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In Vitro Effects of Cadmium and Lead on ATPases in the Gill of the Rock Crab, *Cancer irroratus*

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Both from a public health standpoint and because of their ecological dangers, cadmium and lead have become of increasing concern as pollutants of marine and estuarine environments (DORN et al. 1973, BRYAN 1971). The well-documented case of cadmium contamination in the Jintsu River, Toyama Prefecture, in Japan, has shown the devastating effects this metal can have on a human population (KATO & KAWANO 1968). Lead, entering aquatic environments by dumping, by runoff and by aerosol precipitation, has achieved elevated distribution virtually world-wide (PATTERSON 1965). Laboratory studies of acute toxicity, using high metal concentration for relatively short periods of time, have shown adverse effects on a number of marine organisms (CONNOR 1972, EISLER 1971). However, much needs to be learned of effects on organisms and on the ecosystem of a range of concentrations and of long-term exposures to pollutant metals. Analysis of metal effects requires investigation of routes of uptake, particular organ systems or physiological variables most likely to be affected, and mechanisms by which metals exert their toxic action. In this study with the rock crab, Cancer irroratus, we have focused on the effects of lead and cadmium on enzymes in the gill. This organ is intimately involved in osmoregulation as well as respiration in the estuarine animal (KROGH 1939). Metal uptake studies with crustacea have shown high initial concentrations in gill tissue (EISLER et al. 1972, HUTCHESON 1974). Sodium-potassium-ATPase is a key enzyme in cellular water balance and in osmoregulation in the whole animal (QUINN & LANE 1966). The membrane bound gill Na^+-K^+-ATPase, being intimately exposed to the aqueous environment, seemed an excellent candidate for testing effects of environmentally occurring heavy metals. As part of the enzyme assay procedure, the activity of other ATPases present in the tissue preparations was also determined. Some of this ATPase activity is mitochondrial and important in cellular energy metabolism (PULLMAN et al. 1960). While mitochondrial enzyme exposure to environmental heavy metals may be less direct than that of cellular membrane enzyme, their inhibition could have equally detrimental effects on the functioning of the total organism.

METHODS

Rock crabs, C. irroratus, caught in Raritan Bay, New Jersey by otter trawl or crab rake, were brought to Sandy Hook Laboratory and held in running sea water tanks until sacrificed. For the

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ATPase assay, as modified from the method of BONTING (1970), gill segments were removed from pithed animals, weighed, and homogenized in ice cold deionized water in a motor driven Potter-Elvehjem all-glass homogenizer. One ml aliquots of the gill homogenate were added to incubation mixtures, half of which contained ouabain at a (final) concentration of 1×10^{-3}M. The assay medium also contained the following components, in amounts to give the indicated final concentration in a total incubation volume of 5 ml: Tris buffer (Trishydroxymethylaminomethane-HCl), 90 mM; pH, 7.5; sodium, 60 mM; potassium, 5 mM; magnesium, 4 mM; and ATP*, 4 mM. The metals, as cadmium and lead nitrates, were dissolved in buffer solutions and added to the incubation mixtures to give the appropriate 1 or 10 mg/l concentration (10 mg/l Cd = 8.9×10^{-5}M; 10 mg/l Pb = 4.8×10^{-5}M). Incubation was for 30 minutes at 25°C and the reaction was stopped by adding 1 ml of ice cold 30% trichloroacetic acid (TCA). To a reagent blank control set of mixtures, the TCA was added prior to enzyme addition and these samples were kept cold. After incubation and cooling, the samples were centrifuged to remove the protein precipitated by the TCA, and 2 ml aliquots were added to 2 ml of a solution of 1% ammonium molybdate in 1.15N H_2SO_4 to which $FeSO_4$, 40 mg/ml, had been added immediately before use. For this step, in which phosphate liberated from ATP was determined, approximately one hour was allowed for color development, and the absorbance of the samples was read in a Pye-Unicam spectrophotometer at 700 nanometers. Ouabain is a specific inhibitor of the Na^+-K^+-ATPase, therefore by difference between samples incubated with and without ouabain, activity due to Na^+-K^+-ATPase was calculated and the remaining activity assigned to the Mg^{2+}-ATPase(s). Activity units are given in μmoles PO_4^{3-} liberated per hour per mg gill tissue. Inhibition was measured as the difference in activity between a sample with metal ions added and its individual control, gill tissue from the same animal run concurrently. Statistical analysis was by use of student's t test.

RESULTS

In Table 1, results for both the membrane Na^+-K^+-ATPase and the (mitochondrial) ouabain-insensitive ATPase activities are presented. For convenience, the latter is designated Mg^{2+}-ATPase, although the Na^+-K^+-ATPase also required magnesium ions for activation. For each of the experimental samples, in which cadmium or lead was added, enzyme inhibition was measured as the difference in activity between that sample and its individual control (tissue homogenate from the same animal assayed at the same time). For purposes of statistical analysis the means of each of the treatment groups (16 to 24 measurements, depending on the group) were used. However, since control samples from the four different treatment groups are equivalent, the means and standard error

*ATP obtained from Sigma Chemical Co.; it has recently been reported that vanadium as a trace impurity in some such ATP preparations can be responsible for inhibition of Na^+-K^+-ATPase (CANTLEY et al. 1977).

