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Effects of Embryonic Pre-exposure to Methylmercury and Hg^{2+} on Larval Tolerance in *Fundulus heteroclitus*

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Many reports demonstrate enhanced metal tolerance as a result of previous exposure to low concentrations. BEATTIE & PASCOE (1978) showed that pretreatment of rainbow trout (Salmo gairdneri) eggs with cadmium made the larvae more resistant to subsequent Cd treatment. Similarly, SPEHAR et al. (1978) found that larvae of the flagfish, Jordanella floridae, initially exposed as embryos to Zn and to mixtures of Zn and Cd were much more tolerant than those not previously exposed, indicating acclimation during embryonic exposure. Acclimation to metals after pre-exposure was attributed by PASCOE & BEATTIE (1979) and DIXON & SPRAGUE (1981c) to stimulation of the synthesis of metal-binding proteins, or metallothioneins, in the liver, which form a non-toxic complex with the metal. Metallothioneins are small (6,000-10,000 Dalton) proteins, rich in cysteine, whose free thiol groups readily bind the heavy metal ions and sequester them; in some cases induction by one metal may confer resistance to others (CHERIAN & GOYER, 1978).

In this paper we report on the effects of embryonic pre-exposure to methylmercury (meHg) and Hg^{2+} on larval susceptibility to these toxicants in the killifish, Fundulus heteroclitus.

MATERIALS and METHODS

Adult F. heteroclitus were collected from Bullhead Bay, Southampton, New York, and eggs and sperm were stripped into fingerbowls of seawater. Clutches of eggs from each female were maintained separately rather than pooled. For the pre-exposure experiments, clutches of eggs were treated with 0.02 ppm methylmercuric chloride (I.C.N. Pharmaceuticals, Plainview, N.Y.), or 0.02 ppm Hg^{2+} as $HgCl_2$ (reagent grade, Fisher Scientific), both from stock solutions frequently assayed by atomic absorption spectrophotometry. These were levels which produced negligible amounts of embryonic mortality or malformations. Exposure started after eggs had started to cleave and all non-cleaving eggs had been removed. Embryos were kept in 50 ml filtered seawater (30 o/oo S) at 24°C, and water was changed daily for the first four days of development. Controls were also subjected to water changes. The water was not analyzed for contaminants, but Southampton seawater is considered relatively clean, and several years of previous research had shown that development of embryos and larvae of this species is very successful in that water. A maximum of 50 eggs were in each fingerbowl.

After hatching, which was stimulated by changing the water daily starting on day 12, larvae from each treated and control group were divided into two subgroups which were transferred into either 0.05 ppm

mercury or into clean water. Each treatment group contained ten larvae from the same clutch which had had the same embryonic treatment. Larval meHg experiments were set up from embryonic meHg pre-exposures, and larval Hg^{2+} experiments were set up from embryonic Hg^{2+} pre-exposures. Twenty-nine groups went from control to meHg, 15 from meHg to meHg, 14 from control to Hg^{2+}, 14 from Hg^{2+} to Hg^{2+}, 39 from control to control, 27 from meHg to control, and 9 from Hg^{2+} to control.

Larvae were unfed and were maintained in polystyrene containers at 24° C in 100 ml of unfiltered seawater (30 o/oo S) which was changed and redosed daily, at which time dead larvae were removed and recorded. Larvae were maintained in this way for up to ten days, after which time controls began to die from lack of food.

For each group, an LT_{50} (time to 50% death) was calculated, and a mean LT_{50} for all the groups in each treatment regime was derived. The LT_{50} for different treatment regimes could be compared by a t-test. Also, slopes and Y-intercepts of regressions of number dead vs. day for each regime were compared by analysis of covariance (ANCOVA).

RESULTS

All larvae maintained in clean water (controls), whether pre-treated or not, had very little (0-10%) mortality during the course of the ten days and had not reached an LT_{50} by the time the experiments were terminated.

The LT_{50} values for mercury-treated embryos are shown in Table 1. It can be seen that pre-exposure to meHg reduced the time to 50% death, indicating that these larvae were more susceptible than those which had not been pre-exposed. The larvae which were pre-exposed to Hg^{2+}, however, showed no difference in LT_{50} compared with those which had not been pre-exposed.

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TABLE 1. LT_{50} of groups of larvae in meHg or Hg^{2+} in Fundulus heteroclitus.

