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Investigations into rates and mechanisms of mercury depuration in the Killifish, Fundulus Heteroclitus

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ABSTRACT

Title of Thesis: Investigations into Rates and Mechanisms of Mercury Depuration in the Killifish, Fundulus heteroclitus

Joanne A. Korpanty, Master of Science, Environmental Engineering, (Toxicology Option), 1984

Thesis directed by: Professor Peddrick Weis, Professor Richard Trattner

Metallothionein (MT) and glutathione (GSH) have been shown in mammalian research to play a role in the sequestration and depuration of mercury. The sulfhydryl groups on both these peptides have a strong affinity for mercury; MT for Hg^{2+} and GSH for CH_3Hg^+. In this study, killifish (Fundulus heteroclitus) were force-fed one piece of squid laced with labelled mercuric nitrate to give a final food concentration of 20 ppm $^{203}Hg^{2+}$ and 0.03 -0.05 $\mu g\ g^{-1}$ Hg^{2+} total dose. Most of the mercury, 84.1 $\pm$ 12.1% (mean $\pm$ standard deviation), is depurated within the first 24 hours; of that retained, most was found in the intestine. There was little change over the next 6 days. The feces contained high amounts of mercury and can be considered the main route of excretion. Thin-layer chromatography of bile showed no noticeable amounts of mercury bound to GSH in the bile. But no conclusions regarding bile and enterohepatic circulation can be made since the killifish is too small a model in which to monitor it. MT was found in appreciable amounts in the liver and intestine homogenates by gel electrophoresis but auto-

radiography did not demonstrate the binding of Hg^{2+} to the MT. Most of the mercury which had been retained was associated with both soluble and insoluble proteins of high molecular weight (> 90,000 daltons). Therefore the first line of defense for this organism appears to be the intestine, as 90% of the mercury did not get beyond this organ.

INVESTIGATIONS INTO RATES AND MECHANISMS OF MERCURY DEPURATION IN THE KILLIFISH, *FUNDULUS HETEROCLITUS*

by
Joanne A. Korpanty

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of Master of Science, Environmental Engineering (Toxicology Option)
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APPROVAL SHEET

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I. INTRODUCTION

Mercury poisoning poses a serious health problem in today's environment. Incidents such as those at Minamata Bay and Niiagata in Japan (1) and sites such as Berry's Creek in the Hackensack Meadowlands demonstrate the need to further investigate mercury pollution and its effects on the environment and animal and human health. Studies with the killifish, Fundulus heteroclitus, may give insight into the poisoning of fish due to mercury pollution, their method of dealing with it and the threat to humans through consumption of mercury contaminated fish.

Mercury contamination and poisoning comes from a variety of sources. It is used in the production of thermometers, electric contacts, flourescent bulbs, dental fillings, mirrors and previously in felt for the hat industry. It then becomes a component of the wastewater generated by these industries and is often discharged into the waterways. Mercury vapor is released by degassing from the earth's crust and the oceans (2) and through the burning of fossil fuels containing mercury (3). In terrestrial animals, including humans, the most common paths of entry are through the respiratory tract in the form of dust or vapor, through the skin or ingestion via the gastrointestinal tract. Mercury enters the body of the fish either through the gills, the G-I tract from contaminated food or through the mucous layer and/or skin.

All forms of mercury are toxic to animals and man, but as compared to metallic mercury and inorganic salts, alkyl-

mercuries, especially methylmercury (CH_3Hg^+), are particularly toxic due to their ability to cross cell membranes. CH_3Hg^+ is naturally formed in the aquatic and terrestrial environment from elemental mercury and mercuric mercury (1). It is the predominant form of mercury in aquatic pollution (4). Methylation is likely to occur in upper sedimentary layers of sea or lake bottoms and the methylmercury formed is rapidly taken up by organisms in the aquatic environment (1). Rudd et al. (5) showed that microflora in the intestine of the fish methylated mercury in the gut. This methylation enhances the lipid solubility of mercury causing it to be more quickly absorbed from the G-I tract, to remain stabile within the body, to easily cross the placental barrier and to be attracted to the nervous system (6).

This attraction causes the central nervous system, particularly the brain, to be the critical target organ of mercury poisoning. Neurological disorders dominate the signs and symptoms of humans exposed to long term toxic doses. With increasing doses, a syndrome known as micromercurialism results. Symptoms such as weakness, fatigue and anorexia appear (3). As exposure levels increase, fine trembling of peripheral parts (such as eyelids and fingers) begins. It is intentional and disappears during sleep. This can continue on to a generalized tremor, severe behavioral and personality changes, increased excitability, loss of memory and insomnia which can develop into depression (2). The term "mad hat-

ter" has its roots in the many cases of mercury poisoning among workers in the hat industry.

Although the mechanism of mercury's action on the CNS is still poorly understood, evidence shows that in almost every toxic action there is a reaction between mercury and sulfhydryl groups with a mercaptide complex formed by the two. As predominant sulfhydryl groups are located on cellular proteins, proteins are the primary targets of mercury (7). The proteins lose their reactive properties through chemical destruction of the vital protein structure due to formation of the complex (3). Therefore protein action and synthesis is interfered with. Also, toxic substance blocking thiol enzyme systems alter the metabolism of nerve endings (3).

In this study, we set forth to investigate the pathways mercury takes once ingested by the killifish. The killifish was chosen as a model for various reasons. A report from the National Academy of Science (1) states that the most effective indicator of mercury pollution and the potential hazard to humans and wildlife is the mercury content of fish. Since the killifish is one of the few animal species surviving in the highly mercury contaminated waters of Berry's Creek, it must have some successful detoxification mechanisms worth investigating. Its size and ease of care in the lab also make it a good model.

The threat to humans from fish comes from the ingestion of mercury contaminated fish. Food products, excluding fish, contain between a few micrograms to 50 $\mu g\ kg^{-1}$ of mercury (8).

This is very little in comparison to that which can be found in fish, which often constitutes a large part of the diet (depending on individuals, ethnic groups and countries.) If 30 g day^{-1} of fish flesh is consumed daily, between 1 and 20 $\mu g\ day^{-1}$ CH_3Hg^+ is ingested, under conditions of uncontaminated fish (2). However, under conditions of contamination, the levels of CH_3Hg^+ intake rise rapidly and cause serious problems, as evidenced by two major incidents in Japan. Minamata Bay and the Agano River in Niiagata were the recipients of the industrial release of mercury compounds (1). The mercury then accumulated in edible fish and shellfish, leading to a median total mercury level in fish in Minamata Bay of 11 $\mu g\ g^{-1}$ fresh weight (9). By 1974 more than 700 cases of CH_3Hg^+ poisoning at Minamata and more than 500 in Niiagata were identified (10-12).

