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A Possible Physiological Uptake Mechanism of Methylmercury by the Marine Bloodworm (*Glycera dibranchiata*)

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The uptake of methyl-mercury by fish has been studied extensively(BURROWS & KRENKEL 1973, OLSON et al. 1973, WOBESER 1975a,b; OLSON & FROMM 1973). There have been some studies on marine invertebrates(VERNBERG & O'HARA 1972, VERNBERG & VERNBERG 1972, KOPPLER 1974, CUNNINGHAM & TRIPP 1973). These studies have been concerned with either the effect of methyl-mercury on viability or methyl-mercury distribution among body parts. The physiological uptake mechanisms of methyl-mercury in aquatic organisms has not been studied. The objective of this paper is to examine the uptake mechanism of methyl-mercury from water in a lower-food-chain organism, the marine bloodworm(Glycera dibranchiata).

MATERIALS AND METHODS

Bloodworms were purchased from a bait dealer in Wiscasset, Maine and shipped by air to our laboratory. Upon arrival, the packaged worms were equilibrated to 13.5°C and later transferred to a gravel bottom aquarium maintained at 13.5°C. A solution of Instant Ocean synthetic sea salt (Aquarium Systems, Inc.) was used to maintain the worms in the aquarium. The sea water employed for all experiments was MBL formula no. 4- Marine Biological Laboratory, pH 7.8 (CAVANAUGH 1964).

Whole bloodworms were exposed to 10 ppb ^{203}Hg in the form of $CH_3{}^{203}HgCl$, purchased from ICN of Irvine, California. A series of finger bowls, each containing two bloodworms, were placed in a water bath at 13.5°C. Uptake of mercury was monitored for four days. An ARMAC scintillation detector was used to make repeated whole-body counts of the ^{203}Hg in the bloodworms.

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Elimination of ^{203}Hg from bloodworms was studied. A half-hour exposure of 10 ppb ^{203}Hg in the form of $CH_3{}^{203}HgCl$ was given to double ligated bloodworms. Following exposure, half the bloodworms were transferred to regular sea water and the other half to sea water containing 40 mM cysteine. The body burden immediately after the half-hour exposure was the initial amount available for elimination. Elimination was monitored periodically for 8 h. Mercury-203 remaining was expressed as a fraction of the initial body burden.

In separate experiemnts, body wall sections were pretreated in either 10 mM parachloromercuriphenyl sulfonic acid(pCMBS), 10 mM N-ethyl maleimide(NEM), or 1 mM 1-fluoro-2, 4-dinitrobenzene(FDNB) in sea water for 60 min. After pretreating, body wall sections were mounted in a flux chamber(SCHULTZ et al. 1967) and exposed to 0.5 mL of 10 ppb ^{203}Hg in the form of $CH_3{}^{203}HgCl$ for 3 min. Paired controls were prepared in a similar manner except they were pretreated with regular MBL sea water for 60 min. After exposure to mercury, body wall sections were rinsed with cold sea water, removed from the flux chamber, digested in Protosol(New England Nuclear), and counted by liquid scintillation.

The partition coefficient of $CH_3{}^{203}HgCl$ in the absence and presence of cysteine was studied. Five milliliters of MBL sea water containing 10 ppb ^{203}Hg was placed in a scintillation vial. Five milliliters of octanol was added. The mixture was capped and placed on a shaker for one h. One milliliter aliquots of both the aqueous and the octanol phase were removed from the vial and each was placed in 10 mL of counting cocktail for liquid scintillation counting. One milliliter of 200 mM cysteine in MBL sea water was added to the remaining experimental mixture to give a final concentration of 40 mM in the aqueous phase. The procedure just described was repeated for the mixture after the addition of cysteine. The partition coefficient for each set of data was determined as follows:

$$\text{Partition Coefficient} = \frac{\text{cpm } ^{203}Hg/mL \text{ in octanol}}{\text{cpm } ^{203}Hg/mL \text{ in sea water}}$$

RESULTS AND DISCUSSION

Uptake of mercury by bloodworms was extremely rapid (Figure 1). This is in agreement with other studies on marine invertebrates(KOPFLER 1974, BURROWS & KRENKEL 1973).

Elimination of mercury from the bloodworm was greatly accelerated(Figure 2). Approximately 70% of the ^{203}Hg present after the half-hour exposure to $CH_3{}^{203}HgCl$ remained following an 8 h elimination period in MBL sea water. In comparison,

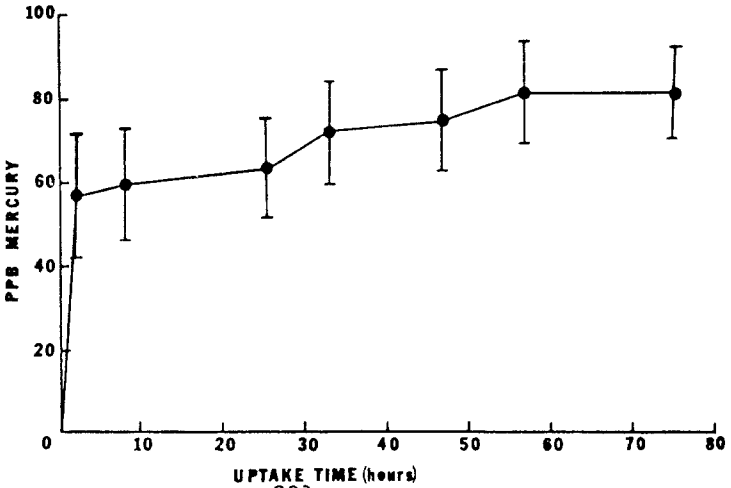

Fig. 1. Uptake of ^{203}Hg by whole bloodworms. Organisms were exposed to 10 ppb ^{203}Hg in the form of $CH_3{}^{203}HgCl$. A rapid uptake of ^{203}Hg by bloodworms is observed(Values are ±S.E.).

