The Effect of Methyl Mercury on Gill Metabolism and Blood Parameters of Rainbow Trout

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The gills of fresh water teleosts function as the primary site for the active absorption of ions from the external media and for the respiratory exchange of gases. Because of the low solubility of oxygen in water large amounts of water must be passed over the gills to meet the oxygen demands of the fish (RAHN 1966) consequently when fish are in contaminated waters their gills can be exposed to large amounts of water soluble pollutants (e.g. methyl mercury). HANNERZ (1968) and RUCKER and AMEND (1969) have shown that mercury is concentrated in gill tissue up to several thousand times the level found in the media. It was therefore the intent of the present investigation to determine if methyl mercury has any effect on the metabolism or physiological function (plasma electrolyte regulation) of the gill.

Materials and Methods

Rainbow trout (Salmo gairdneri) weighing 160-250g were obtained from the Michigan Department of Natural Resources, Grayling, Michigan and maintained in 300 liter fiberglass tanks at 12±1°C with 14 hours of light per day. Experimental design involved exposing a group of starved fish to 10 µg Hg/liter administered as methyl mercuric chloride while another group of starved fish served as controls. The mercury was administered to the experimental fish through the use of a gravity feed system in which a bottle containing a concentrated solution of CH_3HgCl was placed approximately 6 feet above the tank and a piece of polyethylene tubing used to introduce the methyl mercury into the tank. The flow rate of the concentrated mercury solution into the tank was correlated to the flow of water through the tank to maintain a concentration of 10 µg Hg/liter.

At the end of 4, 8, and 12 weeks the fish were killed and assayed for hematocrit, O_2 consumption of gill tissue, protein content of gill sample, and plasma electrolyte concentrations (Na^+, K^+, Cl^-, Mg^{++}, Ca^{++}). Plasma Na^+ and K^+ were determined on a Beckman (Model 105) flame photometer. Mg^{++} and Ca^{++} were

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determined on a Perkin Elmer (Model 290-B) atomic absorbtion spectrophotometer and Cl^- was measured with a Buchler chloridometer. All plasma electrolyte determinations were done in duplicate.

A sample of gill filaments (60-80 mg) was cut from the middle of the second and third branchial arches and suspended in 3 ml of 10% phosphate buffered saline (PBS) and 100% PBS (Grand Island Biological Co., Grand Island, N.Y.). The oxygen consumption was then measured polarographically at 12°C with a Y.S.I. Model 53 biological oxygen monitor. Calculation of oxygen consumption was based on the solubility of oxygen in 10% and 100% PBS at 12°C and the percent of initial O_2 consumed during a 10 minute period. Following O_2 consumption measurements the 3 ml sample of PBS containing the gill filaments was sonified to remove as much tissue as possible from the cartilaginous skeleton. A modified Lowry method (OYAMA and EAGLE, 1956) was used to determine the amount of protein in each sample.

One way analyses of variance were used for all comparisons and Tukey's w - procedure (SOKAL and ROHLF, 1969) was used for multiple comparisons among means. All data are expressed as Mean $\pm$ S.E. and 5% was considered the fiducial limit of significance.

Results

There was no significant difference in plasma electrolyte concentrations (Na^+, K^+, Cl^-, Mg^{++}, Ca^{++}) for paired comparisons between control and experimental rainbow trout after exposure to methyl mercuric chloride (10 µg Hg/liter) for periods of 4, 8, and 12 weeks (Table 1). However, plasma Na^+ levels for mercury exposed fish varied considerably during the course of the experiment while values for control fish remained fairly constant. A marked decrease in plasma K^+ occurred between the fourth and eighth week of exposure for both control and experimental rainbow trout. After 12 weeks exposure control fish exhibited a significant increase in plasma Mg^{++} concentrations while mercury treated fish showed a very substantial increase in hematocrit. Plasma concentrations of Ca^{++} and Cl^- did not change significantly for either control or experimental fish during the experiment.