of the mean (S.E.M.) are also presented in Table 1 for totals of the controls for each enzyme (75 determinations for Na^+-K^+-ATPase and 76 for Mg^{2+}-ATPase). Considerable variability in control activity occurred during the course of time over which these experiments were carried out, reflecting not only differences between individual animals from which gill tissue was obtained but perhaps seasonal variability as well. Control activity for gill Na^+-K^+-ATPase ranged from 0.91 to 5.15 μmoles PO_4^{3-}/mg-gill/hr with a mean and S.E.M., as noted in the table, of 3.09 ± 0.12. However, by whatever method the inhibition of the membrane Na^+-K^+-ATPase by cadmium at a concentration of 10 mg/l, is calculated, this inhibition is statistically highly significant ($P < .005$). This is the case whether the samples are paired with their individual control (as presented in Table 1) or if the mean activity of the treated group is compared with either the mean of its associated controls or the total control mean. Thus with 10 mg/l cadmium added, a 37% inhibition in Na^+-K^+-ATPase activity was observed. Lead at both concentrations and cadmium at 1 mg/l did not cause statistically significant changes in Na^+-K^+-ATPase activity. The measured range of the ouabain-insensitive activity in the controls was 8.6 to 24.0 μmoles PO_4^{3-}/mg-gill/hr with a mean of 14.8 and S.E.M. of 0.47. The Mg^{2+}-ATPase activity was inhibited slightly by both lead and cadmium. The inhibition (ranging up to about 13%) occurred at the lower concentrations, 1 mg/l of added metal ion, as well as 10 mg/l and was statistically significant ($P < .005$) in all four instances.

TABLE 1

In vitro inhibition of gill enzymes by cadmium and lead.

Enzyme	Treatment	Inhibition μmoles PO_4^{3-}/mg-gill/hr
Na^+-K^+-ATPase (Control activity=3.09 ± 0.12 (75)[a])	Lead: 1 mg/l	-0.11 ± 0.30 (16)
	10 mg/l	0.27 ± 0.34 (18)
	Cadmium: 1 mg/l	0.28 ± 0.27 (23)
	10 mg/l	1.13 ± 0.17 (18)*
Mg^{2+}-ATPase (Control activity=14.8 ± 0.47 (76))	Lead: 1 mg/l	0.71 ± 0.23 (16)*
	10 mg/l	2.01 ± 0.15 (18)*
	Cadmium: 1 mg/l	0.60 ± 0.17 (24)*
	10 mg/l	1.00 ± 0.18 (18)*

[a]Mean (μmoles PO_4^{3-}/mg-gill/hr) ± S.E.M. (number of samples)
*Statistically significant, $P < .005$

DISCUSSION

Although *C. irroratus* is not as euryhaline as some other species of crustacea, it does inhabit estuarine as well as marine environments and maintains the osmolality of its hemolymph greater than that of the medium when in salinities below that of sea-

water (CANTELMO et al. 1975). The general body wall of the crab is relatively impermeable; the gills serve as a major organ for osmotic regulation as well as for respiration (POTTS & PARRY 1964). The enzyme Na^+-K^+-ATPase has been shown to play an important role in aquatic organisms which maintain hemolymph ion concentrations different from ion concentrations of their environment. (SKOU 1960, QUINN & LANE 1966).

Metals may alter enzyme activity or function in several ways. They can bind to a number of sites on proteins including imidazol, histidyl, carboxyl and especially sulfhydryl side chains (KENCH 1972). Binding at or near enzyme active sites may interfere with substrate binding; binding at locations away from the active site can still cause conformational changes leading to alterations in enzymatic activity or loss of regulatory function (ULMER 1970). The replacement of a normal co-factor by a different metal ion may alter enzyme activity; this is pertinent for the ATPases which require Mg^{2+} for normal functioning (BRITTEN & BLANK 1973). Lead itself may hydrolyze ATP (WALTON 1973), thus changing the concentration of a component of the ATPase reaction and indirectly altering enzyme activity. Metal ions may also bring about changes in concentrations of co-factors or reactants by altering membrane permeability, including that of mitochondria, again indirectly affecting enzyme activity (PASSOW 1970).

Both lead and cadmium have been reported to inhibit Na^+-K^+-ATPase from mammalian tissues and both metals also interfere with mitochondrial function and inhibit or block oxygen uptake in mitochondrial preparations (CARDONA et al. 1971, CROSS et al. 1970, HASAN et al. 1967). THURBERG and his co-workers (THURBERG et al. 1973; COLLIER et al. 1973) have shown that cadmium can change the rate of oxygen consumption in gill tissue of several species of crabs including C. irroratus.

Vanadium inhibition, if it occurred in our assays, would be in addition to that due to cadmium and thus would not invalidate the conclusion of significant cadmium inhibition of the Na^+-K^+-ATPase.

In this in vitro study, the metal concentrations used were much higher than those found in the heavily utilized waters of the region. Cadmium concentrations in the waters of Raritan Bay have been measured to range from 0.3 to 1.5 ppb and concentrations of lead from 2.6 to 14 ppb (WALDHAUER et al. 1977). Organisms may however concentrate lead and cadmium many times above water column levels (LEATHERLAND & BURTON 1974, DOW & HURST 1972). In subjecting lobsters to 6 ppm cadmium for from 30 to 90 days, values of cadmium in gill tissue reached 3.4 ppm (THURBERG et al. 1977). Organisms from the heavily polluted Jintsu River in Japan had cadmium concentrations of several hundred ppm (FRIBERG et al. 1971), and PRINGLE and his co-workers (1968) noted lead concentrations of 227 ppm in uptake studies with oysters. Sediment metal concentrations from the Raritan Bay region have been measured as high as 985 ppm (dry weight) for lead and 6.5 ppm (dry weight) for cadmium (GREIG & MCGRATH 1977). Thus the con-

centrations used in this study are comparable to what might be expected in tissues of environmentally exposed animals. For the crab, C. irroratus, 10 mg/l cadmium causes considerable inhibition of the gill Na^+-K^+-ATPase. Inhibition of Mg^{2+}-ATPase by both lead and cadmium can be demonstrated at metal levels as low as 1 mg/l. Our results illustrate the potential for damage that these metals have at the biochemical level.

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