As was previously mentioned, the binding of mercury to sulfhydryl groups of proteins is what has been suggested to cause the toxic actions of mercury. However, the binding of mercury to appropriate sulfhydryl groups can also help to detoxify the mercury. There is an affinity constant of CH_3Hg^+ for thiol groups of $\log_{10}K=17$ (13). Usually the major sulfhydryl containing compound in the cell is glutathione. Glutathione (GSH) is a tri-peptide (L -γ - glutamyl - L - cysteinyl glycine) and is known to function either directly or indirectly in many important biological functions. It is an essential cellular component. It is synthesized intracellularly and in mammalian cells is typically found at the milli-

molar level in the cell. Glutathione disulfide (GSSG) is formed upon oxidation of the thiol groups of the cysteine moiety. The regeneration of GSH from GSSG is accomplished by NADPH. Intracellular GSH is normally over 99% reduced and GSH is the major transport form (14).

Binding of CH_3Hg^+ to GSH occurs through the sulfhydryl groups on cysteine. GSH conjugates have the necessary physicochemical properties to be excreted into the intestine via the bile (15). GSH is present in the bile in relatively high concentrations. Refsvik and Norseth(16) found that in rats exposed to CH_3HgCl the predominant resulting methyl mercuric compound found in the bile is methyl mercuric glutathione. Ballatori and Clarkson (17) showed a decrease in CH_3Hg^+ retention in neonatal rats with an increasing ability of the developing liver to secrete GSH into bile. A CH_3Hg-GSH complex has been identified in liver (18) and agents that deplete the hepatic content of GSH inhibit the biliary secretion of CH_3Hg^+ and simultaneously decrease the GSH content of bile (19).

Metallothioneins (MT) are small (6000 - 7000 daltons) proteins low in aromatic amino acids and rich in cysteine, containing thiol groups. They are also capable of binding heavy metals via the sulfhydryl groups. MT's primary function is thought to be in the homeostasis of essential trace elements such as zinc and copper (20). MT synthesis probably occurs in several tissues but is especially significant in the intestinal wall and liver. It is usually found in low concen-

trations but is induced by several heavy metals, including zinc, copper, silver, cadmium and mercury (21). Higher concentrations have been found in animals from metal-rich habitats. MT can protect the organs against injuries caused by mercury absorption (22) by partitioning them away from potential sites of toxic action. Noël-Lambot et al. (23) reported that 35% of the Zn and over 60% of the cytosolic Cu are associated with the MT pool of eel liver tissue. Mercury will displace any other metal bound to the sulfhydryl groups of MT (24).

It is thought that the response of the organisms to heavy metal exposure involving MT is a three stage process: first the binding of the metal to MT, the induced synthesis of more MT to sequester additional metal and finally, since the induction of new protein and binding of metal is finite, "spillover" occurs, in which heavy metal ions are released into the cytosol where they can exert toxic effects and the toxic threshold of the mechanism is reached (25).

Once the mercury has bound to a thiol, it must be excreted in some way. There are various excretion pathways and mechanisms which will bring about elimination of the metal. The excretion of CH_3Hg^+ by the fish is most commonly accomplished through defecation. The rate is determined by three factors: (1) biliary excretion, (2) shedding of the intestinal epithelium and (3) reabsorption; in the rat, biliary excretion is the main route (26). Ballatori and Clarkson (27) showed in rats that there is a close linear corre-

lation between the biliary secretion rates of GSH and CH_3Hg^+. This, the high association constant for the CH_3Hg cation with GSH ($K_1=10^{15.8}$) (28) and the fact that a CH_3Hg^+ complex has been identified in rat bile point to the suggestion that CH_3Hg^+ transport into the bile is determined by the biliary transport system for GSH (26).

However, since the gall bladder secretes bile into the intestine, there is the factor of reabsorption through the intestinal wall to consider. Some of the GSH-CH_3Hg^+ conjugates will be excreted with feces, especially if the mercury exposure was through ingestion. Norseth and Clarkson (26) found that although mercury in bile is most commonly found as CH_3Hg-cysteine it is rapidly reabsorbed. They go on to suggest that CH_3Hg^+ may bind to structural proteins of exfoliated epithelial cells in the lower intestine and undergo biotransformation to inorganic mercury, thus allowing for fecal excretion.

Another possible process of elimination that has been speculated upon is through intestinal corpuscles. White mucous corpuscles, high in calcium and magnesium, have been observed in the intestinal lumen of fish and have been found to bind heavy metals. These corpuscles are regularly evacuated, thereby limiting the entry of metals through the intestinal wall (23).

Thus, it is important to understand the role mercury plays in fish. This study of mercury and the killifish will give information leading to the ability to predict the depuration

rates for mercury in fish and thereby lead to the possibility of predicting the time between clean-up of a mercury contaminated area and its reopening for fishing for safe human consumption.

II. MATERIALS AND METHODS

Killifish (3-5 grams in weight) brought from a relatively unpolluted estuary (Shark River outlet in Belmar, NJ) were used. They were caught in fish traps using cat food as bait. One piece of squid injected with $^{203}Hg(NO_3)_2$ (New England Nuclear, Boston MA) to give a concentration of 20 ppm $^{203}Hg^{2+}$ was force fed to each fish. A total dose of 0.03-0.05 $\mu g\ g^{-1}\ Hg^{2+}$ per fish resulted. Total body counts (Beckman Gamma 4000 Counter) of each fish were taken at 1, 3, 5, 7 and 24 hours, and daily for 7 day studies, after food ingestion. The fish were sacrificed by decapitation at appropriate times and the liver, gall bladder and intestine were quickly dissected out. They were kept on ice to prevent autodigestion. Each organ was weighed and a total organ count was taken. The intestine was rinsed out with an iso-osmotic saline solution prior to counting.

A total of nine separate studies were run using 6 fish in each study with a total of 54 fish used. Fish were sacrificed at 3, 5, 7, 24 hours and at 6 and 7 days. Replications of each study were run to give confident results. Data from replicated studies were combined and means and standard deviations figured for each.

Gall bladders were combined due to small size and 0.5 ml 70% n-propanol was added in preparation for thin-layer chromatography. 0.5 µl dithiothreitol (DTT) was added as a reducing agent for GSH analysis.

