Role of Bacteria in Bioaccumulation of Mercury in the Oyster *Crassostrea virginica*

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An investigation of mercury-resistant bacteria was undertaken to determine their role in the accumulation of mercury in a simplified food chain. Oysters (*Crassostrea virginica*) were maintained in a closed system, sealed aquarium with stirred, aerated water containing 10 µg of **HgCl₂** per liter. Uptake of **Hg** by oysters held under control conditions was compared with that of **Hg** uptake by oysters under similar conditions except that mercury-accumulating and mercury-metabolizing species of *Pseudomonas*, isolated from Chesapeake Bay, were added to the experimental oysters. After incubation for 4 days, the major portion of the **Hg** in the water column was found to be associated with the microparticulate fraction, corresponding to a rise in total viable count. Mercury accumulation in the oysters was significantly higher in the gill and visceral tissue than other tissues. Mercury concentrations were 200 times greater in tissue fractions of oysters dosed with mercury-metabolizing bacteria compared with the oysters held under control conditions without mercury-metabolizing bacteria.

Whether mercury levels in the oceans have risen significantly is a highly controversial issue (1, 2, 16). Undisputable, however, is the fact that marine animals are capable of concentrating mercury and other heavy metals to levels several orders of magnitude above that found in sea water (18), particularly in the proximity of known sources of pollution (7, 11). Regardless of the source, the consequences of the sequestration of mercury are serious because the highly toxic and persistent alkylated forms of mercury comprise most of the mercury in fish tissue (10, 15, 18). The mechanism(s) by which mercury is accumulated in higher organisms is not completely known. Barber et al. found no apparent relationship between mercury concentrations in marine fish and ambient sea water levels of mercury (2). Similarly, Windom et al. (20) found no significant differences in mercury concentrations in inshore and offshore catches of 35 species of North Atlantic fin fish. However, the amount of mercury accumulated in fish appears to be related both to the size (2, 17) and the species of fish involved (2). It is not known, as yet, whether given species of fish, such as tuna or swordfish, acquire relatively large amounts of mercury by unique mechanisms, which are species specific, or from participation in, or association with, a particular mercury-accumulating food web (5). Evidence both for (8, 12) and against (6, 10, 18) the magnification of mercury concentrations through trophic levels has been presented. The question has not been satisfactorily answered since none of the investigations were specifically undertaken to trace mercury flow through a specific food chain, under controlled conditions. Mercury uptake has been studied in individual marine animals such as the oyster (3, 9) and the fiddler crab (15), but in neither case were the microbiological parameters of the experiment measured or controlled.

We have investigated the ecology of mercury-resistant bacteria in Chesapeake Bay and have concluded that bacteria, by virtue of their ability to accumulate mercury and reduce Hg²⁺ to Hg⁰, are influential in the mobilization and transformation of mercury in the Chesapeake Bay habitat. Consequently, an investigation of the role of bacteria in the introduction of mercury into a simple estuarine food chain was initiated. Based upon a comparative survey of benthic and pelagic animals in Hawaiian waters, Klemmer and Luoma (8) concluded that mercury is probably most readily transported through short food chains directly linked to benthic organisms. After considering the available information and the fact that mollusks are known to accumulate mercury and other heavy metals (3), we selected, for our study, a food chain incorporating bacteria and a benthic filter feeder, the *American* oyster, *Crassostrea*...
virginica. A series of experiments was conducted to elucidate the routes and mechanisms of mercury introduction into aquatic food chains at the lowest trophic level.

MATERIALS AND METHODS

Aquarium system. The apparatus used in the experiments consisted of a flanged, 14-liter Pyrex fermenter jar equipped with a stainless-steel lid which was sealed with a rubber O-ring and fastened with one-eighth-inch (0.32 cm) bolts and wing nuts (Fig. 1). The lid contained threaded brass and nylon hose fittings wrapped with Teflon tape to prevent leaks. A rubber serum cap was placed on the central one-half-inch (1.27 cm) fitting to permit sampling of the closed system with a 12-inch (30.48 cm) sterile cannula and syringe. A smooth enamel support was used to suspend the oysters from the bottom of the jar, above a Teflon-coated magnetic stirring bar. A gas dispersion tube was connected to an outside air pump via tygon tubing and a hose fitting in the stainless-steel lid. The entire aquarium, complete with a stainless-steel, fiber glass-packed air filter, was sterilized by autoclaving at 121 C for 15 min. Ten liters of 0.45-µm filter-sterilized, dilute, artificial seawater (Seven Seas Marine Mix, Utility Chemical Co., Paterson, N. J.; 33% strength sea water) was aseptically pumped in and the aquarium was immersed in a 15 C constant temperature bath (American Instrument Co., Silver Spring, Md.). Circulation was provided by an external, submersible water-driven magnetic stirrer. Aeration was accomplished by pumping air through the external glass-fiber filter and the submerged gas dispersion tube. Effluent air from the aquarium was transported through tygon tubing to two mercury scrubbers, in series, each containing 100 ml of HgBr₂-KBr solution, 1.5 g/100 and 10 g/100, respectively, to trap volatilized mercury (14).

