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Specificity of Acquired Cadmium Tolerance in the Fiddler Crab, *Uca pugilator*

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In some invertebrates, tolerance to metals may be increased by prior exposure to lower sublethal concentrations. Le Blanc (1982) found that Daphnia magna pre-exposed to copper, lead, or zinc became more resistant to the toxic effects of these metals. Roesijadi et al (1982) reported that mussels (Mytilus edulis) pre-exposed to 0.5 ug/l mercury became more tolerant to higher concentrations of the metal, but that a higher pre-exposure concentration did not enhance the subsequent Hg tolerance. They attributed the effectiveness of a particular pre-exposure concentration to the induction of metal-binding proteins.

Metal-binding proteins are generally rich in cysteine, whose free thiol groups readily bind the metal ions. It is thought that they normally function in storing essential trace metals (e.g. Zn and Cu) and only incidentally can function in heavy metal detoxification. In many organisms the metal binding proteins are metallothioneins; however, the metal-binding proteins of mollusks are different in structure, and specific Cd-binding proteins have been found in several invertebrates (Roesijadi and Hall 1981). In crustaceans in particular, Wiedow et al. (1982) have isolated Cd-binding proteins from the midgut gland of the blue crab, Callinectes sapidus. There is a finite ability of the metal-binding proteins to detoxify metals, beyond which the toxicants "spill over" and react with nonspecific proteins, thereby causing the toxic effects.

Organisms have also developed a variety of mechanisms for depurating heavy metal pollutants. Calcium deposits, both intracellular and extracellular, can sequester cations (Fowler et al 1981). Since crustaceans deposit calcareous compounds in exoskeletons which are cast off at ecdysis, the metals may be removed at the time of molt.

In Brachyuran crabs, limbs can be autotomized at a preformed breakage plane and can be regenerated. The regenerating limb grows in a folded position and unfolds when the animal molts. Thus, regeneration and the molt cycle are closely connected, The regeneration of many limbs as a result of multiple autotomy will accelerate the molt cycle (Skinner and Graham 1972). Multiple autotomy generally serves to synchronize the molt cycle in crabs that may be initially in different stages of intermolt, so that they generally will all begin regenerating and will molt within a comparable period of time.

The processes of regeneration and molting in crustacea have been shown to be affected adversely by the presence of various contaminants in the water. Weis (1976, 1977) demonstrated a retardation of regeneration and ecdysis in Uca by exposure to Cd and methylmercury (meHg). Weis (1985) found that pre-exposure to 0.5 mg/L Cd for one week enabled male (but not female) U. pugilator to become more resistant to the effects of 1.0 mg/L Cd during regeneration. In the current study, we wished to learn whether this same pre-exposure to Cd would enable the crabs to become more resistant to HgCl or to meHg.
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MATERIALS AND METHODS

Male crabs 12-19 mm in carapace width were collected in July and August 1985 from Scallop Pond, Southampton, N.Y. and brought into the laboratory. They were placed in clean Southampton sea water (25o C, 25 o/oo salinity) for one week or in 0.5 mg/L Cd (as CdCl2, Fisher Scientific) in sea water. Solutions were changed once during this week of pre-exposure. At the same time, another set of crabs were pre-exposed to the same concentration of Cd for a period of one-half week. After this period, autotomy of six limbs was induced by pinching the merus with a hemostat, and the crabs were placed in individual containers with either 0.5 mg/L Hg as HgCl2, (Fisher Scientific), 0.5 mg/L meHg, (ICN Pharmaceuticals Plainview, N.Y.), or clean water. The following groups were set up: Control - control, control - Hg, control - meHg, Cd - Hg, Cd - meHg. Metal- control groups were not included because earlier work (Weis 1985) showed no effect. Groups contained 15 crabs each and were arranged to have a comparable distribution of crabs of different sizes. Animals were fed, measured, and the water was changed and re-dosed twice weekly. The concentrations of metals were thus "nominal", that is, measured out of a stock solution that was assayed by atomic absorption spectrophotometry, and changed regularly. The measurements of limb buds were made through a dissecting microscope with a calibrated ocular micrometer. The measurements were converted to R-values

(limb bud length x 100/carapace width) (Bliss, 1956). The first walking leg was used as the representative limb for each crab. Mean R-values on each day were compared by t-test. Time to molt was recorded for each crab. The small number of crabs that failed to begin regeneration were removed from the experiment. Any crab that was in proecdysis at the time of limb removal would molt shortly thereafter without having formed any limb buds, and was also not included in the data. The experiment was repeated once with a full week of pre-exposure to Cd.