The livers and intestines were homogenized in a 0.1M

PO_4 and 0.25M sucrose solution. The homogenates were centrifuged for 20 minutes at 18,300 rpm (= $40.3x10^3$ x G). Counts were taken of each subsequent fraction (pellet = membranes, supernatant = cytoplasm and surface = lipid.) The supernatant fraction was heated for one minute in a 70°C water bath for the denaturation of enzymes and other heat-labile proteins. After cooling in ice, it was centrifuged for 15 minutes at $20x10^3$ x G. These steps correspond to the initial steps in the MT isolation procedure (29). Counts of the fractions were again taken.

1. Thin-Layer Chromatography

Thin-layer chromatography was run on combined gall bladders. Eastman Chromagram Sheets layered with 100 μ cellulose were used. The sheets were developed at room temperature in the solvent system n-propanol/H_2O 70:30 (16). The spots were localized by spraying ninhydrin reagent. Reference compounds of GSH, GSH-CH_3Hg and cysteine were run with each chromatogram.

2. Protein Estimation

Estimation of protein was determined by the Lowry method (30) on the fractions before and after heat treatment. This was to determine amounts of each fraction to apply to gels. Standards of bovine serum albumin (1 mg ml^{-1}) were used and an equal amount of sodium dodecyl sulfate (SDS) was added to both the standards and the blanks.

3. Gel Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was run on the

sediment and soluble fractions of the liver and intestine homogenates both after homogenization and after heat-treatment. A 3% stacking gel and 15% resolving gel were used. SDS was added to break the disulfide linkages of the proteins and render them anionic. DTT was added as a reducing agent. The gels were stained in a 0.2% Coomassie Brilliant Blue in 50% methyl alchol and 7% acetic acid solution. Molecular weight markers were run with each gel. No more than 100 µl of protein were applied to each well.

The gels were dried on a Hoefer Slab Gel Dryer and scanned on a Hoefer Scanning Densitometer.

4. Autoradiography

Thin-layer chromatograms and dried gels were placed against x-ray film (Kodak SB-5) for a suitable time of exposure (2-4 weeks) in a cold room. They were developed in Kodak GBX and scanned on a Hoefer Scanning Densitometer.

III. RESULTS

1. Depuration

Counts of the whole fish taken hourly after feeding show that only 15.9 ± 12.1% (mean ± standard deviation) of the dosed ^{203}Hg remains in the fish 24 hours after feeding. (See Figure 1) The other 84% is excreted, probably primarily through the feces. After 24 hours there is a drop in the body burden of ^{203}Hg of approximately 4% of the original amount in the next 24 hours and at 72 hours an additional decrease of approximately 3%. For the remaining 4 days, the body burden remains fairly constant. (See Figure 2).

Mercury content in feces was determined periodically throughout each study. There was a relatively high content of mercury in the feces, both soon after feeding when the fecal matter was high in organics and later on when the feces were white and sac-like (possibly intestinal corpuscles.)

2. Distribution in Organs

The highest concentration of ^{203}Hg was found in the intestine. (See Figure 3) There is a sharp decrease at 7 hours but then the intestinal content again increases. The liver (Figure 4) also shows a gradual increase in ^{203}Hg content after 5 hours, although the total content is much less than that found in the intestine. The gall bladder content remained fairly constant for the 7 day period and was approximately 0.1% of the total body content.

3. ^{203}Hg and GSH

The thin-layer chromatograms on bile from the combined

Figure 1

Total body retention of ^{203}Hg during the first 24 hours after feeding. Values are means of four to six fish at each time interval. The one hour reading is arbitrarily taken as 100%. The means and standard deviations are given in Table 1 in Appendix 1.