There was also no significant difference between the *in vitro* oxygen consumption of gill filaments from control and mercury treated fish determined in 10% or 100% PBS after 4, 8, and 12 weeks exposure to CH_3HgCl (Table II). However, both groups show a higher rate of oxygen consumption in 100% PBS than in 10% PBS. Control and mercury exposed fish also demonstrated a significant decrease in oxygen consumption between the fourth and eighth week of exposure for gill filaments measured in 10% PBS but no change occurred in filaments

measured in 100% PBS.

TABLE I

Values for Some Blood Parameters of Control and Mercury Exposed (10 μg Hg/liter) Rainbow Trout.

Blood Parameter	Length of Exposure (Weeks) 4	8	12
Plasma Na^+ (meq/L)			
Control	140.08±1.35(12)	142.68±3.48(8)	139.37±3.13(8)
Hg-treated	142.58±1.93(12)	149.20±1.83(12)	133.75±7.66(8)
Plasma K^+ (meq/L)			
Control	2.74±0.17(12)	1.36±0.22(8)	1.70±0.27(8)
Hg-treated	2.61±0.13(12)	1.42±0.19(12)	1.77±0.36(8)
Plasma Cl^- (meq/L)			
Control	123.36±0.95(12)	122.02±5.13(8)	121.75±4.00(8)
Hg-treated	123.27±2.07(12)	128.73±1.71(12)	110.85±11.14(8)
Plasma Ca^{++} (meq/L)			
Control	4.36±0.28(12)	4.00±0.15(8)	3.76±0.15(8)
Hg-treated	3.97±0.25(12)	4.15±0.26(12)	3.73±0.25(8)
Plasma Mg^{++} (meq/L)			
Control	1.82±0.05(12)	1.96±0.04(8)	2.15±0.09(8)
Hg-treated	1.66±0.09(12)	2.02±0.06(12)	1.92±0.23(8)
Hematocrit (% RBC)			
Control	19.44±1.28(12)	19.59±2.63(8)	21.31±1.46(8)
Hg-treated	18.00±0.88(12)	21.52±1.38(12)	30.34±2.40(8)

Mean±S.E.(N)

TABLE II

In Vitro Oxygen Consumption of Gill Tissue from Control and Mercury Exposed (10 μg Hg/liter) Rainbow Trout in 100% and 10% Phosphate Buffered Saline (PBS). Data expressed as μl O_2 consumed/hr/mg protein.

Sample	Length of Exposure (Weeks) 4	8	12
100% PBS			
Control	17.35±0.74(12)	15.56±0.98(8)	16.47±2.07(8)
Hg-treated	18.53±1.37(12)	14.48±1.15(12)	17.27±1.15(8)
10% PBS			
Control	14.13±0.78(12)	8.74±0.53(8)	9.78±0.77(8)
Hg-treated	13.13±0.64(12)	8.64±0.50(12)	9.57±0.68(8)

Mean±S.E.(N)

Discussion

Results from the present investigation indicate that up to 12 weeks exposure to methyl mercuric chloride (10 μg Hg/liter) does not significantly affect the in vitro metabolism of the gill or the concentration of plasma electrolytes in rainbow trout. The only deleterious effect exhibited by fish used in the present study was a significant increase in hematocrit after 12 weeks exposure. LARSSON and LEWANDER (1973) reported no significant change in the hematocrit of the European eel (Anguilla anguilla L.) after 145 days of starvation, while KAWATSU (1966) and KRISTOFFERSON and BROBERG (1971) have reported that fish normally show a decrease in hematocrit during starvation. Therefore the increase in hematocrit observed in our experimental fish cannot be attributed to the effects of starvation. Furthermore, OLSON (1972) reported vacuolization of red blood cells (RBC) after exposure for periods of 4 and 8 weeks to 0.3 μg Hg/liter administered as CH_3HgCl. WEBB (1966) also points out that many mercurials are known to cause hemolysis of RBC. Accordingly one would expect that mercury exposed fish would show a decrease in hematocrit as a result of RBC hemolysis rather than the very significant increase which we found after 12 weeks exposure. Possibly methyl mercury directly stimulated erythropoiesis causing an increase in hematocrit or the fish may have over compensated erythropoietically in response to RBC loss resulting from exposure to mercury.