RESULTS AND DISCUSSION

The results of the half week and one week pre-exposures to Cd are seen in Figures 1 and 2. In both cases, crabs that were pre-exposed to Cd did not grow any faster in Hg or in meHg than crabs that had not been pre-exposed. In fact the pre-exposed groups grew somewhat more slowly and also did not molt as soon as the groups that had not been pre-exposed to the cadmium. This trend reached statistical significance in the crabs that were pre-exposed to Cd for a full week and regenerated in Hg. On days 14, 17, and 21, the pre-treated group had significantly lower R-values than the group that had not been pretreated ($t = 2.16$, 2.27, and 2.25 respectively, P 0.05). When the experiment was repeated, no protective effects were again observed. For example, on day 14, the R value for the C - Hg group was 6.1 ± 1.7 (S.E.), the Cd - Hg group was 8.3 ± 1.0, the C - meHg group was 7.0 ± 1.6, and the Cd - meHg group was 7.1 ± 1.2.

In the current experiments, the Cd exposure, rather than producing increased resistance, seems to have made the animals if anything, more susceptible to the growth and molt retarding effects of the mercury compounds. This is in contrast to the results of Weis (1985) in which, in similar experiments, crabs that had been pre-exposed to the same concentration of Cd developed increased resistance to Cd and regenerated more rapidly than those that had not been pre-exposed. The present experiments indicate that the increased resistance developed as a result of the pre-exposure is specific to Cd and not generalizable to other metals (at least not mercury).

When fiddler crabs were pre-exposed to meHg, their tolerance to higher levels was unchanged (Callahan & Weis, 1983). Killifish pre-exposed to meHg showed a decrease in tolerance to higher levels (Weis et al., 1985). This may indicate that the methylated form of mercury is not able to activate physiological protective mechanisms in these organisms.

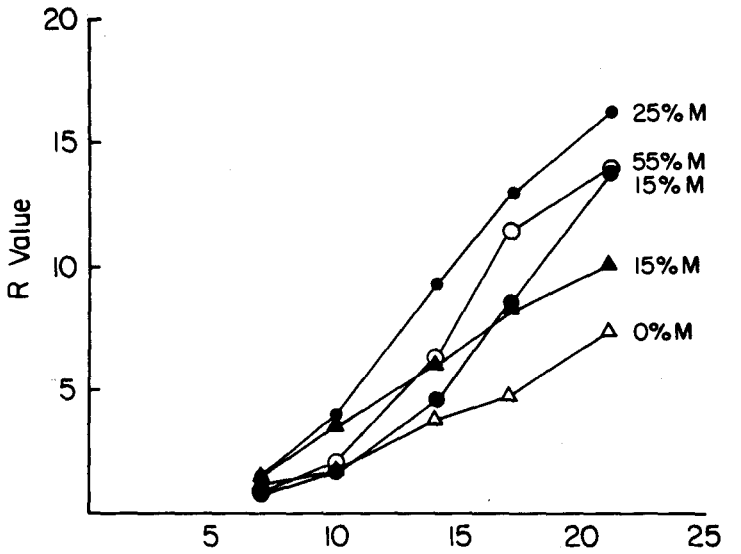
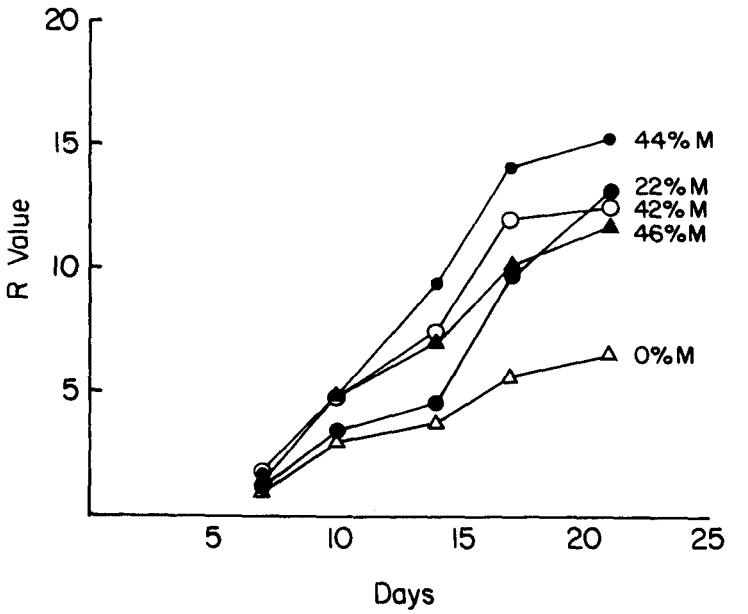