Figure 1

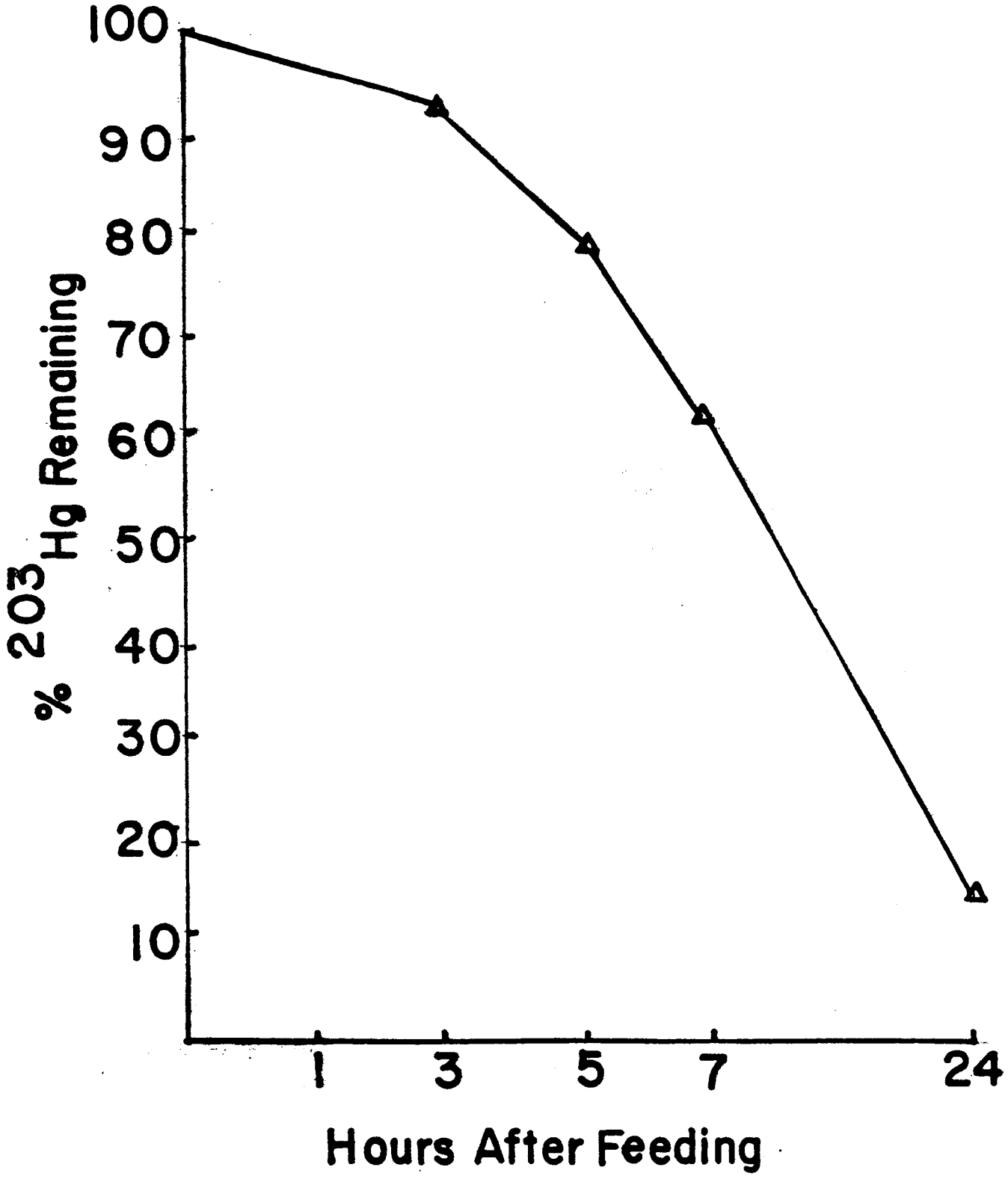

100
90
80
70
60
50
40
30
20
10
% 203Hg Remaining
1
3
5
7
24
Hours After Feeding

Figure 2

Total body retention of ^{203}Hg during 7 days after feeding. Values are means of four to six fish at each time interval. The one hour reading is arbitrarily taken as 100%. The means and standard deviations are given in Table 1 in Appendix 1.

Figure 2

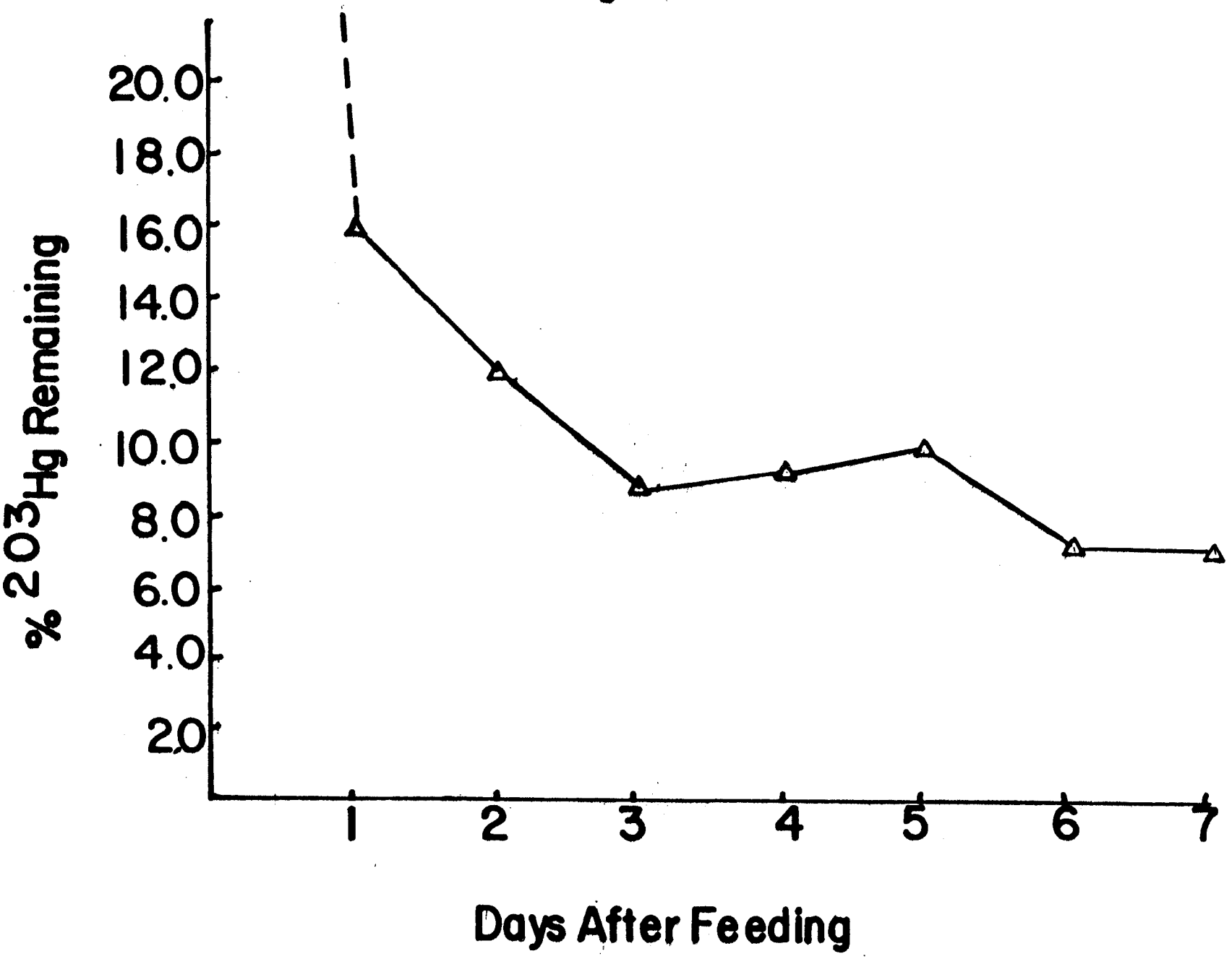

20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
% 203Hg Remaining
1
2
3
4
5
6
7
Days After Feeding

Figure 3

Intestinal retention of ^{203}Hg as compared to the total body burden of ^{203}Hg in 7 days after feeding. Values are means of three to six fish at each time interval. Results are expressed as the percentage of body burden at time of autopsy. The means and standard deviations are given in Table 2 in Appendix 1.

