COOPER (1974, 1976) appear to be consistent with our findings, there is a clear need for future comparisons of in situ methylation in sediments with varying levels of known salinity.

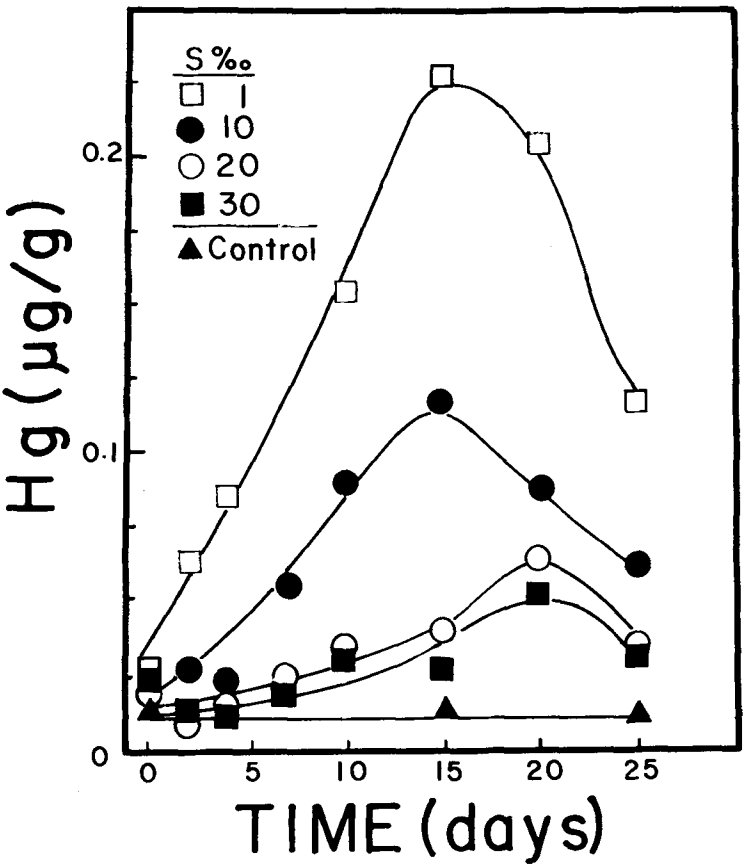

Figure 1. Effect of salinity on the anaerobic methylation of 10 ppm Hg^{++} (as $HgCl_2$) in an estuarine sediment. Salinities (S °/₀₀, see key) were adjusted from 1 to 30 parts per thousand. Methylmercury concentrations [Hg(µg/g)] are plotted on the Y axis as µg methylmercury-Hg per g dry sediment. Controls without $HgCl_2$ and also with $HgCl_2$ plus 0.2% NaN_3 were included at several salinity levels. Since none of these controls showed detectable mercury methylation, for clarity only a single control curve is shown.

Further work will be necessary to prove that the observed salinity effect is indeed due to the sulfate component of sea salts, and if so, the mechanism of interference will need to be defined. However, the inverse relationship between salinity and mercury methylation is clear from the work accomplished to date, and this finding should have some significant practical implications. If it can be confirmed that conversion of inorganic mercury to methylmercury is lower in saltwater than in freshwater environments, this could affect the siting of inevitable sources of mercury pollution and, perhaps, result in differential mercury pollution standards for freshwater and saltwater environments.

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