Behavior of Mercury in Bio-Systems
II. Depuration of $^{203}Hg^{2+}$ in Various Trophic Levels

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Introduction

Interconversion of mercurials by chemical and microbiological processes results in the accumulation of mercury in fish and other aquatic organisms (BURROWS and KRENKEL, 1973). Methylmercury compounds, the form of mercury in fish, appear to arise from microbial action on inorganic or other organic mercury compounds present in sediments (WOOD et al., 1968; VONK and SIJPESTEIJN, 1973; HAMDY and NOYES, 1975). Many published investigations of mercury in biological systems have been primarily directed towards the determination of whole-body Hg^{2+}-residues. A question that immediately arises with respect to the mercury content of an organism is how long the mercury will be present in the system. The biological half-life of a pollutant has been described previously as the time required for 1/2 of the accumulated tissue pollutant to be removed as a result of biological processes (CUNNINGHAM and TRIPP, 1975a). KRENKEL (1973) stated that the biological half-life depends on the environmental conditions, the species of organism and the type of mercurial involved. Although the concept of a biological half-life for a compound has been questioned (LEWIS, 1971) we felt it would be instructive to examine the biological depuration of mercury in selective organisms of a simple model food chain. Factors affecting the biological elimination as well as the retention of $^{203}Hg^{2+}$ following washing were also examined.

Materials and Methods

Trophic systems. Representatives of three trophic levels were used to ascertain the depuration rates of radioactive mercury. These are: cells of *Bacillus licheniformis* representing the lowest trophic level, mosquito larvae (*Aedes aegypti*) and guppies (*Lebistes reticulatus*) as the next highest trophic systems, respectively.

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Isotopic labeling. All experiments were performed using $^{203}Hg^{2+}$ isotope in the form of mercuric nitrate, $^{203}Hg(NO3)2$, or phenylmercuric acetate, $C_6H_5{}^{203}HgOOCCH_3$, obtained from New England Nuclear Corp. (Boston) and International Chemical and Nuclear Corp. (Cleveland), respectively. Cells of B. licheniformis, grown (for 18 h) in fluid thioglycollate were harvested by centrifugation and washed with sterile saline. Approximately 2-3 g of bacterial cells were resuspended (for 48 h) in 60 ml glucose basal salts broth with 110 ng $^{203}Hg^{2+}$ per g medium as $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ (HAMDY and NOYES, 1975) and incubated at 37 C. The labeled cells were then harvested by centrifugation after washing once with distilled water. Eggs of A. aegypti were surface washed with dilute Liqui-Nox[R] and sterilized by immersion in 75% ethanol for 5 min (LEA et al., 1956). The eggs were then transferred to a beaker containing sterile diet (Purina rabbit chow). They were then hatched at 27 C yielding the sterile larvae. Three day old larvae were placed in sterile distilled water containing the desired form of radioactive mercury (22 ng $^{203}Hg^{2+}$/g water). Following exposure for 48 hr at 23 C, the labeled larvae were harvested, washed once with distilled water, and used. Guppies (L. reticulatus) reared in aquarium tanks (27 C) were exposed to the desired radioactive mercury by placing them in a beaker containing 2.2 ng $^{203}Hg^{2+}$/g water. The guppies were incubated for 48 h at 23 C after which time they were rinsed once with distilled water, blotted dry, and used.

Counting system. A known weight of water or sample (bacterial cells, larvae or tissue homogenate of guppies) was placed in a standard scintillation vial containing 10 ml of toluene-based scintillation fluor and counted in a Beckman LS 100 C liquid scintillation. The samples were counted to $\pm$ 1% error and all data were corrected for retention of $^{203}Hg^{2+}$ onto walls of containers and/or volatilization (HAMDY and NOYES, 1975; NOYES et al., 1976) as well as for background and half-life.

Biological depuration. The washed $^{203}Hg^{2+}$ cells (2.5 g) of B. licheniformis, forty-eight $^{203}Hg^{2+}$-labeled 3 day old larvae and thirty-six $^{203}Hg^{2+}$-guppies were each transferred to isotope-free sterile distilled water and incubated for the desired time. The biological depuration of $^{203}Hg^{2+}$ by the bacterial

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cells was studied at different temperatures (4, 23, 37 and 45 C) whereas temperatures of 27 and 23 C were used for the larvae and guppies respectively. During the incubation period, representative samples of labeled bacteria, larvae and guppies, as well as the distilled water, were obtained periodically, weighed and then assayed for their radioactivities. The average of six experiments, reported as % of initial activities, were plotted as a function of time of incubation (days) and then the rate of depuration, k (slope of the line) was calculated. The 50% biological retention time (i.e. biological half-life) of $^{203}Hg^{2+}$ was also determined and reported as the time, in days, required for 1/2 of the radioactivities of $^{203}Hg^{2+}$ (cpm/g) to be eliminated from the trophic system as a result of biological processes.