Figure 3

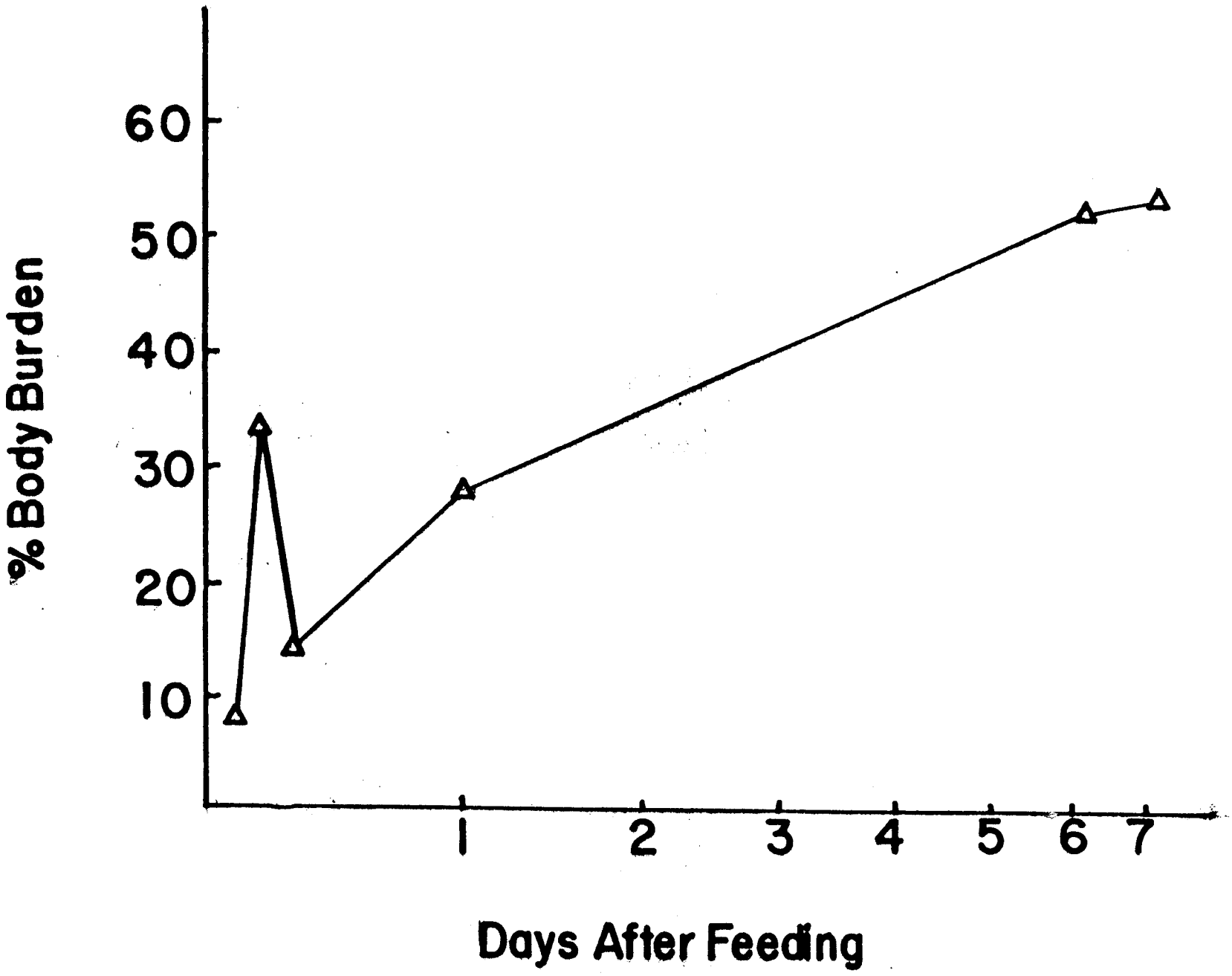
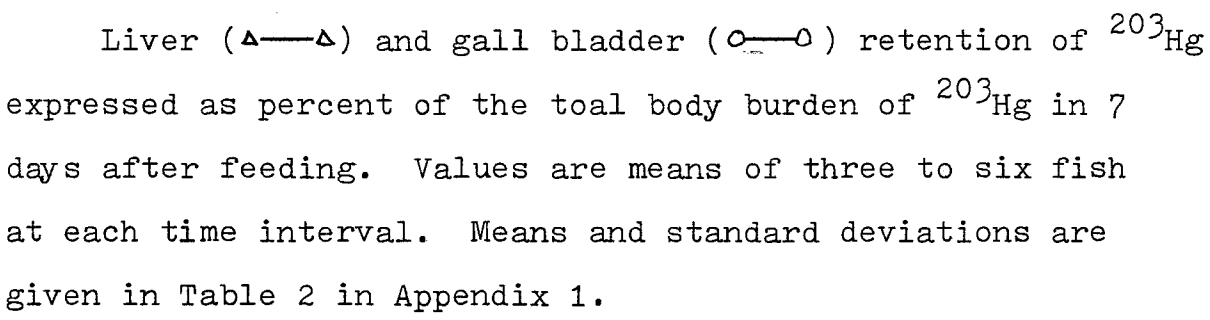
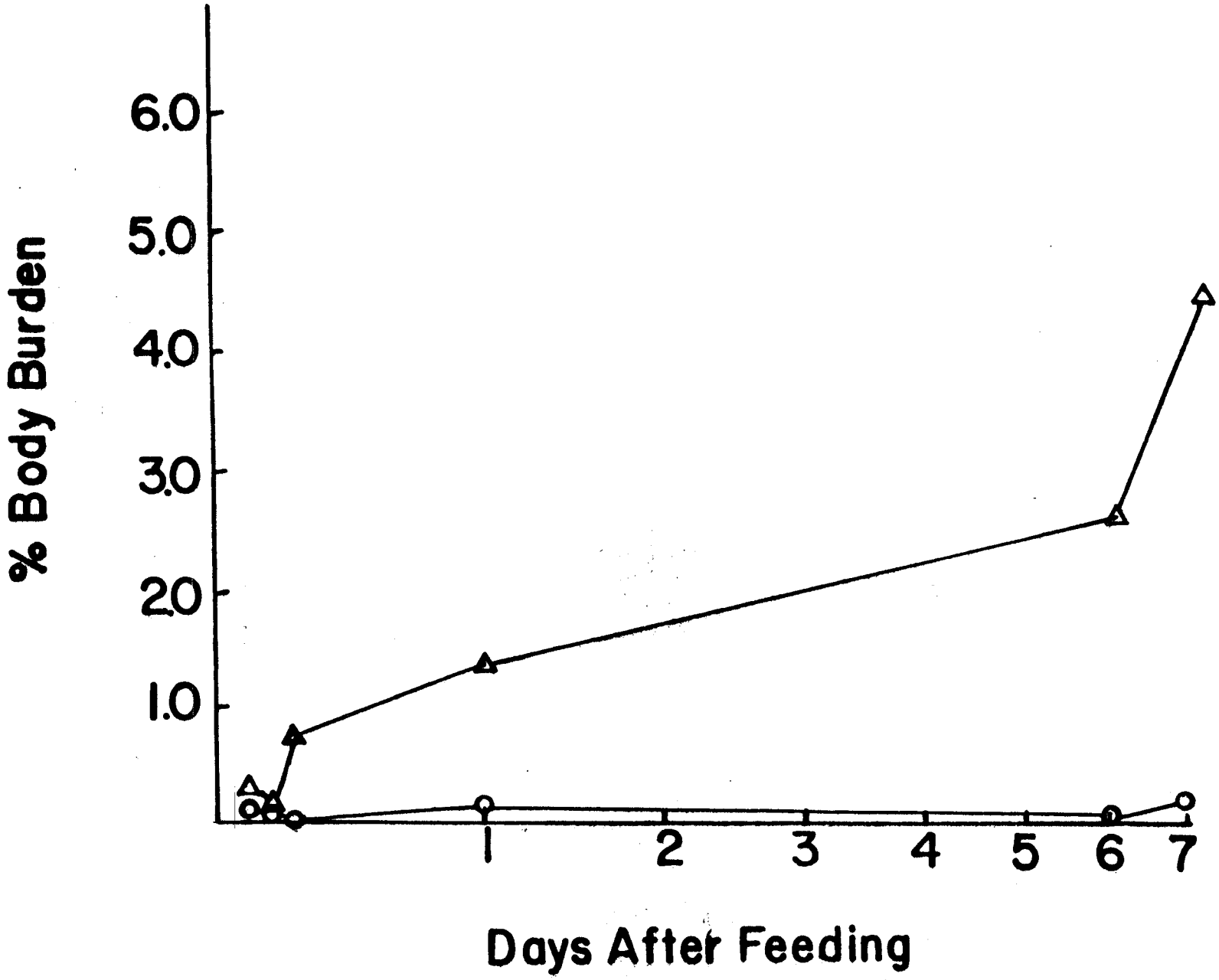