Figure 1. R-values (mm x 100/ carapace width) of U. pugilator with ½ week pre-exposure to Cd. • = control, O = C → meHg, ● = Cd → meHg, ▲ = C → $HgCl_2$, △ = Cd → $HgCl_2$. The %M values are percentage molted by day 25.

Figure 2. R-values of U. pugilator with one week pre-exposure to Cd.



There have been other studies in which pre-exposure to one metal has been found to decrease resistance to another metal. Corner & Sparrow (1956) found that short pre-exposure of Artemia salina nauplii to copper lowered their resistance to mercury exposure. The same was found when they were pretreated with mercury and then placed in copper. Dixon & Sprague (1981) found that Salmo gairdneri pre-exposed to copper lowered their resistance to zinc. LeBlanc (1982) found that Daphnia magna pre-exposed to copper became more resistant to copper, but did not become more resistant to other metals.

The particular concentration of a metal used for pre-exposure is critical in determining whether increased resistance will be produced (Roesijadi et al. 1982). Since the pre-exposure concentration used in the present experiments was chosen as a result of its effectiveness in enhancing Cd resistance in the earlier work, it is clearly an appropriate concentration, at least for Cd resistance. The fact that resistance to mercury was not enhanced can be attributed to the specificity of the protective mechanism.

In Weis (1985) the Cd-pre-exposed groups tended to accumulate higher amounts of Cd in their tissues than groups not pre-exposed, indicating that the pre-exposure was activating a storage mechanism, rather than increasing depuration of Cd . This latter protective mechanism has been observed in pre-exposure studies with the killifish, Fundulus heteroclitus (Weis & Weis, in press). In that study, groups of fish pre-exposed to Cd and then exposed to higher Cd levels, demonstrated increased resistance to the effects of the Cd and accumulated lower concentrations of Cd than fish which had not been pre-exposed, Such a depuration mechanism does not appear to occur in the Uca. Metallothioneins induced by exposure to one metal can often confer protection against other metals. However, specific cadmium binding proteins have been found in crustacea (Wiedow et al, 1982). Otvos et al. (1982) have noted that metallothionein produced by the crab Scylla serrara by Cd exposure bound only Cd and did not bind Zn. This may imply that that protein would not bind Hg as well and may also explain our unpublished data indicating that Hg pre-exposure did not enhance tolerance of fiddler crabs to Hg, meHg, or Cd. Stone & Overnell (1985) have reviewed non-metallothionein Cd-binding proteins and have found the existence of such proteins in many taxa. In some cases, the protein structure resembles metallothionein, and in other cases it is very different. It is quite possible that specific Cd-binding proteins were induced in Uca by the Cd pre-treatment and these conferred no increased resistance to mercury compounds. The trend, in fact, was towards decreased

resistance to the Hg and meHg, indicating that the Cd pre-treatment acted as an additional stress.

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