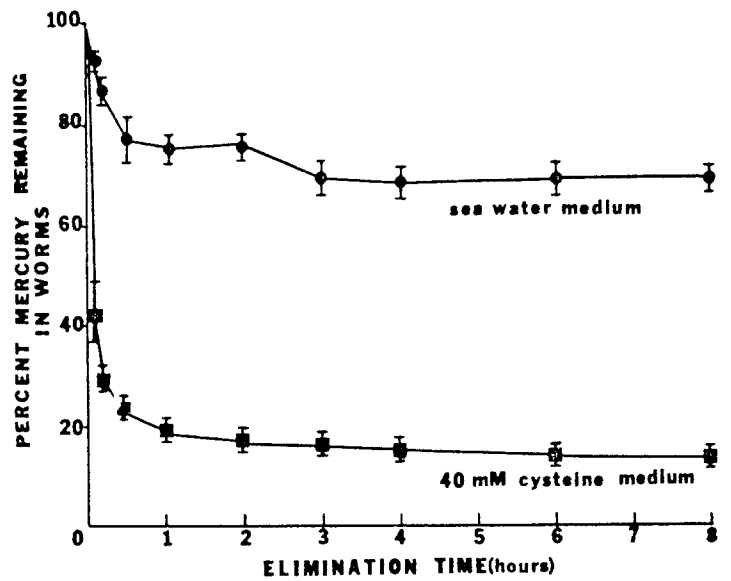

Fig. 2. Elimination of ^{203}Hg from Glycera. Organisms were exposed to 10 ppb ^{203}Hg in the form of $CH_3{}^{203}HgCl$ for a half-hour. The organisms were then incubated in either a sea water medium or a cysteine medium. Mercury remaining after a certain incubation period is expressed as a percent of the initial body burden(Values are ±S.E.).

only 14% remained in the 40 mM cysteine in MBL sea water group. The effect of cysteine suggested uptake of methyl-mercury was due to availability of sulfhydryl groups.

Cysteine was used in the efflux medium because it is a complexing agent for mercury(ROTHSTEIN 1959). Some methyl-mercury accumulated by the organism could be replaced by cysteine. Our original hypothesis was that the increased elimination of mercury in the presence of cysteine was due to the ability of cysteine to remove methyl-mercury that bound to surface sulfhydryl groups by breaking mercury-sulfur bonds.

To test this hypothesis, body wall sections were pre-treated with sulfhydryl inhibitors and then exposed to $CH_3{}^{203}HgCl$ in a flux chamber. We anticipated that these compounds would bind with the available sulfhydryl groups and thus reduce uptake of mercury during subsequent exposure. However, the results(Table 1) failed to show any significant reduction in mercury uptake after pretreating body wall segments with the three sulfhydryl inhibitors used.

TABLE 1

Effect of sulfhydryl blocking reagents on $CH_3{}^{203}HgCl$ uptake by body wall sections.

Inhibitor	N	cpm control ±S.E.	cpm inhibitor ±S.E.	$p \leq 0.05$[a]
pCMBS	12	72 ±13.2	65 ±5	N.S.[b]
NEM	24	21 ±2	30 ±3	N.S.
FDNB	20	16 ±2	12 ±2	N.S.

[a] Statistical significance at 0.05 level

[b] N.S. indicates no statistical significance

An alternative mechanism for initial mercury uptake by body surfaces was lipid solubility. Several workers have demonstrated that the lipid component of an organism is important in influencing the uptake of methyl-mercury(WOOD 1975, ELDER & GAUFIN 1974). Could the enhanced elimination of mercury from body walls by cysteine be explained on the basis of lipid solubility? Partition coefficient experiments conducted with $CH_3{}^{203}HgCl$ utilized octanol, a non-polar solvent to examine the influence of cysteine on the mercury solubility. The reduction in the partition coefficient from 2.0 ±0.1 to 0.025 ±0.003 by the addition of cysteine represented a decrease of 99%. A t-test on the means showed them to be significantly different($p \leq 0.0005$). Cysteine appears to reduce the lipid

solubility of $CH_3{}^{203}HgCl$. Thus the mechanism for initial uptake could be absorption of methyl-mercury that has been solubilized by lipids at or near the external body wall surface.

Our results suggest that the initial rapid uptake phase could be due to the lipid solubility of methyl-mercury in Glycera. However, the partiton coefficient of $CH_3{}^{203}HgCl$ in octanol and water is not large enough to explain uptake solely on the basis of lipid solubility. Thus sulfhydryl groups may play a role in the uptake of methyl-mercury in certain slow uptake components.

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