Figure 4



Liver (Δ——Δ) and gall bladder (O——O) retention of ^{203}Hg expressed as percent of the toal body burden of ^{203}Hg in 7 days after feeding. Values are means of three to six fish at each time interval. Means and standard deviations are given in Table 2 in Appendix 1.

Figure 4



gall bladders showed the presence of GSH and cysteine. However when autoradiography was performed on these chromatograms no exposure from ^{203}Hg was seen at these points. There was also no exposure at any points along the migration of bile (although a small amount was seen at the point of origin.)

4. ^{203}Hg and MT

Gel electrophoresis on the soluble and sediment fractions of the homogenate and the heat-treated samples produced some interesting results. MT should particularly be found in the heat-treated soluble fractions of the intestine and liver. This was not the case, however. Distinct bands corresponding to MT were seen with each fraction of liver and intestine. Densitometric scanning of the gels showed high peaks of Coomassie Blue stained material (protein) at the levels corresponding to the molecular weight marker of 14,000 daltons (See Figures 5 and 6.) Gel electrophoresis showed the MT band at the level of 14,000 daltons, caused by the dimerization of MT. Although the molecular weight of MT is around 7,000 daltons, the dimeric form resulting from post-mortem changes raises the weight to around 14,000 daltons (31).

Interestingly, the homogenate soluble fractions of the intestine show a concentration of high molecular weight proteins at the beginning of and in the stacking gel and at the start of the migration into the resolving gel. This indicates the presence of insoluble membranous material.

When the gels were subjected to autoradiography there

Figure 5

Gel electrophoresis of liver and intestinal homogenates. Arrows MT indicate the 14,000 dalton level at which MT bands appear. Arrows AR indicate areas which came up positive on autoradiograms of gels, showing binding of ^{203}Hg.

