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Ecological Research Series

Pharmacokinetics of Toxic Elements in Rainbow Trout

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EPA-660/3-74-027
December 1974

PHARMACOKINETICS OF TOXIC ELEMENTS IN RAINBOW TROUT

I. Uptake, Distribution and Concentration of Methylmercury by Rainbow Trout (*Salmo gairdneri*) Tissues

II. The Mechanism of Methylmercury Transport and Transfer to the Tissues of the Rainbow Trout (*Salmo gairdneri*)

No. 3

by

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Program Element 1BA022

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ABSTRACT

^{203}Hg-methylmercury (MeHg) was administered intragastrically in a single dose (0.5 mg Hg/kg=3.3μCi/kg) to trout (av. wt. 250g). The fish were sacrificed from 1.0 hr. to 290 days postadministration. Twenty tissues were analyzed for MeHg content by gamma scintillation spectrometry. The label was taken up rapidly by blood, gills, spleen, liver and kidney and more slowly by muscle, brain and lens. After 290 days: (a)blood, spleen, kidney, liver and lens had concentration factors (C.F.=tissue Hg conc./Hg dose x final tissue wt./ initial tissue wt.)=1.0;(b)in general, C.F.s had dropped off by at least 2/3 from their maxima except for muscle, brain and lens in which the C.F.s remained=their maxima; (c)~64% of the dose still remained in the fish and skeletal muscle (comprising ~55% of body weight) contained >40% of the residue. Assuming MeHg excretion to be a first order process, there are at least two rates of excretion--a rapid initial rate resulting in a biological half-retention time (HRT) of ~200 days and a slower, subsequent rate yielding an HRT of >1000 days which, apparently, is governed by the rate of release of MeHg from the skeletal muscle.

Hemoglobin (Hb) is the main methylmercury (MeHg) transport protein in trout blood. *In vitro*, MeHg is taken up rapidly in 3 minutes by red blood cells. MeHg binding in the RBC is reversible *in vitro* as demonstrated by the efflux of Hg from

RBCs suspended in protein solutions. MeHg binding in the RBC also is reversible *in vivo* as gel filtration chromatography of liver soluble proteins yielded identical elution profiles for MeHg administered as the free salt or bound in RBCs. The number of reactive sulfhydryl (-SH) groups per molecule of Hb was found to be 4 by amperometric titration with MeHgCl. The reactive -SH concentration in the RBC was calculated to be at least 20mM. A mechanism for the efflux of MeHg from the RBC is proposed involving the dissociation of MeHg from Hb initiated by -SH groups outside the RBC and migration of MeHg across the membrane as MeHgCl.

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CONTENTS

Section		Page
I	Conclusions	1
II	Recommendations	3
III	Uptake, Distribution and Concentration of Methylmercury by Rainbow Trout (*Salmo gairdneri*) Tissues	4
	Introduction Materials and Methods Results and Discussion	
IV	The Mechanism of Methylmercury Transport and Transfer to the Tissues of the Rainbow Trout (*Salmo gairdneri*)	14
	Introduction Materials and Methods Results and Discussion	
V	References	28

FIGURES

No.		Page
3-1	Relative Abilities of Trout Tissue to Concentrate MeHg	7
3-2	MeHg Uptake and Elimination Pattern of Blood, Heart and Gonads	8
3-3	MeHg Uptake and Elimination Pattern of Blood, Brain and Lens	9
3-4	MeHg Uptake by Skeletal Muscle	11
3-5	MeHg Dose Contained in Total Weight of Each Tissue	12
4-1	The in vivo Removal of $Me^{203}Hg$ from Washed, Intact, Rainbow Trout RBCs by Protein Solutions	21
4-2	The Decrease in Hg Concentration Factor in Rainbow Trout Whole Blood after the Intracardiac Injection of $Me^{203}HgCl$ (0.5mg Hg/kg body weight)	22
4-3	Gel Filtration Elution Profiles of Rainbow Trout Soluble Liver Proteins 8 Days after Intracardiac Injection of $Me^{203}HgCl$ (0.5mg Hg/kg body weight)	23
4-4	Determination of the Number of Sulfhydryl Groups of Dialyzed Rainbow Trout Hemolysate (0.40×10^{-3} mmoles) by Amperometric Titration with $Me^{203}HgCl$	25
4-5	Proposed Mechanism of the Transfer of MeHg from Trout RBCs to Tissues	27

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SECTION I

CONCLUSIONS

Following administration of a single, intragastric dose (0.5mg Hg/kg = 3.3μCi/kg) of MeHg to the rainbow trout, maximum concentration factors (C.F.s) were reached in the gills after one hours, and the skeletal muscle, brain and lens after 34, 56 and > 290 days, respectively. Maximum C.F.s were reached in most other tissues at ~7 days. Skeletal muscle appeared to function as a reservoir for MeHg and accumulated ~50% of the dose from 34 to 100 days postadministration. At 290 days postadministration, skeletal muscle contained > 40% of the dose. MeHg accumulation in the brain never rose above 0.1% of the dose. The rate of mercury excretion appeared to be biphasic as a result of a slow elimination from the skeletal muscle relative to the other tissues. Assuming excretion to be a first order process, the half-retention time (HRT) for MeHg for the first 100 day postadministration was calculated to be ~200 days. Over the last 190 days (days 100-290), a HRT of > 1000 days was obtained.

MeHg is taken up rapidly, *in vitro* and *in vivo*, by trout RBCs. The uptake of MeHg by the RBC is dependent, to a large extent, on the number of reactive -SH groups per hemoglobin molecule which was found to be 4 for the trout. In the trout

RBC, MeHg binds almost exclusively to hemoglobin. The binding within the cell is freely reversible, *in vitro* and *in vivo*, by -SH groups located outside the cell. The ability of MeHg to rapidly penetrate the RBC membrane and to reversibly bind to hemoglobin is responsible for its rapid transport and transfer to tissues.

SECTION II

RECOMMENDATIONS

Experience has shown that:

i. Mercury in any form can be converted in the natural aqueous environment into a readily bioavailable, highly toxic organic form, methyl mercury.

ii. The biological half-life of methyl mercury in fishes is extremely long (> 1000 days in the case of the rainbow trout, *Salmo gairdneri*).

iii. There is no comprehensive, systematic knowledge of:
   a. the biological effects in man of low-level, long-term (environmental) exposure to any single toxic or potentially toxic environmental pollutant.
   b. the synergistic effects of multiple environmental pollutants on living systems.

Therefore:

i. Environmental release of toxic or potentially toxic substances should be monitored and controlled rigidly to prevent:
   a. (potentially irreversible) damage to aquatic food webs and, therefore, man's dwindling food food supply.
   b. the reoccurrance of Minamata-like disasters: episodes of environmentally derived human intoxication of epidemic proportions.

ii. The EPA should undertake the development of a comprehensive program of extramural support of basic research in environmental toxicology: especially in those areas dealing with low-level, long-term; synergistic; and behavioral effects of pollutants.

SECTION III

UPTAKE, DISTRIBUTION AND CONCENTRATION OF METHYLMERCURY BY RAINBOW TROUT (SALMO GAIRDNERI) TISSUES

INTRODUCTION

It has been known for some time that fishes are able to take up organic mercury from their environment and concentrate it in their tissues (1,5,8,11,12,15,20) to levels that are toxic to humans (4,6,14,17,18). Our research is directed toward elucidating the mechanisms of organic mercury concentration in the tissues of the rainbow trout, *Salmo gairdneri*. Presently our attention is focused on establishing the relative affinities of the various tissues of this species for methylmercury (MeHg); that is, the capacity of the tissues to take up, concentrate and store MeHg, and the half-retention time of this compound in the tissues.