Elimination of $^{203}Hg^{2+}$ from bacterial cells by washing. B. licheniformis cells (2.5 g) previously labeled with either $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ were washed five times with distilled water. In another experiment the labeled cells were washed five times with 0.05 M sodium phosphate buffer (pH 7.0). The initial activity in cells labeled with $^{203}Hg(NO_3)_2$ was 3.68×10^5 cpm/g wet cells while the initial activity was 3.72×10^6 cpm/g wet cells for bacteria labeled with $C_6H_5{}^{203}HgOOCCH_3$. After washing, the cells were centrifuged and aliquots of cells and supernatant were counted. The retention of $^{203}Hg^{2+}$ in the cells as a function of number of washing was determined and reported as % of initial counts.

Results

Factors affecting the depuration of $^{203}Hg^{2+}$ by various trophic levels

Effect of time, temperature and form of $^{203}Hg^{2+}$. It appears that the biological elimination of radioactive mercury was affected by many factors (Table 1). At 4 C the biological elimination of $^{203}Hg^{2+}$ by the bacterial cells labeled with $^{203}Hg(NO_3)_2$ was slow, as evidenced by the rate of $^{203}Hg^{2+}$ elimination k of -0.44 day^{-1} and by the biological half-life (BHL) of 22.3 days. At other temperatures, 23, 37 and 45 C, the biological excretion rates of $^{203}Hg^{2+}$ were much faster than at 4 C. At 23 C, the value for k was -0.23 day^{-1} and the BHL was 11.8 days whereas at both 37 and 45 C, k was the same (-0.26 day^{-1}) and the values for BHL were

Table 1

Effect of time, temperature and form of $^{203}Hg^{2+}$ on the biological half-life (BHL) and rate of elimination (k) of $^{203}Hg^{2+}$ from various trophic systems. $C_6H_5{}^{203}Hg OOCCH_3$ and $^{203}Hg(NO_3)_2$ represented the organic and inorganic forms used, respectively.

Labeled Trophic System	Temp. C.	BHL (days)		k ($days^{-1}$)[a]	
		Organic	Inorganic	Organic	Inorganic
Bacterial cells	4	11.8	22.3	-0.24	-0.44
	23	11.8	11.8	-0.23	-0.23
	37	4.5	12.5	-0.09	-0.26
	45	6.7	12.1	-0.13	-0.26
Mosquito larvae	27	11.2	4.1	-0.22	-0.08
Guppies	23	7.2	6.5	-0.14	-0.13

[a] Slope of the line

12.5 and 12.1 days, respectively. When bacterial cells were labeled with $C_6H_5{}^{203}HgOOCCH_3$, the depuration rate of $^{203}Hg^{2+}$ at 4 C was much faster (k = -0.24 day^{-1}) and the BHL was 11.8 days, which is almost half that obtained at 4 C. At 37 and 45 C, the biological elimination of $^{203}Hg^{2+}$ was very rapid. The slope of the initial activities as a function of time were -0.09 and -0.13 day^{-1}, respectively, and the BHL of $^{203}Hg^{2+}$ at these temperatures were 4.5 and 6.7 days, respectively.

When the larvae were labeled with inorganic form of $^{203}Hg^{2+}$ and kept at 27 C, the rate of elimination of $^{203}Hg^{2+}$ was rapid as indicated from the k value of -0.08 day^{-1} and the BHL was 4.1 days whereas larvae labeled with $C_6H_5{}^{203}HgOOCCH_3$ exhibited a slower rate (k = -0.22 day^{-1}) and the BHL increased almost threefold.

Guppies labeled with inorganic or organic form of mercury exhibited a fast depuration of their $^{203}Hg^{2+}$. The k value for the $^{203}Hg(NO_3)_2$ was -0.13 day^{-1} and

the BHL was 6.5 days. Again, guppies labeled with $C_6H_5{}^{203}HgOOCCH_3$ showed a similar pattern and the k value was -0.14 day^{-1} and the BHL was 7.2 days.