Oysters and bacteria. Oysters were dredged from Tolly Bar outside Annapolis Harbor, Annapolis, Md., in March through April and September through December 1974. The oysters were maintained in a flow-through raw Chesapeake Bay water system at in situ temperature and salinity; before initiation of the experiments, the oysters were scrubbed and placed in artificial sea water (12% salinity) at 6 C for 1 week. The temperature of the water in which the oysters were held was brought up to 15 C gradually and was held for 3 days at 15 C before placing them in the sterilized aquarium.

Bacterial isolates from the Chesapeake Bay were grown in a liquid basal medium containing: HgCl₂, 6 mg; Casamino Acids (Difco Laboratories, Detroit, Mich.), 5.0 g; yeast extract (Difco), 1.0 g; and glucose, 2.0 g per liter of artificial estuarine salts solution (10 g of NaCl, 2.3 g of MgCl₂·6H₂O and 0.3 g of KCl per liter of distilled water). Freshly transferred 18-h cultures were grown in mercury-free broth for 3 to 5 h, after which the cultures were centrifuged, washed, and resuspended in sterile phosphate-buffered saline and added to the aquarium water. Total viable bacterial counts of the aquarium water were determined every 24 h by spreading suitable dilutions of the water on solid mercury-free basal medium and incubating the inoculated plates at 25 C for 2 weeks.

Experimental procedures. Six oysters of uniform size were selected for each experiment. Two were shucked and freeze dried for total mercury analysis. The remaining four oysters were placed in the sterilized aquarium. Bacterial suspensions were added through a port in the lid, followed by 2.0 ml of artificial sea water containing 10 to 50 µCi of ³²³HgCl₂ (Amersham Searle Corp., Arlington Heights, Ill.) to give a final concentration of 10.2 ± 0.2 µg/liter. After an equilibration period of 15 min, duplicate 1.0-ml samples of aquarium water and scrubber solution were removed for counting. A second pair of 1.0-ml aliquots of aquarium water was separately filtered through 0.45-µm membrane filters which were then rinsed with 3 volumes of artificial sea water. The filters were placed in gamma counting tubes and stored for counting. The sampling procedure was repeated on four successive days. At the conclusion of
each experiment, the oysters were removed from the aquaria and shucked. The mantle fluid was collected and the tissues were dissected into four fractions: mantle, gills and labial palps, viscera, and adductor muscle. The tissue fractions were rinsed with artificial sea water and placed in gamma tubes for scintillation counting. Fecal material, which was allowed to settle to the bottom of the aquarium, was collected on 8.0-μm membrane filters, rinsed with sea water, and then counted.

Radioactivity of the tissue was measured in a Packard Tri-Carb spectrophotometer (Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with an auto-gamma spectrometer. Efficiency was measured by the channel ratios method: the standard deviation was found to be approximately 1% of the total counts for all oyster tissues. A two-way analysis of variance was performed to detect significant increases in relative mercury accumulations in experimental oysters. All treatment means were tested for significant increases (α = 0.05) using a least significant range comparison.

RESULTS AND DISCUSSION

Results of three replicated experiments, each using four oysters per experiment, are shown in Tables 1 to 5. Oysters chosen for the experiments were harvested before oyster spawning and following oyster spawning from the same location. There was some variation in oyster size (Table 1); however, no correlation between oyster size and the amount of mercury accumulated was noted. Total wet weight mercury concentrations in the oysters before each experiment were approximately equal to the initial, 10 μg/liter, concentration of mercury in the aquarium water (Table 2). Untreated oysters spiked with methyl mercury and reanalyzed for total mercury indicated complete recovery of all tissue-bound mercury (Table 2). Therefore, the significantly greater concentrations of mercury in treated oysters (Tables 3 and 5) indicated that the uptake of $^{203}$Hg was a true measure of accumulation and not exchange.