MATERIALS AND METHODS

Hatchery reared rainbow trout (*Salmo gairdneri*) of similar genetic background were obtained from the New York State Hatchery at Caledonia, New York. The natural mercury content of their skeletal muscle was found to be ~0.08ppm by neutron activation analysis (10). The average weight of the fish at the beginning of the experiment was 250 g. A weight increase of up to 59% was recorded during the course of the experiment (290 days).

Maintenance of Fish

The fish were maintained in aged, aerated tap water in 200 gallon galvanized steel tanks in a temperature controlled room. Water temperature fluctuated between 5 and 9°C. The water was continuously filtered through activated charcoal and cotton and was replaced with fresh water every other day. The fish were fed a diet of commercial fish food pellets (Strike Fish Feed: Agway, Inc., Syracuse, New York).

^{203}Hg labeled MeHg was obtained by isotope exchange between ^{203}Hg labeled mercuric nitrate (radiochemical purity 98%: ICN, Waltham, Mass.) and methyl mercuric chloride (assay > 95%: obtained as the hydroxide from Alfa Chemical Co., Beverly, Mass., and subsequently dissolved in 1 N HCl). It was administered to the fish in an accurately measured, single, intragastric dose. This was accomplished via a stomach tube constructed from small bore polyethylene tubing attached to a calibrated glass syringe. The fish were starved for two days prior to the MeHg feeding and were anesthetized with tricaine methanesulphonate (MS 222: Sigma Chemical Col, St. Louis, Mo.) prior to intubation. Each fish was given 0.5 mg Hg/kg body weight corresponding to a radioactive dose of 3.3μCi/k body weight. It was determined that the fish lost less than 5% of the dose by regurgitation.

At least two fish were sacrificed at 15 different time periods ranging from one hour to 290 days after the MeHg

feeding. Approximately 20 different tissues were isolated each time, placed in preweighed plastic vials and counted in a Packard Model 5319 Gamma Scintillation Spectrometer. Tissue MeHg content was determined by comparison with a MeHg standard counted along with the samples.

The term "Concentration Factor,"

$$C.F. = \frac{\text{ng Hg / g wet wt.}}{\text{500 ng Hg / g}} \times \frac{\text{final tissue wt.}}{\text{initial tissue wt.}},$$

is employed to express the data. It reflects the ability of the tissues to concentrate MeHg on a gram per gram wet weight basis. Since the fish increase in weight significantly over 290 days, tissue MeHg concentrations appear to decrease even in the absence of MeHg excretion. To account for this, the first term of the equation is multiplied by a factor equal to the final tissue weight divided by the initial weight.

RESULTS AND DISCUSSION

Fig. 3-1 illustrates the relative abilities of the trout tissues to concentrate MeHg on a gram per gram basis (2,15,19). The open bars represent the maximum C.F. found for each tissue over the 290 day period. The solid bars represent the MeHg C.F. at the end of the experiment. As can be seen, the maximum C.F.s for the blood and spleen were at least twice as

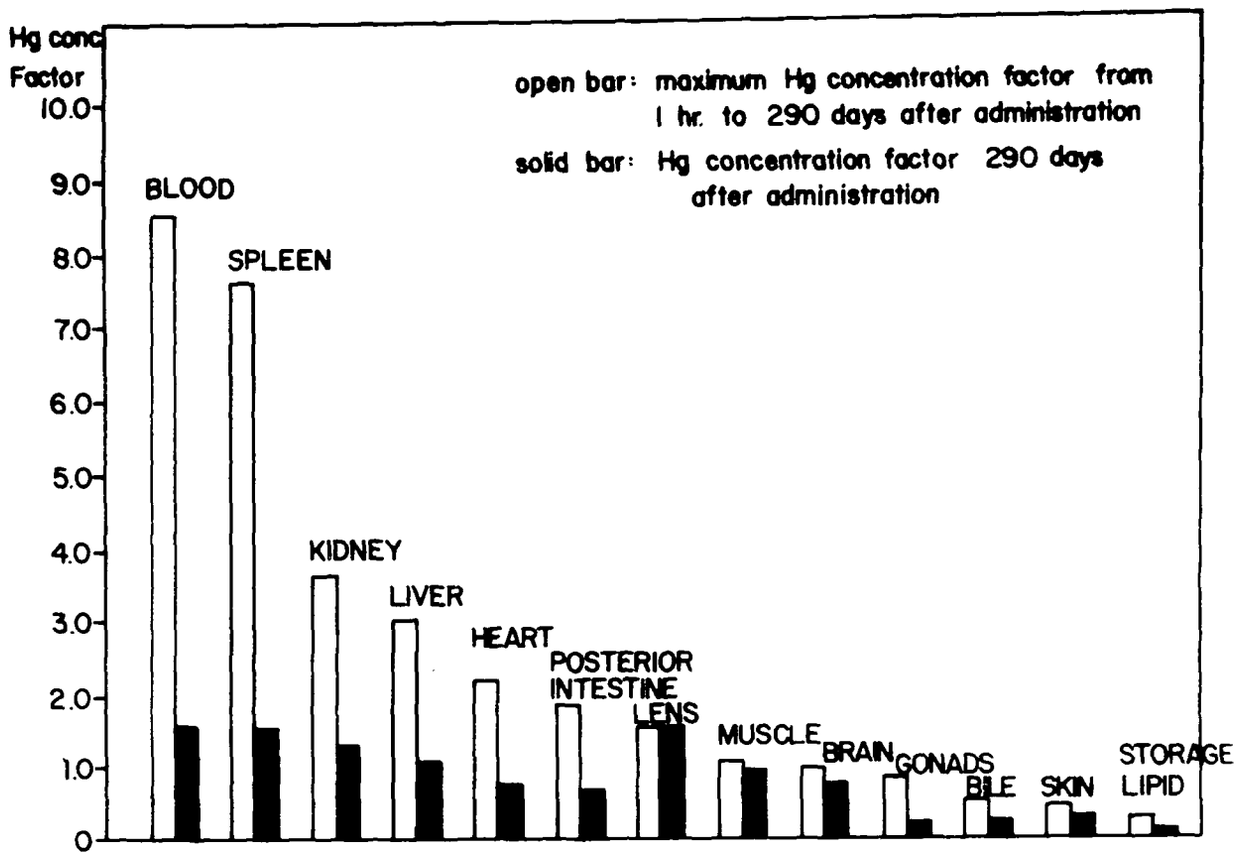

Figure 3-1. Relative Abilities of Trout Tissue to Concentrate MeHg

high as those of any of the other tissues. The kidney and the liver also were able to concentrate MeHg to a high degree. The lowest concentrations of MeHg throughout the study were found in the skin and storage lipid. Due to its high lipid solubility, it might be expected that MeHg would be concentrated in the storage lipid of the fish as is DDT, but this was not observed to be the case.

At the end of the 290 day study, the only tissues having MeHg C.F.s greater than 1.0 were the blood, spleen, kidney, liver and lens. In general, most of the tissue MeHg C.F.s had decreased by at least 2/3 from their maximum values. However, this was not true for the skeletal muscle, brain and lens, where the MeHg concentrations after 290 days were nearly equal to the maximum values.