Retention of $^{203}Hg^{2+}$ by Bacillus licheniformis following washing. Figure 1 depicts the data for retention of $^{203}Hg^{2+}$ by cells of B. licheniformis following repeated washing. Cells labeled with $^{203}Hg(NO_3)_2$ retained 67.3% of the mercury after the first wash using sterile distilled water and the % retention decreased rapidly after each subsequent wash to reach a value of 26.2% after the 5th wash. For cells labeled with $C_6H_5{}^{203}HgOOCCH_3$ and washed with distilled water (Fig. 1), 98.2% of the $^{203}Hg^{2+}$ was retained after the 1st wash out gradually decreased after subsequent washing to reach 34.7% of initial activity after the 5th wash. When cells labeled with either organic or inorganic $^{203}Hg^{2+}$ were washed with 0.05 M Na-phosphate buffer (pH 7.0), most of the radioactivities were retained by the bacterial cells particularly those labeled with $^{203}Hg(NO_3)_2$. However, the greatest elimination occurred after the 4th and 5th washes, respectively. Following the last each, the $^{203}Hg^{2+}$ activities retained by the cells reached an average of 53.6 and 52.2% for cells labeled with inorganic and organic forms of $^{203}Hg^{2+}$, respectively.

Discussion

Several investigators reported that temperature affects the BHL of $^{203}Hg^{2+}$ due to its influence on many physiological and metabolic processes. CUNNINGHAM and TRIPP (1975a) stated that the BHL of mercuric acetate in adult oysters, Crassostrea virginica, is shorter at higher temperature than at lower ones. This was found to be true for bacterial cells labeled with $^{203}Hg(NO_3)_2$ and kept at low temperature (4 C) where the BHL was 22.3 days as compared to 11-12 days for the same bacterial cells kept at 23, 37 and 45 C. Again, increasing the temperature within the range from 23 to 37 or to 45 C significantly reduced the BHL for cells labeled with $C_6H_5{}^{203}HgOOCCH_3$ but not with $^{203}Hg(NO_3)_2$. CUNNINGHAM and TRIPP (1975b) further stated that with higher temperature and increased metabolism, rapid turnover of all tissue constituents including mercury or other contaminants would be expected. PRINGLE et al. (1968) also stated that temperature, salinity, dosage, duration of exposure to a specific metal, as well as physiological condition of the organism may influence heavy metal depuration. FRIBERG and VOSTAL (1972) reported that mercurial compounds can undergo a

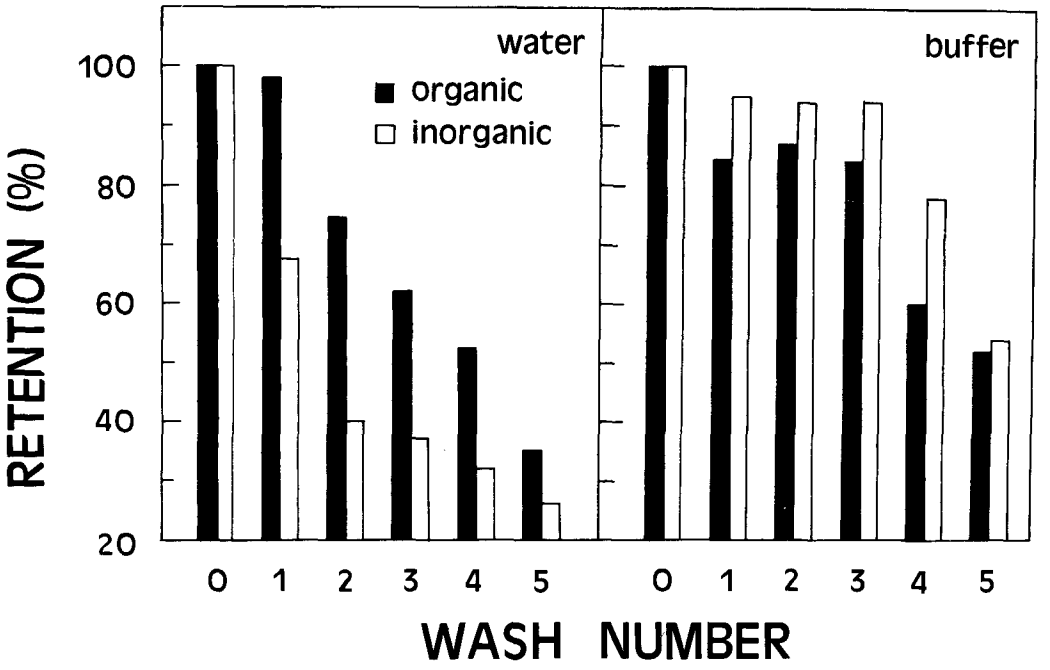

Figure 1. Retention of $^{203}Hg^{2+}$ by bacterial cells labeled with $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ as a function of wash number using either water or Na-phosphate buffer. Average of 3 experiments are reported as % of initial activity.

variety of biotransformations and that organic mercury compounds such as ethyl and phenyl mercuries are biotransformed to the inorganic mercury. NELSON et al. (1973) reported that phenyl mercury acetate may be degraded to elemental mercury vapor by mercury-resistant microorganisms. It is possible that bacterial cells labeled with $C_6H_5{}^{203}HgOOCCH_3$ degraded this compound to elemental (Hg^o), which is volatile, and hence the short BHL of $^{203}Hg^{2+}$ noted for these cells at 37 and 45 C.