The purpose of the first experiment (no. 1) was to establish a base line for measuring the effect of mercury-resistant, estuarine bacteria on the uptake of mercury by oysters. This was accomplished by limiting the bacterial population in the aquaria to those organisms associated with the scrubbed oysters. Total viable counts of oyster-associated microflora rose from $3.7 \times 10^4$ bacteria/ml to $1.2 \times 10^5$ bacteria/ml, during the course of the experiment.

In the second experiment (no. 2), a Pseudomonas strain 5, isolated from the Chesapeake Bay, was added to the aquarium. Strain 5 actively reduced Hg$^{2+}$ to volatile Hg$^0$ (25.4 μg of

| Table 2. Mercury concentrations in untreated oysters before treatment with mercury |
|----------------------------------|---------------------|---------------------|---------------------|
| Expt | Total mercury concn (μg/kg) | | |
|       | Dry wt* | Wet wt* | Methyl mercury spike recovery (%) |
| 1     | 171.0 ± 132.5 | 26.5 ± 19.9 | 100 |
| 2     | 132.7 ± 59.3  | 19.9 ± 8.9  | 86.5 |
| 3     | 175.0 ± 90.5  | 26.3 ± 13.5 | 95  |

*Two oysters were selected from each experimental group of six before mercury dosing.

**Mean of four oysters ± standard deviation.

***Extracted from dry weight. (Dry weight equals 15% of wet weight.)

| Table 3. Relative distribution* of $^{203}$Hg after exposure of oysters to 10 μg of HgCl₂ per liter |
|----------------------------------|---------------------|---------------------|
| ***Hg fraction | Total mercury recovered (%)* |
|                   | (1) Control | (2) Mercury reducer | (3) Mercury accumulator |
| Oyster uptake     | 19.5 ± 9.0  | 28.2 ± 15.8  | 49.6 ± 14.4 |
| Feces             | 0.3 ± 0.2   | 2.8 ± 0.9    | 3.9 ± 2.6   |
| Water             | 2.2 ± 1.0   | 0.9 ± 0.1    | 0.8 ± 0.4   |
| Microparticulate  | 3.8 ± 2.0   | 6.6 ± 0.9    | 6.6 ± 3.5   |
| Volatile          | 7.3 ± 2.8   | 14.6 ± 8.3   | 18.0 ± 14.0 |
| Total recovery    | 36.9 ± 2.2  | 53.1 ± 6.4   | 78.9 ± 27.8 |

*As percent of $^{203}$Hg in solution at time zero.

Incubation time, 4 days.

Average of replicated experiment ± standard deviation.
HgCl₂ reduced to Hg²⁺/mg of cells per 15 min from a solution containing 6 mg of HgCl₂/liter.

In experiment no. 3, a *Pseudomonas* sp. (strain 14), which accumulates mercury (12 μg of HgCl₂ accumulated per mg of cells per 15 min from a solution containing 6 mg of HgCl₂/liter), was added to the experimental aquarium. In experiments 2 and 3, the number of mercury-resistant bacteria added to the aquarium was observed to be approximately equal to the mixed, oyster-associated flora of the aquaria. Total viable counts of the aquarium water rose from 3.8 x 10⁴ to 4.3 x 10⁴ bacteria/ml and 1.2 x 10⁵ to 1.3 x 10⁵ bacteria/ml in experiments in which oysters were exposed to mercury-reducing or mercury-accumulating *Pseudomonas* spp., respectively.

The results of the experiments are summarized in Table 3. Mercury accumulated by oysters dosed with mercury-volatilizing bacteria was greater by a factor of 1.5 than oysters without added mercury-resistant bacteria. Oysters exposed to mercury-accumulating bacteria were found to accumulate more than two times the concentration of 203Hg compared to the control oysters. A twofold increase in mercury volatilization was observed in experiment 2, compared with the control oysters tested in experiment 1. However, in experiment no. 3 where oysters were exposed to mercury-accumulating bacteria, a 2.5-fold increase in the percentage of mercury recovered in the volatile phase was noted. Approximately 7.3% of the total mercury added to the system was volatilized, even in the absence of mercury-resistant bacteria. This volatilization and the apparent excretion of 203Hg into the feces suggests metabolism of mercury by the oysters or by bacteria associated with the oysters.