Fig. 3-2 is a log-log plot of the MeHg uptake and elimination pattern exhibited by the blood, heart and gonads. Each point represents the average value for at least two different fish. The shape of the patterns is essentially identical, and

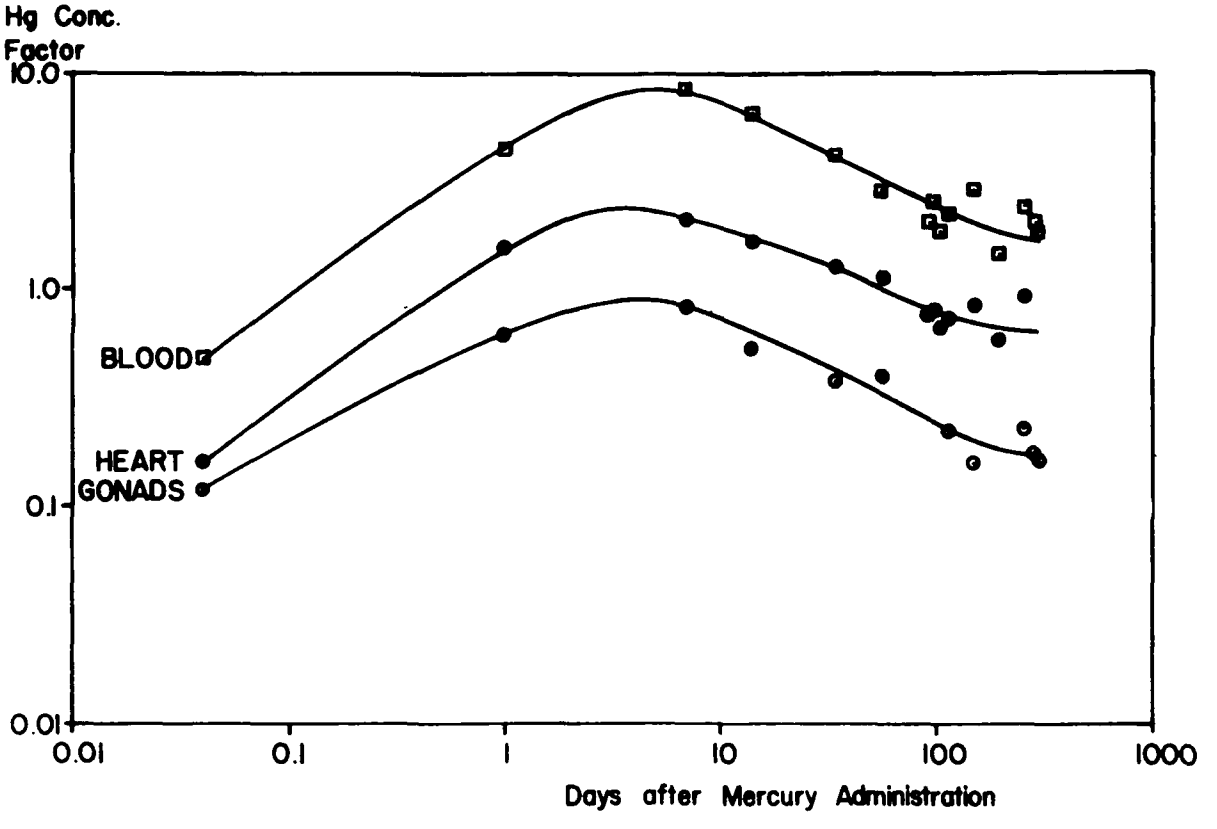

Figure 3-2. MeHg Uptake and Elimination Pattern of Blood, Heart and Gonads

similar patterns, differing only in their maxima, were exhibited by the liver, kidney, spleen, swim bladder and bile. Thus, for most of the tissues investigated, the maximum MeHg concentration was reached approximately seven days after the MeHg feeding. This, in turn, was followed by an approximately linear decrease in concentration over the next 100 days and a leveling-off of the rate of decrease.

A distinctly different type of uptake and elimination pattern was exhibited by the brain and lens. As illustrated in Fig. 3-3, the brain of the trout was observed both to accumulate and release

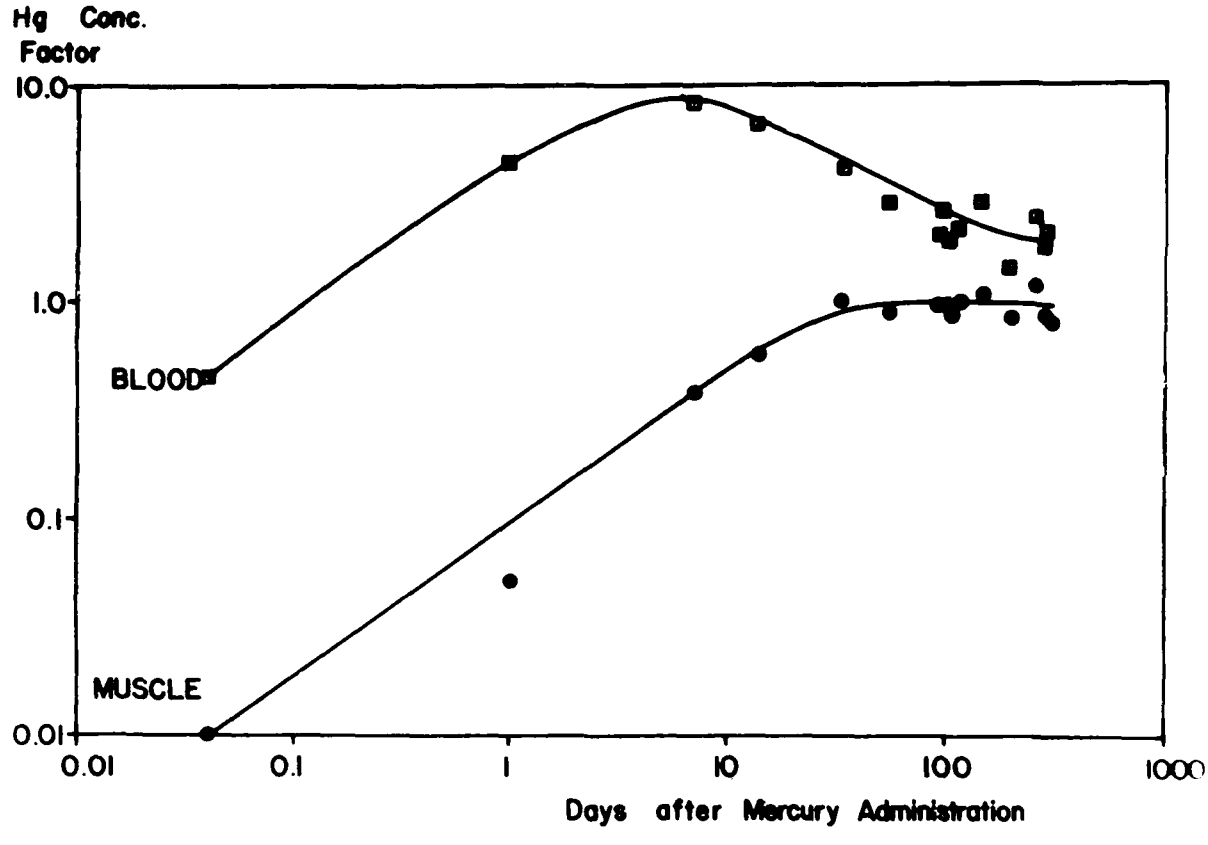

Fig. 3-3. MeHg Uptake and Elimination Pattern of Blood, Brain and Lens

MeHg at a very slow rate (2,5,19). The maximum MeHg concentration was reached 50 days later in the brain than in the blood. During the last 200 days of the experiment only a very slight decrease was observed in the brain MeHg levels. Methylmercury in the brain never rose above a C.F. of 1.0. It may be that the blood-brain barrier of the trout is able to retard, to a certain degree, the influx of MeHg. However, once MeHg has entered the brain, transport out is very slow.