For mosquito larvae labeled with $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$, the BHL of $^{203}Hg^{2+}$ were 4.1 and 11.2 days, respectively. BURKETT (1975) stated that the

avidity of organomercurials for the algae Cladophora glomerata resulted in slow excretions of these compounds. YOSHIDA et al. (1967) stated that BHL of $^{203}Hg^{2+}$ in the midgut gland of shellfish exposed to $^{203}HgCl_2$ and methyl mercury chloride was 2.8 days and 4.8 days, respectively, which agrees with our study where $^{203}Hg^{2+}$ from $^{203}Hg(NO_3)_2$ had a shorter BHL compared to $C_6H_5{}^{203}HgOOCCH_3$.

In the present investigation, guppies labeled with $^{203}Hg(NO_3)_2$ had a BHL of 6.5 days at 23 C. WEISBART (1973) found that goldfish (Carassius auratus) injected intraperitoneally with $^{203}Hg(NO_3)_2$ exhibited a BHL of 23.6 days at room temperature. The differences between the two experiments may be related to species-specific differences and to the mode of administration of the $^{203}Hg(NO_3)_2$. However, BACKSTROM (1969) and JARVENPAA et al. (1970) stated that differences in BHL cannot be attributed to the site of Hg^{2+} administration. FANG (1974) found that the BHL of $^{203}Hg^{2+}$ from ethyl mercury chloride in the guppy was 20-23 days. In our study using phenyl mercury acetate, the BHL of $^{203}Hg^{2+}$ was 7.2 days. The discreprancy may be due to differences in the chemical form of $^{203}Hg^{2+}$ used. Fecal excretion may account for the low BHL of the inorganic form of $^{203}Hg^{2+}$ for both larvae and guppies as compared to bacterial cells kept at the same temperature (23 C). It is also possible that the size of the trophic system used as well as the differences in metabolic rates may be other factors to be considered. BARBER et al. (1972) reported that the amount of mercury accumulated in fish appeared to be related to the size and the species of fish.

It should be stated at the present time the chemical form of $^{203}Hg^{2+}$ actually stored within the biological systems used is not known, nor is the form which was eliminated during the biological depuration. However, data obtained from our lab (HAMDY and NOYES, 1975) revealed that the uptake of $^{203}Hg^{2+}$ by cells of Hg^{2+}-resistant Enterobacter aerogenes showed that the movement of $^{203}Hg^{2+}$ from media into the bacterial cells and the distribution of mercury among various cellular fractions of this culture were affected by both incubation time and growth conditions.

The results for the retention of $^{203}Hg^{2+}$ by bacterial cells following washing indicated that when the cells were washed with water, the $^{203}Hg^{2+}$

activity was retained to a greater extent (34.7%) in cells labeled with $C_6H_5{}^{203}HgOOCCH_3$. When washed with 0.05 M phosphate buffer, approximately equal activities were retained by cells labeled with $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$. When washed with 0.05 M phosphate buffer, approximately equal activities were retained by cells labeled with $^{203}Hg(NO_3)_2$ or $C_6H_5{}^{203}HgOOCCH_3$ (53.5% and 52.2%, respectively). The greater retention of PMA by the bacterial cell may be due to strong bonding to the cell. YOSHIDA et al. (1967) studied the effect of four washes with water on $^{203}Hg^{2+}$-labeled cells of Pseudomonas flourescens and stated that transference of mercury might be mainly a kind of chemical bonding to bacterial cell substances instead of mere physical adsorption. GLOOSCHENKO (1969), however, stated that all populations of Chaetoceros costatum accumulated $^{203}Hg^{2+}$ presumably by surface adsorption. BRUNKER and BOTT (1974) showed that mercury appears to be associated with the cell wall, membrane, and intercellular vacuoles of the cytoplasm in a Hg^{2+}-resistant yeast which belongs to the genus Cryptococcus. GIBLIN and MASSARO (1975) stated that organic mercury compounds have a strong tendency to bind to sulfhydryl groups in cells. This may be the reason for the greater retention of PMA by the cells on washing with water. The approximately equal retention values obtained for cells labeled with $^{203}Hg(NO_3)_2$ and $C_6H_5{}^{203}HgOOCCH_3$ on washing with 0.05 M phosphate buffer may be due to complexation of the mercury with the phosphate ions.

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