There was no significant difference in mercury uptake between the spring and fall oysters as determined by analysis of variance (α = 0.05). Less than quantitative recovery of the tracer was attributed to the adsorption of 203Hg to the inner surface of the aquarium wall, the stainless-steel lid, and the surfaces of the oyster shell and ceramic support, which were relative to all experimental dosings. Total 203Hg recovery, although variable, approached 80%.

During the course of each experiment, it was observed that the total concentration of 203Hg in the water column approached the concentration of 203Hg in the microparticulate fraction (Fig. 2) obtained by filtration of the aquarium water through 0.45-μm membrane filters. Particulate mercury increased most rapidly during the first 24 h, as did the amount of 203Hg volatilized. Mercury volatilization during the initial 24 h most probably reflects a shift in the chemical equilibrium from the aqueous phase to the vapor phase or the ionic to elemental state, accelerated by the action of the gas dispersion tube (Fig. 3).

The relative accumulation of 203Hg in individual oyster tissues is shown in Table 4. The largest amount of accumulated mercury was found in the gills and labial palps. There was an insignificant decrease (α = 0.05) in the relative

| Oyster tissue | Mean percentage of uptake and 95% confidence limits |
|---------------|---------------------------------------------------|
|               | (1) Control | (2) Mercury reducer | (3) Mercury accumulator |
| Mantle fluid  | 0.9 ± 0.8   | 2.8 ± 2.3            | 1.5 ± 0.5               |
| Mantle        | 13.3 ± 7.6  | 26.6 ± 7.4           | 16.4 ± 7.5             |
| Gills         | 50.4 ± 9.0  | 44.2 ± 7.2           | 65.0 ± 8.8             |
| Viscera       | 26.3 ± 11.2 | 30.3 ± 7.8           | 15.7 ± 5.0             |
| Adductor muscle | 2.5 ± 0.9  | 2.7 ± 0.9            | 2.9 ± 0.9              |
| Whole oyster* | 3.1 ± 1.7   | 6.7 ± 3.2            | 12.4 ± 3.7             |

* Average of eight oysters. Underlined values are significantly different from controls.

* Computed on ²⁰³Hg concentration in aquarium at time zero.

| Oyster tissue | ²⁰³Hg (μg)/kg wet tissue, * 95% confidence limits |
|---------------|---------------------------------------------------|
|               | (1) Control | (2) Mercury reducer | (3) Mercury accumulator |
| Mantle fluid  | 3.5 ± 2.2   | 9.7 ± 8.0            | 11.8 ± 7.3             |
| Mantle        | 199.9 ± 173.0 | 511.4 ± 357.8     | 405.4 ± 215.0          |
| Gills         | 647 ± 538.7 | 1747.3 ± 880.7      | 2849.9 ± 1282.0        |
| Viscera       | 216.4 ± 177.7 | 465.2 ± 393.0      | 335.7 ± 210.2          |
| Adductor muscle | 56.1 ± 42.5 | 133.0 ± 129.3       | 161.2 ± 75.5           |
| Whole oyster  | 201.4 ± 183.5 | 312.4 ± 238.0      | 483.0 ± 258.4          |

* Average of eight oysters. Underlined values are significantly different from controls.
in oyster tissue (Table 5), both treatments with mercury-resistant bacteria resulted in higher levels of mercury in all oyster tissues. The concentration of mercury in the gill tissue of oysters receiving the mercury-reducing and mercury-accumulating bacteria was approximately three to four times greater, respectively, than the concentrations of mercury in gill tissue of control oysters. A significant increase ($P < 95\%$) in the whole oyster concentration of mercury was also observed for oysters treated with mercury-accumulating bacteria.

The distribution of mercury in oyster tissue in this study is consistent with the data published by others. Pentreath (13) showed that the stomach and gills were the primary sites of both concentration and exchange of heavy metal nuclides in the mussel, *Mytilus edulis*. Kopfler (9) reported that in oysters continuously exposed to 50 $\mu$g/liter of Hg as HgCl$_2$, mercury was accumulated in the gills at a rate 10-fold higher than any other tissue. Mercury may be accumulated in the gills in the microparticulate or dissolved form via mucoid secretions of the gills.