The slowest initial uptake of any of the trout tissues was exhibited by the lens of the eye (Fig. 3). The lens also was unique in that it exhibited no peak MeHg concentration. Throughout the duration of the experiment the concentration continued to increase even as the blood MeHg level declined. The continuous uptake of MeHg by the lens may be related to the high sulfhydryl content reported for this tissue and to the reported occurrence of cataracts in MeHg intoxicated fishes (5).

Skeletal muscle, which comprises the bulk of the edible portion of the trout, accounts for ~55% of its total body weight. Fig. 4 illustrates the uptake of MeHg by the skeletal muscle. The pattern is similar to that of the brain. Maximum concentrations are reached ~34 days after administration and then decline only very slightly over the next 250 days. The point at which uptake plateaus represents the storage of ~ 50% of the MeHg dose. The slow rate of release of MeHg from skeletal muscle will govern, to a large extent, the rate of excretion of MeHg from the whole fish.

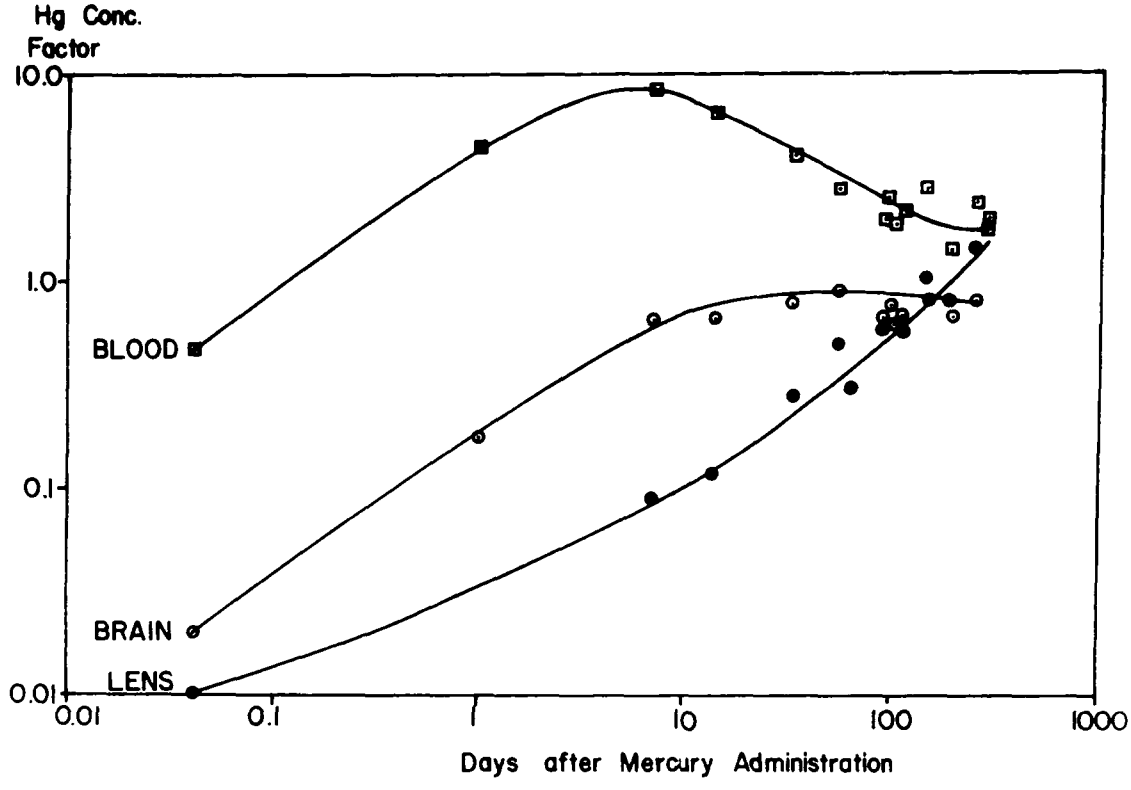

Figure 3-4. MeHg Uptake by Skeletal Muscle

The percentage of the MeHg dose contained in the total weight of each tissue 100 days after MeHg administration is illustrated in Fig. 5. Each value was obtained by multiplying the concentration of MeHg in the tissue by the weight of the tissue, and dividing by the weight of MeHg originally administered to that particular fish. Each bar is the average value for four different fish. The significance of the skeletal muscle as a storage reservoir for MeHg in the rainbow trout is obvious. The skeletal muscle binds 50% of the MeHg dose 100 days after administration. This amounts to ~70% of the MeHg present in the entire fish.

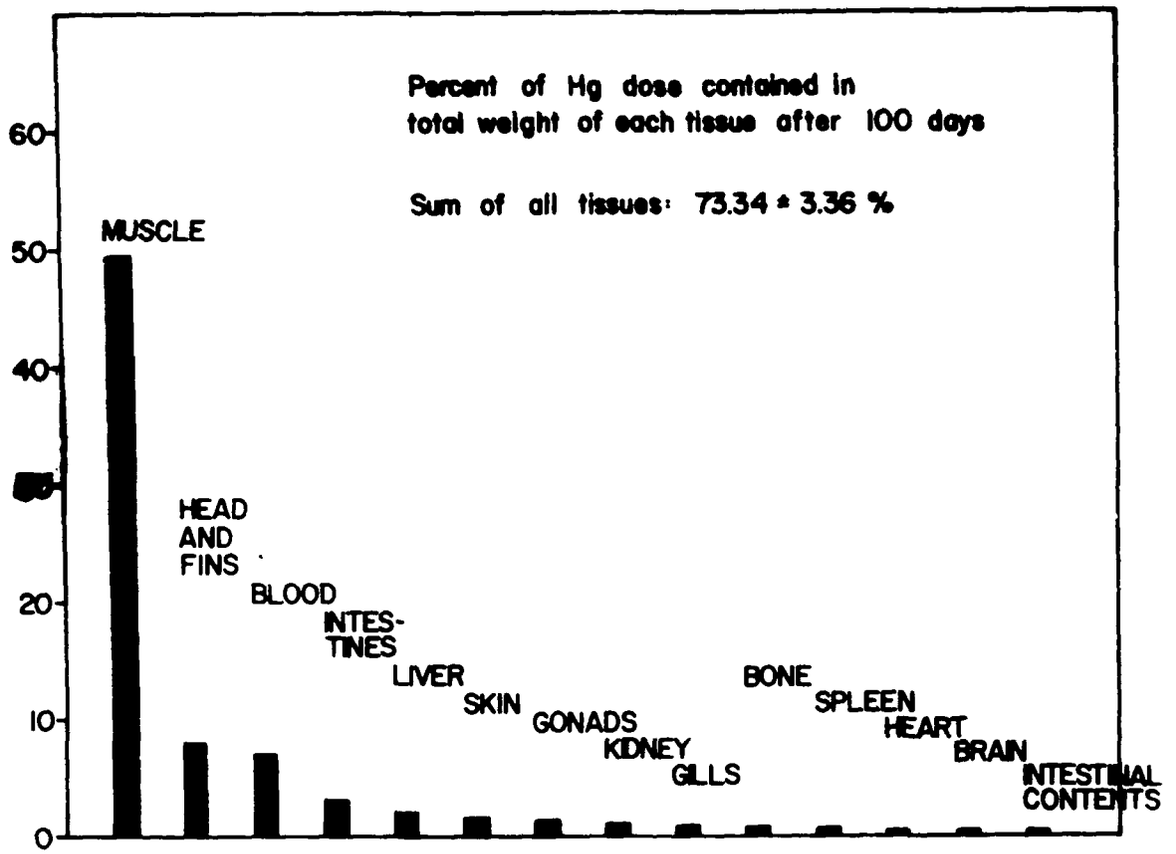

Figure 3-5. MeHg Dose Contained in Total Weight of Each Tissue

Other tissues, some of which had relatively high C.F.s, contributed very little to the total amount of MeHg stored. An example is the spleen, which had a C.F. twice as high as that of muscle after 100 days;but, due to its relatively small mass, it contained only 0.1% of the total MeHg dose. It is of interest to note that the brain also accumulated only 0.1% of the dose. The intestinal contents were found to contain 0.05% of the dose with over 95% of this amount located in the posterior intestine (that portion of the intestine leading from