Lane 1 Intestinal heat-treated

Lane 2 Intestinal homogenates

Lane 3 Liver heat-treated

Lane 4 Liver homogenates

Lane 5 Molecular weight markers

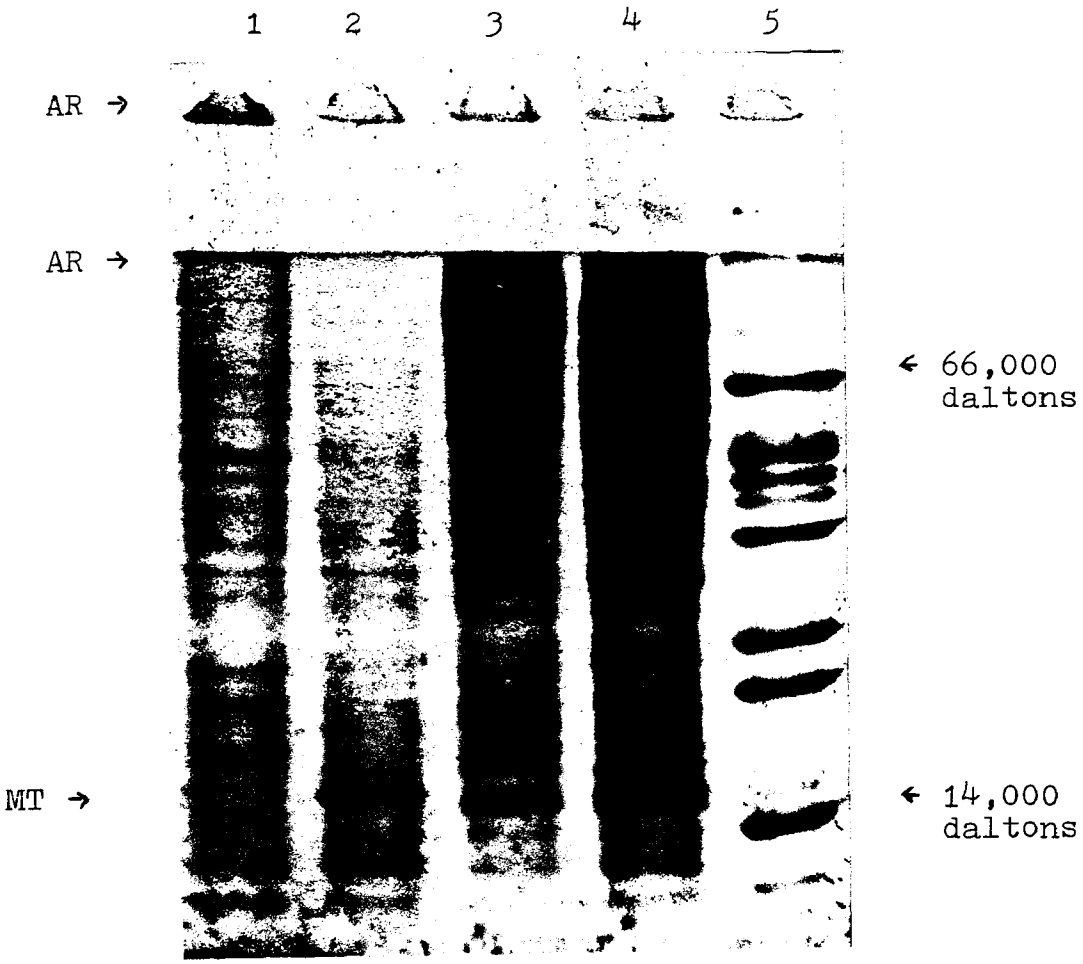
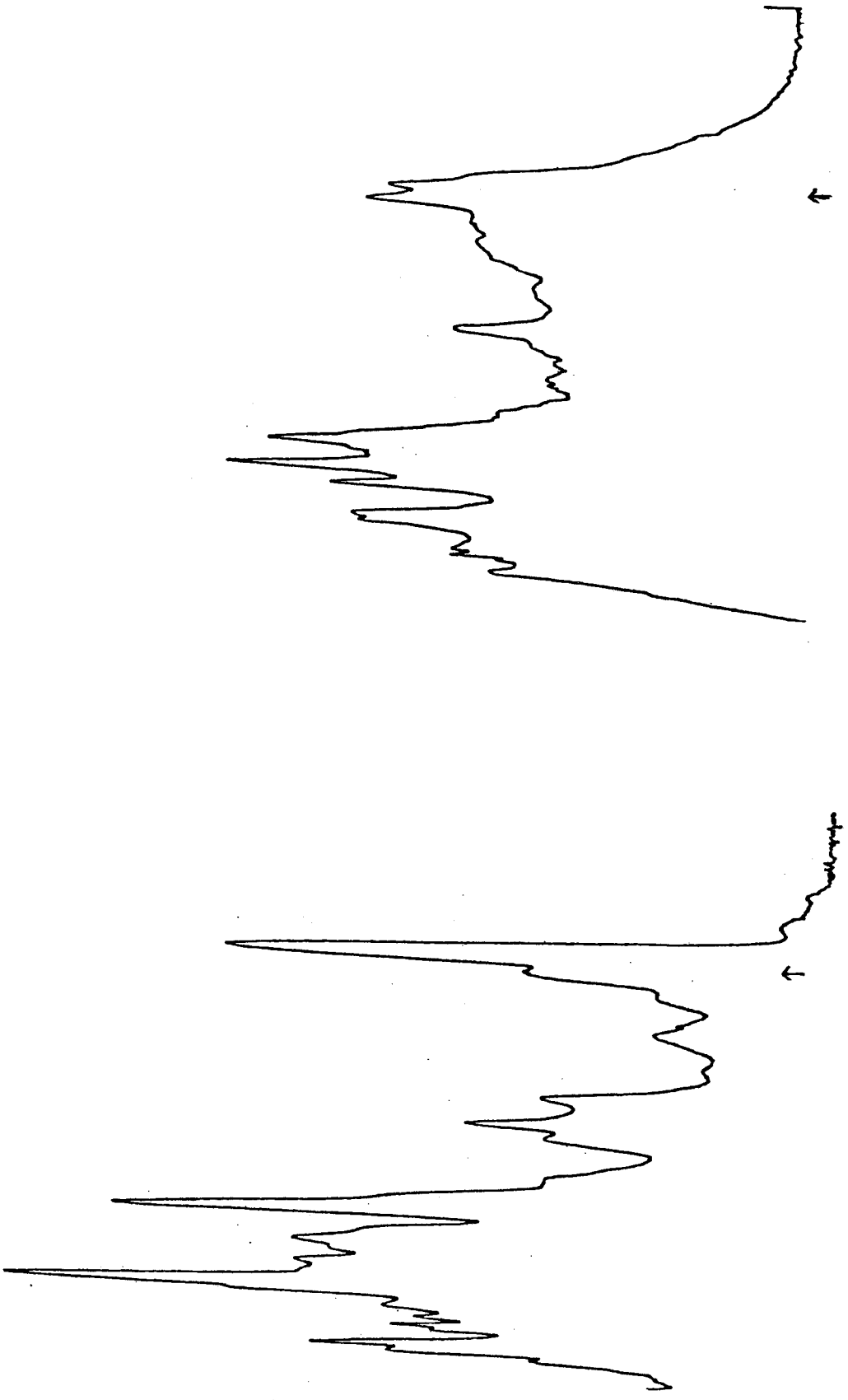

1
2
3
4
5
AR →
AR →
MT →
← 66,000 daltons
← 14,000 daltons

Figure 6

Densitometric scanning of gel electrophoresis of liver (left) and intestine (right) homogenates of fish. Migration of gel is from left to right with molecular weight decreasing from left to right. Arrows indicate the 14,000 dalton level at which peaks of MT can be seen.



was little or no exposure at points corresponding to the MT bands. Most of the mercury is associated with the insoluble membranous material that was not able to migrate into the gel, particularly with the homogenate soluble fractions.

IV. DISCUSSION

The results of this study provide some interesting facts about the depuration of mercury in the killifish. The gall bladder, although not appearing to play a large role, could nevertheless be important in depuration, as demonstrated by Refsvik and Norseth (16). A small amount of mercury was found in the gall bladder, but considering the size of the organ (the average weight was 10 mg) it might be deemed important. The turnover of bile in the gall bladder is constant and rapid, so that at the time of autopsy only one datum is obtained. The constant counts per minute obtained demonstrate this constant turnover without storage. Enterohepatic circulation (intestine → liver → gall bladder → intestine) is an important and active phenomenon. Norseth and Clarkson (26) demonstrated that in rats there is selective reabsorption of methylmercury excreted in bile while inorganic mercury is problably completely excreted in feces. However, there are considerable species differences in the amount of bile to which mercury binds, even among rats and mice (32). But no conclusions can be made regarding bile and enterohepatic circulation from this study as the killifish is too small a model in which to monitor it (weights were 3-5 g).

The liver showed an increase in mercury content as time after feeding went on. This may be caused by the mercury that is absorbed through the intestinal wall then entering the liver via the enterohepatic circulation system.

Most of the mercury remains in the intestine. Very little penetrates the intestinal wall, but that which does has a long residence time in the body as shown by Figure 3. Bäckström (33) found that after ingestion of methylmercury nitrate by salmon the gastrointestinal mucosa was seen to be one of the organs with the highest concentration of mercury. The gut lumen and walls have the highest level of divalent heavy metals as compared to other soft tissues after ingestion of contaminated food (34-36).