Although there were no significant differences in plasma electrolyte levels for paired comparisons between control and mercury treated fish after 4, 8, and 12 weeks exposure, over the 12 week period several of the electrolytes did vary considerably. Both control and experimental rainbow trout showed a significant decrease in plasma K^+ concentrations between the fourth and eighth week of exposure. LARSSON and LEWANDER (1973) believed the change in the plasma Cl^- of the eel during starvation was due to the increased activity of adrenocorticoid tissue. These authors point out that increased gluconeogenesis during starvation is stimulated by glucocorticoids and that these hormones can affect plasma electrolyte levels. Increased glucocorticoid levels might also be responsible for the decrease in plasma K^+ which was noted in the present study. However, another explanation for the decrease in plasma K^+ is offered by URIST and VAN DE PUTTE (1967). They reported a significant hypokalemia in migrating salmon and attributed this decrease to the depletion of tissue stores of exchangeable potassium as a result of prolonged starvation. Plasma concentrations of Na^+ for control fish remained fairly constant at 140 meq/liter while Na^+ levels for experimental fish

varied considerably between 133 meq/liter -149 meq/liter. Although no significant difference for paired (control vs. experimental) comparisons was observed, the greater variability in plasma Na^+ concentrations among experimental fish may represent a deleterious effect of methyl mercury on the active transport of Na^+, but further work is needed to substantiate this hypothesis. No plausible explanation can be offered for the increase in plasma Mg^{++} levels of control fish after 12 weeks exposure.

Control and mercury exposed gill samples measured in 100% PBS showed a significantly higher rate of O_2 consumption than samples measured in 10% PBS. This could be the result of the sudden transition of gill tissue from fresh water (5-10 mOsm) to 100% PBS (285-290 mOsm) which represents both an osmotic shock and a probable increased salt load for the tissue. Chloride cells present in the gill filaments could have responded with an increased rate of active transport to maintain osmotic equilibrium with a concomitant increase in O_2 consumption; and in this way account for the increased rate of O_2 consumption in 100% PBS. LANGE (1968) reported the greatest oxygen consumption in 20-28% sea water for the common mussel Mytilus edulis. He believed that 20-28% sea water stimulated the respiratory enzymes maximally and accounted for the optimum O_2 consumption at that salinity. A similar stimulation of respiratory enzymes in the present study might also be responsible for the increased O_2 consumption in 100% PBS. The oxygen consumption of both experimental and control fish measured in 10% PBS showed a significant decrease between the fourth and eighth week of exposure. Perhaps this decrease in the O_2 consumption of gill tissue measured in 10% PBS is a reflection of the general decrease in metabolism observed in whole animals during prolonged starvation (BEAMISH, 1964; SMITH,1935). It is also interesting to note that the decrease in O_2 consumption for control and mercury treated fish in 10% PBS occurred during the same period of time that a decrease in plasma K^+ concentration was seen. It would appear that these changes represent some form of accommodation to the effects of starvation since they were observed in both control and experimental fish.

The failure to see any significant difference between the O_2 consumption of gill tissue or plasma electrolyte concentrations of control and mercury treated fish seems to indicate that longterm exposure to methyl mercuric chloride (10 µg Hg/liter) does not alter the metabolism of the gill or affect its role in plasma electrolyte regulation. However, such a generalization at this time would at best be premature. McKIM et al. (1970) reported significant changes in seven blood parameters of brook trout after exposure to three

different concentrations of copper for periods of 6 and 21 days; but when the experiment was extended to 337 days five of the seven blood parameters were similar to control values. It is possible that methyl mercury exerted a similar transient effect on the fish in the present study before the first samples were taken at 4 weeks or possibly a longer period of exposure is necessary before significant changes in O_2 consumption of plasma electrolyte concentrations occur. Furthermore, the possibility also exists that the magnitude of the changes in plasma electrolytes and O_2 consumption was too small to detect with the techniques employed in the present investigation; but could still be large enough to represent a significant physiological burden to the fish. The need for longer term studies with shorter time intervals between samplings is evident and would do much to further our understanding of the physiological effects of mercurials on fish.

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