the stomach). This suggests that defecation may be an important route of exretion of MeHg from the fish (3,5).

The total amount of MeHg measured in the whole fish after 100 days amounted to ~73% of the dose. The biological half-retention time (HRT) for MeHg was calculated for the first 100 days. Assuming excretion to be a first order process and that 100% of the dose was retained at day 0 and 73% was retained at day 100, an HRT of ~200 days (7,9) was obtained.

Total MeHg in the fish also was measured after 290 days. At this time ~64% of the dose was present, amounting to a decrease of ~12% over the last 190 days of the experiment. The HRT calculated for this period (day 100 to day 290) was >1000 days (7). Apparently there are at least two rates of excretion of MeHg: a rapid initial rate, followed by a much slower rate (7,13,16), which apparently is governed by the rate of release of MeHg from the skeletal muscle.

SECTION IV

THE MECHANISM OF METHYLMERCURY TRANSPORT AND TRANSFER TO THE TISSUES OF THE RAINBOW TROUT (SALMO GAIRDNERI)

INTRODUCTION

The blood of the rainbow trout (*Salmo gairdneri*) concentrates methylmercury (MeHg) to a greater extent than any other tissue (24). This has been reported to occur in a wide variety of other species and, apparently, is a general phenomenon. The erythrocyte (RBC) is the blood element responsible for concentrating MeHg (30,31). Relatively little is known concerning the binding of MeHg within the RBC and the transfer of Hg from the RBC to tissues. Recently, White and Rothstein (33) have demonstrated, *in vitro*, the reversibility of the binding of MeHg to human and rat RBCs. The studies we describe were undertaken to elucidate the mechanism of distribution of MeHg in the rainbow trout; in effect, to determine the extent of binding of MeHg to trout hemoglobin and the mechanism of MeHg transport from the blood into the tissues/organs.

MATERIALS AND METHODS

Animals, tissue sampling, Hg analysis: Hatchery reared rainbow trout were obtained from the New York State Hatchery at Caledonia, New York and maintained as described previously (24). The collection of tissue samples and the determination of their Hg content by gamma scintillation spectrometry also

have been described (24),

Reagents: ^{203}Hg-labeled MeHgCl, > 95% radiochemical purity, was obtained from New England Nuclear, Boston, Mass. MeHgOH, 100.8% by titration, was obtained from Alfa Inorganics, Beverly, Mass.

Collection and fractionation of whole blood: The fish were anesthetized with ethyl m-aminobenzoate methanesulfonate (MS-222; Sigma Chemical Co., St. Louis, Mo.), the caudal fin was severed and a volume of blood was collected in an equal volume of modified Alsever's solution (22). The RBCs, isolated by centrifugation for 10 minutes at 2000g, were washed once by resuspension in 2 volumes of Alsever's solution and lysed in 2 volumes of distilled water. The lysate was incubated for 6 hours at $5^{o}C$ with stirring and the stroma removed by centrifugation at 40,000g for 10 minutes. The stroma was discarded except in the study in which its Hg content was measured. In that case, it was freed of residual hemoglobin by washing 4 times in 5 volumes of 0.001 M NaH_2PO_4/Na_2HPO_4 buffer, pH 7.3 containing 0.1% NaCl.

Tissue soluble protein extract: Livers were homogenized in a Virtis Model 60K Homogenizer (Virtis Co., Inc., Gardiner, N.Y.) at 45,000 rpm for 2 minutes. The tissue:buffer ratio was 1:1.5 (wt:vol). The buffers employed were 0.1 M Tris-citrate, pH 6.8, in preparation for gel filtration chromatography and 0.02 M NaH_2PO_4/Na_2HPO_4, pH 7.3, containing 0.14 M NaCl, for the study of the efflux of MeHg from the RBCs. A soluble protein

extract was prepared by incubating the homogenate at 5°C for 10 minutes and centrifuging for 20 minutes at 40,000g.

Protein concentration measurements: Total soluble protein concentration was measured by the colorimetric procedure of Lowry, et al.(9). Stromal protein concentration was calculated from total nitrogen values determined by the Kjeldahl method (21). The concentration of hemoglobin was determined spectrophotometrically employing the absorbance of cyanmethemoglobin at 540nm. (23). This method assumes a molecular weight for hemoglobin of 66,000.

MeHg administration: MeHg was administered intragastrically by stomach tube (24) to fish averaging 350g in weight. The dose solution (^{203}Hg-MeHg plus carrier MeHg in 0.14 M NaCl) contained 4.0 mg Hg/kg body weight (B.W.) and 3.3μCi/kg B.W.

Uptake of MeHg by RBCs in vitro: One ml of a solution of ^{203}Hg-labeled MeHg in 0.14 M NaCl containing 50μg Hg and 3μCi was added to 10 ml of whole blood in an equal volume of Alsever's solution. The suspension was incubated at 3°C with occasional stirring. At various time intervals, duplicate aliquots were taken and separated into RBCs and plasma by centrifugation. The RBCs were washed and the Hg content of the RBCs and plasma was measured as described (vide supra).

Efflux of MeHg from RBCs in vitro: RBCs containing MeHg were prepared as described (vide supra). They were isolated

by centrifugation, washed twice with Alsever's solution and suspended in 0.02 M NaH_2PO_4/Na_2HPO_4, pH 7.3, containing 0.14MNaCl. The suspension contained 85mg hemoglobin/ml and 5.7μg Hg (0.3μCi)/ml (equivalent to 13μg Hg/ml RBCs packed at 2000g). Efflux of Hg from the RBCs was investigated by adding 3ml of the suspension to 10ml of a 75mg/ml solution of trout liver soluble protein in the same buffer. The RBC suspension added to buffer alone served as the control. At various intervals, 0.5ml aliquots were withdrawn and the RBCs isolated by centrifugation and washed. The Hg content of the RBCs was determined and calculated as the percentage of the total Hg in the aliquot. There was no indication that the RBCs had lysed in the liver protein solution (no leakage of hemoglobin into solution).

The identical procedure was followed using trout hemolysate (made 0.14 M in NaCl) containing 55mg/ml of hemoglobin in place of the liver protein solution. In this case, 0.6ml of the RBC suspension was added to 9.5ml of hemolysate.

Efflux of MeHg from RBCs in vivo: $Me^{203}Hg$ labeled RBCs were prepared as described (*vide supra*). The RBCs were suspended in Puck's saline solution (Grand Island Biological Co., Grand Island, N.Y.) in preparation for injection into the fish. The MeHg concentration of the suspension was 20μg Hg/ml.