Farmanfarmaian and Socci (37) showed that in an in vitro assay $CH_3{}^{203}Hg^+$ penetrates Fundulus intestinal tissue surface rapidly and accumulates within the tissue exponentially over a 60 minute period (as opposed to $^{203}Hg^{2+}$ which rapidly binds to the tissue surface at high concentrations but only weakly penetrates the tissue over the same time period.) This is ascribed to the lipophilic properties of CH_3Hg^+. The more lipophilic a compound the more rapidly it can penetrate the lipid bilayer of the cell membrane. The longer residence time of mercury in the intestine may also be due to the binding of CH_3Hg^+ to negative charge sites on the mucosal side of the gut (37).

However, Farmanfarmaian and Socci used an aqueous solution of $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$ and hence got an immediate response. In this study, dosed food was used and therefore digestion had to first take place before $^{203}Hg^{2+}$ could be available. It is possible that Hg^{2+} binds to the protein of the meal and is then transported into the intestine concommitant with

amino acid absorption. Or Hg^{2+} could be cleaved from the amino acids, methylated by intestinal flora and then absorbed into the intestine.

Intestinal cells may temporarily retain mercury in either form, and then reexcrete it via several proposed mechanisms. Mucosal epithelial cells in the intestine containing MT-Hg complexes or Hg-large protein and CH_3Hg-membrane complexes are desquamated and excreted. These cells mix with the fecal content, causing the high mercury content found in the feces. The insignificant levels of MT bound-^{203}Hg makes this route involving MT unlikely as a significant route. The latter route (involving large protein and membrane complexes) is more probable; accumulation prior to desquamation could account for the 5 hour (temporary) rise in the intestinal Hg content.

Lysosomes, as suggested by Weis and Weis (38), may also act in the excretion of ^{203}Hg from the intestine. Lysosomes are membrane-bound cytoplasmic bodies containing hydrolytic enzymes that function in cell metabolism. Substances taken into the cell by pinocytosis or phagocytosis are digested by lysosomes. Insoluble materials may be stored in lysosomes indefinitely or may be removed from the cell by exocytosis of the lysosome. Fowler et al. (39) discussed the compartmentalization of cations in lysosomes. George and Pirie (40-41) found that large lysosome-like vesicles in Mytilus kidney which contain 38% of the body burden of Cd^{2+} were shed into the lumen of the kidney.

Noël-Lambot (23) demonstrated the presence of carbonate-

rich intestinal corpuscles that bind cadmium and some other heavy metals very rapidly. From intoxicated fish, Anguilla anguilla, the intestinal corpuscles contained a large fraction of the total cadmium content in the fish. These corpuscles were regularly evacuated.

Although Rudd et al. (5) and Bäckström (31) found that fish methylated most of the mercury ingested, Clarkson (42) found that in rats the carbon-mercury bond of CH_3Hg is apparently split by cellular processes and by certain microorganisms present in the intestine. Burrows and Krenkel (43) presumed that demethylation of methylmercury occurred in the liver of the blue-gill sunfish and it has also been shown that demethylation occurs in both whole body samples and gill tissue of Fundulus (44).

This may not be the case in this study as the mercury did not bind to MT. MT will bind preferentially with a divalent ion. Mercury as Hg^{2+} would bind to it, but mercury in the intestine is presumed to be predominantly in the methylated form; hence no significant amount or radioactivity was associated with the MT.

It was found that much of the mercury retained was bound to both large soluble and insoluble proteins. When ^{203}Hg was given to rainbow trout, chromatography of cytosol fractions showed most of the mercury to be associated with fractions of high molecular weight (>75,000 daltons) in all tissues. However, the liver cytosol did have appreciable association of methylmercury with MT (45). These authors concluded that MT does not account for the majority of mercury

bound in the soluble fractions.

Roesijadi and Hall (46) described a protein in studies with the mussel Mytilus edulis that was isolated from the gills which was twice the size of MT and contains relatively little cysteine. This protein bound mercury but contained aromatic amino acids and histidine, not characteristic of MT.

Weis (47) found that MT is not a significant factor in mercury tolerance. Only trace amounts of mercury found in the cytosol fractions of Fundulus livers were associated with MT. Most is associated with larger proteins and approximately 25% is dialyzable. Norseth and Clarkson (26) postulated that in rats injected with labelled methylmercury chloride most of the fecal mercury was bound to water-insoluble structural proteins.

The killifish in this study demonstrated a relatively fast rate of depuration of mercury. Within 24 hours more than 80% of the ingested mercury was eliminated. This is in contrast to the half-life of methylmercury given orally to four species of fish which varied between 25 and 600 days (48). This may be attributed to a possible higher basal metabolism in the killifish, less efficient absorption or to the type of administration. This other study involved aqueous doses of Hg^{2+} and CH_3Hg^{+}, which therefore did not involve digestion. It should be noted that the common mode of uptake of mercury in fish is through contaminated food.

In conclusion, one can see that the mercury given orally to killifish does not bind significantly to two peptides,

GSH and MT, which have been shown in mammalian research to act as detoxifying mechanisms against mercury. However, some mechanism is acting to prevent the uptake of mercury from the intestine. Using the killifish as a model one could say that mercury is not a major concern to the fish since most of it is excreted. Differences between fishes must be taken into account and further research must be done on different families of fishes, particularly edible fish, before one can make conclusions as to the threat to humans by consumption of fish once contaminated by mercury.

APPENDIX I

Table 1

Percentage of Mercury Remaining in the Body After Ingestion of ^{203}Hg Laced Squid*

Time After Feeding	% Mercury
3 hours	93.22 ± 8.9
5 hours	79.88 ± 21.8
7 hours	60.78 ± 28.6
24 hours	15.93 ± 12.1
2 days	11.76 ± 5.06
3 days	8.85 ± 4.47
4 days	8.97 ± 7.22
5 days	9.35 ± 4.20
6 days	7.57 ± 3.25
7 days	7.60 ± 3.40

*Table values are given as means ± standard deviation. Results are expressed as percent of a one hour reading, arbitrarily taken as 100%.

Table 2

Mercury Distribution in Gall Bladder, Liver and Intestine As Percentages of Total Body Mercury Content*

Time After Feeding	Gall Bladder	Liver	Intestine
3 hours	0.144 ± 0.160	0.280 ± 0.56	7.9 ± 1.9
5 hours	0.075 ± 0.067	0.095 ± 0.10	32.4 ± 42.7
7 hours	0.042 ± 0.002	0.796 ± 1.55	14.7 ± 8.9
24 hours	0.132 ± 0.133	1.315 ± 1.40	28.1 ± 27.6
6 days	0.064 ± 0.024	2.788 ± 1.05	52.9 ± 36.4
7 days	0.171 ± 0.123	4.416 ± 1.21	53.6 ± 24.9

* Table values are given as means ± standard deviation.

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