Embryonic Treatment	Larval Treatment	(n)	LT_{50}
Control	0.05 ppm meHg	(22)	5.32 ± 0.31 (S.E.)
0.02 ppm meHg	0.05 ppm meHg	(15)	4.33 ± 0.24 *
Control	0.05 ppm Hg^{2+}	(14)	7.40 ± 0.51
0.02 ppm Hg^{2+}	0.05 ppm Hg^{2+}	(14)	7.93 ± 0.37

*Significantly different from control to meHg by t-test, P=0.05.

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The analysis of covariance (Table 2) shows that the Y-intercepts of the regression lines for meHg are significantly different, reflecting a change in the time at which larvae began to die in the pre-

exposed groups as compared to the controls. This is the same change reflected in the LT_{50} data. Slopes (death rates) were not significantly different, however. In the Hg^{2+} experiments, pre-exposure did not change the slope or the Y-intercept.

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Table 2. Analysis of covariance comparing Y-intercepts and slopes for number dead larvae vs. days *E. heteroclitus*.

Treatment	vs	Treatment	DF	F for Y-intercept	F for Slope
C ---meHg		meHg ---meHg	173	4.565 *	1.994
C---Hg^{2+}		Hg^{2+}---Hg^{2+}	166	0.150	0.116

*F value significant to 0.05

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DISCUSSION

Our data indicate that pre-exposure of embryos to meHg decreased larval tolerance to this toxicant, while pre-exposure to Hg^{2+} caused no change in larval tolerance to that form of the metal. It would appear that neither meHg nor Hg^{2+} activated a protective mechanism. Metallothionein probably is not involved with meHg in any case, since we have found that meHg is not associated with the MT fraction of adult killifish liver homogenates (Weis, 1983). Unlike Hg^{2+}, pre-exposure to meHg appeared to have a cumulative effect, resulting in weakening of the larvae.

In many previous studies, pre-exposure to metals has produced increased tolerance. PASCOE & BEATTIE (1979) pre-treated adult *Salmo gairdneri* with low levels of Cd, exposed the pre-treated and control fish to higher levels, and found the LC_{50} for the pre-treated fish was significantly higher. DIXON & SPRAGUE (1981a) found that *S. gairdneri* pre-exposed to arsenic gradually increased their tolerance as measured by incipient lethal level. These investigators (1981b) also found that fish pre-exposed to Cu increased their tolerance, but that pre-exposure to Cu caused a decrease in tolerance to Zn. LEBLANC (1982) found that Daphnia pre-exposed to Cu, Pb, or Zn developed increased resistance, but that exposure to one metal did not convey resistance to others. On the other hand, DUNCAN & KLAVERKAMP (1983) found that pre-exposure to Cd, Hg, or Zn increased the tolerance of white suckers (*Catostomus commersoni*) to cadmium.

There have also been reports in the literature of pre-exposure failing to produce an increase in tolerance. For example, GREEN et al. (1976) found that pre-exposing white shrimp (*Penaeus setiferus*) to 0.5 to 1.0 ppb mercury for 57 days had no effect on the LC_{50} or on growth and molting in response to higher concentrations of Hg. Similarly, CORNER & SPARROW (1956) found that pre-exposure of *Artemia salina* nauplii to copper actully lowered their resistance to mercury and that pre-exposure to mercury lowered their resistance to copper.

The concentration chosen for pre-exposure can have important effects on the outcome. ROESIJADI et al. (1982) found that while pre-ex-

posure of Mytilus edulis to 0.5 ppb Hg enhanced their subsequent tolerance to higher levels due to synthesis of mercury-binding proteins, a higher pre-exposure concentration (5ppb) did not enhance subsequent mercury tolerance. BUCKLEY et al (1982) found that Oncorynchus kisutch pre-exposed to 140 ppb Cu became three times more Cu tolerant than controls, while those pre-exposed to 70 ppb were only slightly more tolerant than controls. Our pre-exposure concentration of mercury may not have been appropriate for inducing mechanisms for increasing tolerance. Perhaps a sublethal larval test rather than death might have revealed increased tolerance.

Thus, pre-exposures can have major effects on the subsequent tolerance of an organism in ways that cannot necessarily be predicted. This can cast doubt on the reliability of routine bioassays, since the outcome is a function of the tolerance of the organisms tested. The often-reported increase in tolerance after pre-exposure is not a general principle, but a phenomenon which happens only under certain circumstances.

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