Fish averaging 485g in weight were employed. They were anesthetized, tagged and injected into the heart in groups of three either with: (i) the RBC bound MeHg to provide a MeHg dose of 0.05mg Hg/kg B.W. (3.5μCi/kg B.W.) or (ii) an identical dose of free $Me^{203}Hg$ in Puck's saline.

Whole blood (0.2ml) was withdrawn from the heart of each fish at various intervals after injection and analyzed for Hg concentration. Each sample was corrected to account for the slight amount of Hg removed from the blood in prior samplings.

RESULTS AND DISCUSSION

Uptake of MeHg by trout blood components: *In vitro*, RBCs take up MeHg very rapidly. Approximately 3 minutes after the addition of 5ppm MeHg to whole blood in an equal volume of Alsever's solution, 84% of the Hg was found in the RBCs. This figure reached 89% after 1 hour and remained constant for the next 2 hours.

In vivo, < 2% of whole blood Hg was found in the plasma 2 weeks after an intragastric dose of $Me^{203}HgCl$ (Table 1). Almost 95% was found in the soluble contents of the RBC and < 4% was bound to the stroma. In a volume of whole blood, the hemolysate was shown to contain 7 times more protein than the plasma and to have a Hg binding capacity 10 times that of plasma on a mg Hg/g protein basis. These factors impart

TABLE 4-1

Methylmercury and Protein Content of Fractions of Rainbow Trout Blood 14 Days After A Single Intragastric Dose (4 mg Hg/kg Body Weight) of $Me^{203}HgCl$

Fraction	Hg concentration mg/100ml whole blood	% of total whole blood Hg	Protein concentration[c] g/100ml whole blood	Binding capability mg Hg/g protein
Plasma[a]	0.07	1.40	1.43	0.05
Stroma-free hemolysate[b]	4.70	94.80	9.67	0.49
Stroma	0.18	3.80	1.57	0.12

a. Includes RBC washes

b. Includes stroma washes

c. Calculated from the average of duplicate Kjeldahl analyses by multiplication by 6.25 (1.)

to the hemolysate the ability to accumulate 70 times as much MeHg as plasma.

Stroma-free hemolysate from the *in vivo* study (*vide supra*) was fractionated by gel filtration chromatography employing Bio-Gel 0.5m agarose. The protein fraction corresponding to hemoglobin was found to contain 95% of the ^{203}Hg present in the hemolysate.

Efflux of MeHg from the RBC: $Me^{203}HgCl$ labeled RBCs were suspended in protein solution (ref. "Materials and Methods") to investigate, *in vitro*, the reversibility of the MeHg-hemoglobin bond. Figure 4-1 shows that the bond is reversible. In a 75mg/ml solution of trout liver protein 21% of the RBC Hg was removed in a 6.5 hour incubation period. Trout hemolysate, containing 55mg/ml of hemoglobin, removed 36% of the total Hg in a 12 hour incubation period. Control RBCs place in buffered saline showed < 4% loss of Hg after 12 hours of incubation.

The rates of disappearance of MeHg from whole blood in the experiment in which MeHg was injected into the rainbow trout heart as free MeHgCl or as MeHg bound within washed, intact RBCs were identical (Figure 4-2). Since MeHg is taken up rapidly and almost exclusively by RBCs, the rate at which it is lost from whole blood depends on the net rate of its efflux through the RBC membrane plus the net rate of RBC removal from the circulation (RBC replacement). Gel filtration chromatography of the liver soluble proteins (which contained

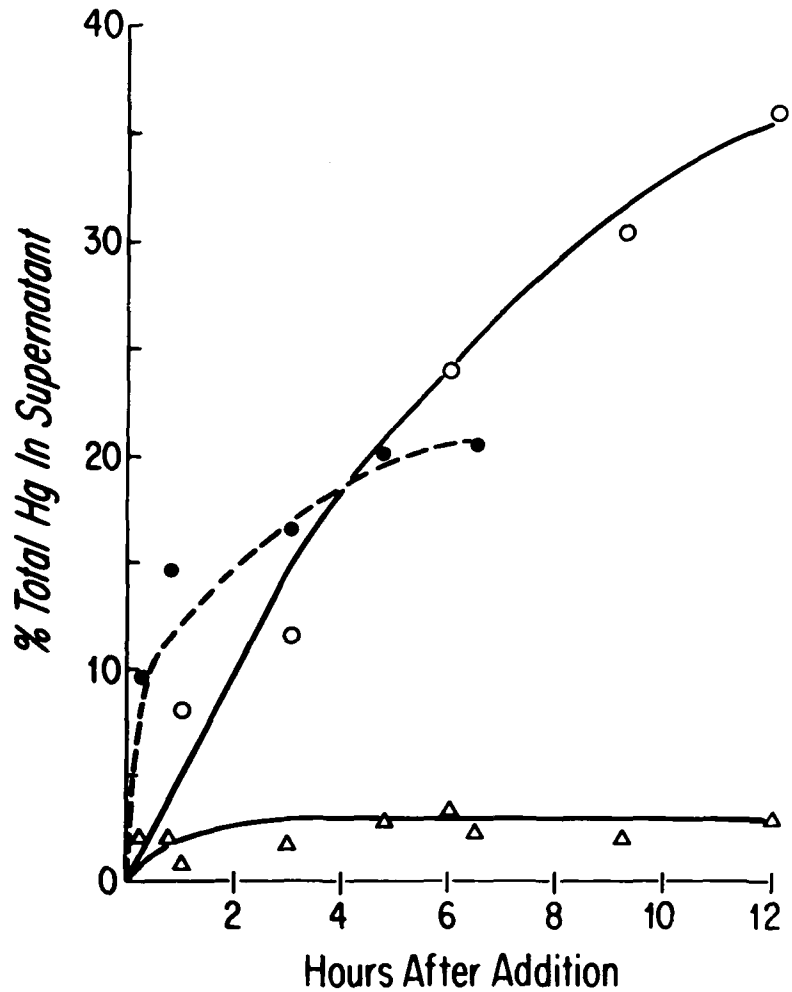

Figure 4-1. The *in vitro* removal of $Me^{203}Hg$ from washed, intact, rainbow trout RBCs by protein solutions. Buffer: 0.02 M $NaPO_4$, pH 7.4, containing 0.14 M saline. RBC MeHg concentration: 13μg Hg/ml packed cells.