The data resulting from the short-term, static-flow experiments reported here indicate a highly significant role of bacteria in the mobilization and incorporation of mercury in a simplified food chain. Whole oyster accumulation of mercury was more than doubled in the presence of mercury-resistant bacteria. Increases in gill and labial palp concentrations of mercury were found to be in excess of 200 times greater than the concentration of mercury in the water column, compared with a 60-fold concentration factor in the control oysters. Judging from the increase in $^{203}$Hg in the microparticulate fraction in the water column and the corresponding increase in total viable counts, it can be concluded that bacteria have a demonstrable effect upon mercury accumulation in those food chains that include filter feeding and, possibly, deposit feeding components.

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**LITERATURE CITED**

1. Anderson, A. A., J. M. Anderson, and L. E. Mayer. 1973. System simulation to identify environmental research needs: mercury contamination. *Oikos* 24:231-238.
2. Barber, R. T., A. Vijayakumar, and F. A. Cross. 1972. Mercury concentrations in recent and ninety-year-old benthopelagic fish. *Science* 178:636-639.
3. Cunningham, P. A. and M. R. Tripp. 1973. Accumulation
and depuration of mercury in the American oyster, 
Crassostrea virginica. Marine Biol. 20:14-19.
4. Galtsoff, P. 1964. The American oyster, 
Crassostrea virginica (Gmelin). Fishery Bull. Fish. Wildlife Service 
64:1-40.
5. Holden, A. V. 1973. Mercury in fish and shellfish. A 
review. J. Food Technol. 8:1-25.
6. Jernelov, A., and H. Lann. 1971. Mercury accumulation 
in food chains. Oikos 22:403-406.
7. Klein, D., and E. Goldberg. 1970. Mercury in the marine 
environment. Environ. Sci. Technol. 4:765-768.
8. Klemmer, H., and S. N. Luoma. 1973. Mercury levels in 
marine biota. Project Bull. no. 6. Water Resources 
Research Center, University of Hawaii, Honolulu.
9. Kopfler, F. C. 1974. The accumulation of organic and 
inorganic mercury compounds by the Eastern oyster 
(Crassostrea virginica). Bull. Environ. Contam. Toxi-
col. 11:275-280.
10. Leatherland, T. M., J. D. Burton, F. Culkin, M. J. 
McCartney, and R. J. Morris. 1973. Concentrations of 
some trace metals in pelagic organisms and of mercury 
in Northeast Atlantic Ocean water. Deep Sea Res. 
20:673-685.
11. Parsons, T. R., C. A. Bawden, and W. A. Heath. 1973. 
Preliminary survey of mercury and other metals con-
tained in animals from the Fraser River mud flats. J. 
Fish. Res. Board Can. 30:1014-1016.
12. Peakall, D. B., and R. J. Lovett. 1972. Mercury: its 
occurrence and effects in the ecosystem. BioScience 
22:20-25.
13. Pentreathe, R. J. 1973. The accumulation from water of 
**Zn, **Mn, **Co, and **Fe by the mussel, 
Mytilus edulis. J. Mar. Biol. Assoc. U.K. 53:135-153.
14. Spangler, W. J., J. L. Spigarelli, J. M. Rose, R. S. Flippin, 
and H. H. Miller. 1973. Degradation of methyl mercury 
by bacteria isolated from environmental samples. 
Appl. Microbiol. 25:488-493.
15. Vernberg, W. B., and J. O'Hara. 1972. Temperature-
salinity stress and mercury uptake in the fiddler crab, 
Uca pugilator. J. Fish. Res. Board Can. 29:1491-1494.
16. Vostal, J. 1972. Transport and transformation of mercury 
in nature and possible routes of exposure, p. 15–27. In 
L. T. Friberg and J. Vostal (ed.), Mercury in the 
environment. Chemical Rubber Co. Press, Cleveland, 
Ohio.
17. Weiss, H. V., M. Koide, and E. D. Goldberg. 1971. 
Mercury in a Greenland ice sheet: evidence of recent 
input by man. Science 174:692-694.
18. Westoo, G. 1973. Methylmercury as percentage of total 
mercury in flesh and viscera of salmon and sea trout of 
various ages. Science 181:567-568.
19. Williams, P. M., and H. V. Weiss. 1973. Mercury in the 
marine environment: concentration in sea water and in 
a pelagic food chain. J. Fish. Res. Board Can. 
30:293-296.
20. Windom, H., R. Stickney, R. Smith, D. White, and F. 
Taylor. 1973. Arsenic, cadmium, copper, mercury, zinc 
in some species of North Atlantic fin fish. J. Fish. 
Res. Board Can. 30:275-279.