85% of the liver Hg) showed identical elution profiles for MeHg administered as the free salt or bound in RBCs (Figure 4-3). Each profile had 4 main peaks of radioactivity plus a number of

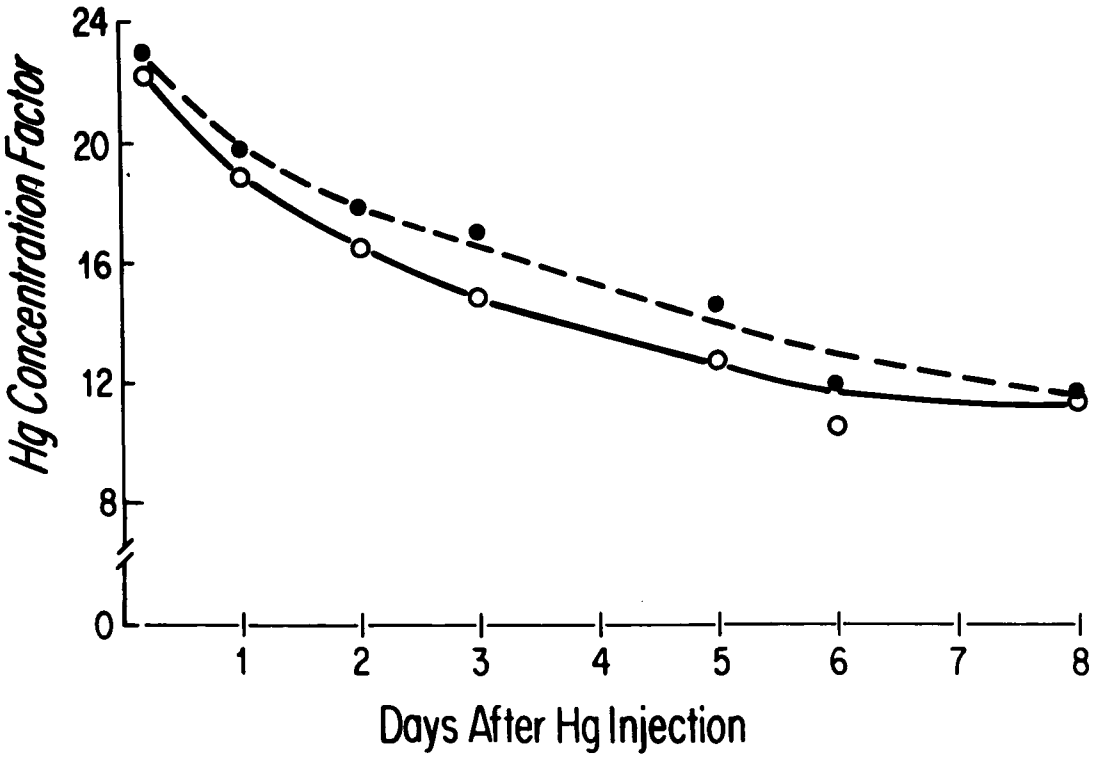

Figure 4-2. The decrease in Hg concentration factor in rainbow trout whole blood after the intracardiac injection of $Me^{203}HgCl$ (0.05mg Hg/kg body weight). Each value is the average for 3 different fish. Injection of $Me^{203}HgCl$ bound in washed RBCs: -o-o-o Injection of Me HgCl in saline solution: -o-o-o

minor peaks. The Hg was found mainly in the void volume, the hemoglobin fraction (at an elution volume of 300ml) and two other fractions of molecular weight < 60,000 (based on molecular weight

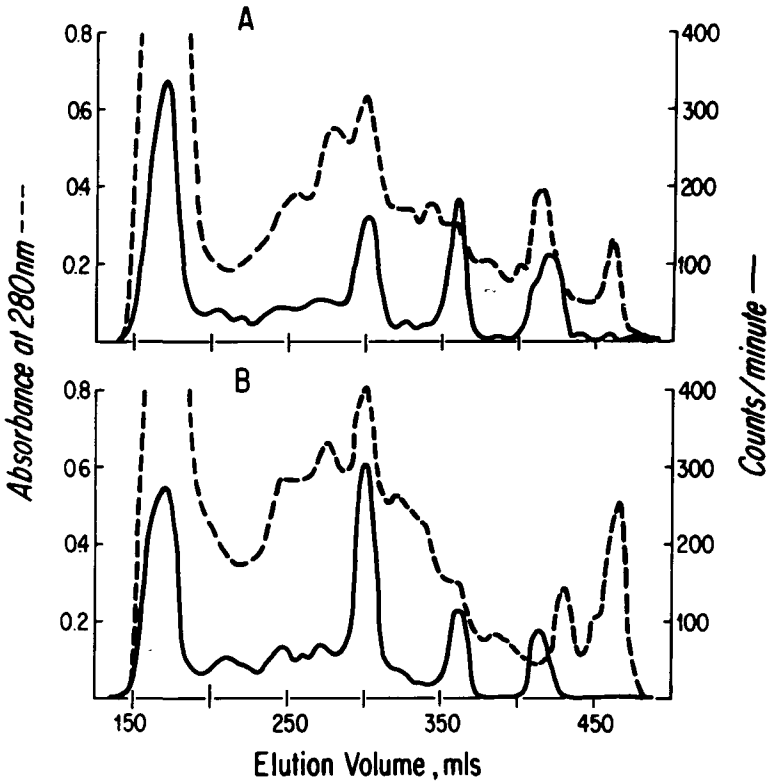

Figure 4-3. Gel filtration elution profiles of rainbow trout soluble liver proteins 8 days after intracardiac injection of $Me^{203}HgCl$ (0.05mg Hg/kg body weight). CPM: ———————— Absorbance at 280nm: ---------- Injection of $Me^{203}HgCl$ in saline solution: A Injection of $Me^{203}HgCl$ bound in washed RBCs: B Bio Gel A 0.5m; column dimensions: 1.8 x 160cm; flow rate: 13ml/hr; buffer: 0.1M Tris-citrate, pH 6.8.

calibration of the column with standard proteins). The distribution of radioactivity in these elution profiles was fundamen-

tally identical to that obtained following intragastric administration of MeHg (Giblin and Massaro, unpublished data).

The demonstration, in vivo and in vitro, of the efflux of MeHg from RBCs is significant because it is the reversibility of the binding of MeHg within the RBC which allows for the transfer of Hg to the tissues.

Determination of hemoglobin -SH content: The number of -SH groups per molecule of rainbow trout hemoglobin was determined by amperometric titration at the dropping mercury electrode (28). MeHg is a highly specific reagent for protein -SH groups (25) and was used to titrate the hemoglobin of freshly prepared hemolysates which had been dialyzed for 20 hours against 2 changes of 100 volumes each of distilled water. Titrations were performed in 0.1 M Tris-HCL, pH 7.4, and in the same buffer made 8 M in urea. The values obtained were 3.7 and 4.3 moles -SH per mole hemoglobin, respectively (Figure 4-4). Titrations of standard solution of glutathione and bovine serum albumin yielded literature -SH values. Since the rainbow trout hemoglobin consists of 3 major and approximately 9 minor types (27,32), the -SH value obtained for hemolysates must be taken as the average of all the types.

When MeHg is added to a solution of rainbow trout hemoglobin near neutral pH the solution becomes cloudy at a concentration of approximately 3 moles of MeHg per mole hemoglobin. This does not occur in 8 M urea at the same pH. Except for making the

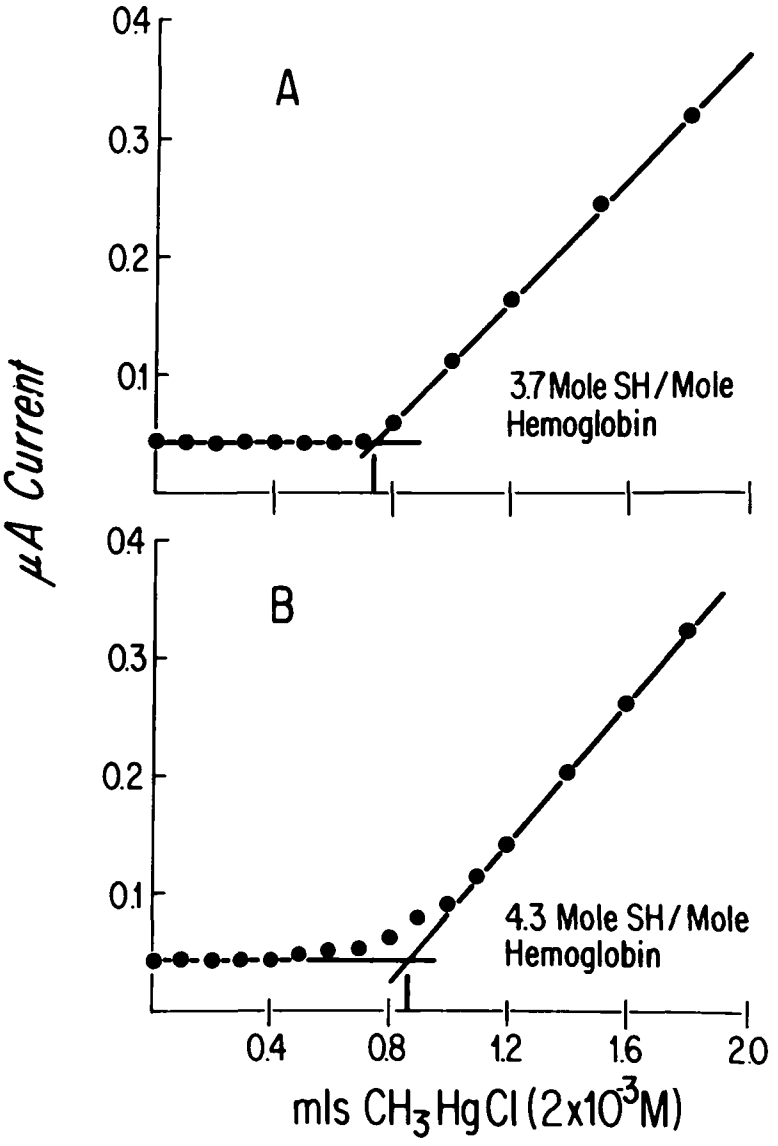

Figure 4-4. Determination of the number of sulfhydryl groups of dialyzed rainbow trout hemolysate (0.40×10^{-3} mmoles) by amperometric titration with $Me^{203}HgCl$. Temperature: 22°C; potential: -0.6V (vs. saturated calomel electrode); total volume: 19 ml; solutions contained 0.015% gelatin and 0.04 ml octanol; MeHgCl dissolved in a 25% solution of dimethylformamide in water.
0.1 M Tris-HCl, pH 7.4, made 8 M in urea: A
0.1 M Tris-HCl, pH 7.4: B

end point less sharp, the clouding did not appear to affect the amperometric data. The number of -SH groups obtained with and

without 8 M urea was approximately 4 which would indicate that, under physiological conditions, all of the -SH groups of trout hemoglobin are available to bind MeHg.

The existence of 4 reactive -SH groups per hemoglobin molecule makes the trout RBC at least 20mM in reactive -SH (based on a measured RBC hemoglobin content of 300-350 mg/ml RBCs packed at 2000g). It is this highly localized concentration of -SH which draws MeHg into the interior of the RBC and binds it.

As we have demonstrated (vide supra), a high concentration of -SH outside the RBC induces the efflux of MeHg from the cell and forms the basis for the transfer of MeHg from the RBC to tissues. A possible mechanism for the efflux is illustrated in Figure 4-5. The mechanism is based on the fact that a small, but definite proportion of MeHg in the presence of excess thiol will be associated with halide as dictated by the Mass Law (26). Highly lipid soluble MeHgCl traverses the cell membrane to -SH groups outside the cell. Abundant membrane -SH groups (11) facilitate transport through the membrane. The exit of MeHgCl causes a shift in the equilibrium inside the cell which is reestablished by disassociation of MeHg from hemoglobin. Eventually a dynamic MeHg equilibrium is established based on the relative concentrations of hemoglobin, RBC membrane, plasma and tissue -SH groups.

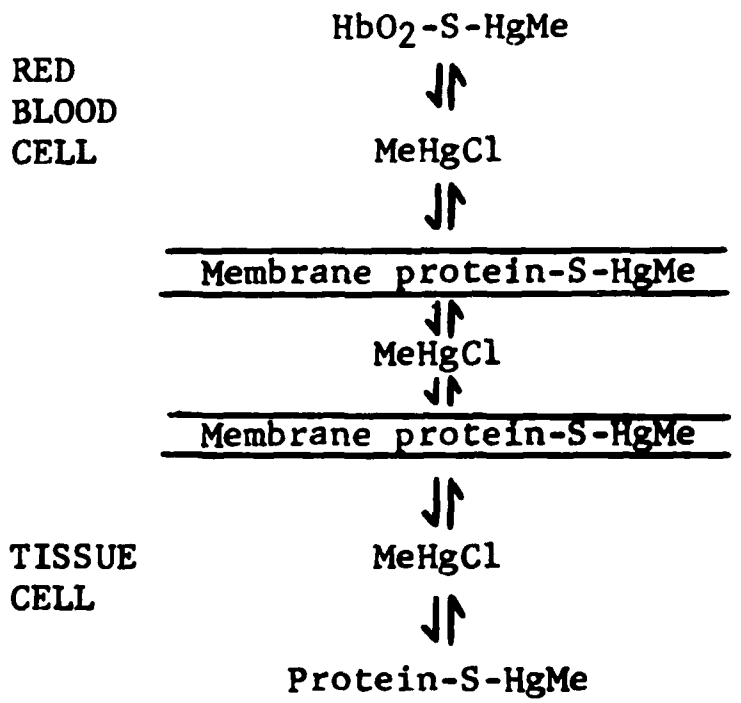

Figure 4-5. Proposed mechanism of the transfer of MeHg from trout RBCs to tissues.

SECTION V

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16. ABSTRACT ^{203}Hg-methylmercury (MeHg) was administered intragastrically in a single dose (0.5mg Hg/kg=3.3μCi/kg) to trout (av. wt. 250g). The fish were sacrificed from 1.0 hr. to 290 days postadministration. Twenty tissues were analyzed for MeHg content by gamma scintillation spectrometry. The label was taken up rapidly by blood, gills, spleen, liver and kidney and more slowly by muscle, brain and lens. After 290 days: (a)blood, spleen, kidney, liver and lens had concentration factors (C.F.=tissue Hg conc./Hg dose x final tissue wt./initial tissue wt.)=1.0;(b)in general, C.F.s had dropped off by at least 2/3 from their maxima except for muscle, brain and lens in which the C.F.s remained=their maxima; (c)~64% of the dose still remained in the fish and skeletal muscle (comprising ~55% of body weight) contained >40% of the residue. Assuming MeHg excretion to be a first order process, there are at least two rates of excretion--a rapid initial rate resulting in a biological half-retention time (HRT) of ~200 days and a slower, subsequent rate yielding an HRT of> 1000 days which, apparently, is governed by the rate of release of MeHg from the skeletal muscle.

Hemoglobin (Hb) is the main methylmercury (MeHg) transport protein in trout blood. In vitro, MeHg is taken up rapidly in 3 minutes by red blood cells. MeHg binding in the RBC is reversible in vitro as demonstrated by the efflux of Hg from RBCs suspended in protein solutions. MeHg binding in the RBC also is reversible in vivo as gel filtration chromatography of liver soluble proteins yielded indentical elution profiles for MeHg administered as the free salt or bound in RBCs. The number of reactive sulfhydryl (-SH) groups per molecule of Hb was found to be 4 by amperometric titration with MeHgCl. The reactive -SH concentration in the RBC was calculated to be at least 20mM. A mechanism for the efflux of MeHg from the RBC is proposed involving the dissociation of MeHg from Hb initiated by -SH groups outside the RBC and migration of MeHg across the membrane as MeHgCl.

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