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Effects of *In Vivo* Cadmium Exposure on ATPases in Gill of the Lobster, *Homarus americanus*

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The increasing pollution of estuaries and coastal marine waters poses a threat to living organisms, to commercial and recreational fisheries, and ultimately a potential hazard to man himself. Especially serious are materials having long residence times, such as organochlorine compounds and heavy metals. Marine and estuarine organisms can concentrate metals many times above ambient levels (EISLER et al. 1972, LEATHERLAND & BURTON 1974, MARTIN & FLEGAL 1975). In our laboratory, we have recently measured metal concentrations in waters of Raritan Bay, New Jersey and New York, higher than previously reported for any other estuary (WALDHAUER et al. 1978). The toxicity of such metals as mercury, copper, silver and cadmium has been well established. Metal ions may damage organisms, at the cellular levels, by several possible mechanisms which disrupt cellular structure or interfere with metabolic function. For example, metal ions may substitute for required metabolic cofactors; e.g. a different divalent cation may complete with Mg^{2+} in binding to ATP. The metal ions may be electrostatically attracted to negatively charged cell membranes, competing for or interfering with transport function or bringing about detrimental structural alteration. Metals may bind to macromolecules inside cells, particularly with enzymes, altering their activity (BRITTEN & BLANK 1973, VALLEE & ULMER 1972).

In vitro tests of cadmium on ATPase activity in gills of the rock crab, *Cancer irroratus*, showed an inhibition of Na^+K^+ATPase (TUCKER & MATTE 1979). However, preliminary experiments with gills of lobsters, *Homarus americanus*, exposed to low levels of cadmium *in vivo*, showed small increases in ATPase activity (THURBERG et al. 1977). In order to clarify *in vivo* effects of cadmium on gill ATPase, further experiments were carried out.

METHODS

As previously described (THURBERG et al. 1977) lobsters were exposed in a flowing seawater system to a 6 ppb cadmium (=5.3 x 10^{-8}M $CdCl_2 \cdot 2\frac{1}{2}\ H_2O$) for 30 days at the National Marine Fisheries Service Laboratory, Milford, Connecticut. Salinity of the sand-filtered Long Island Sound water ranged from 24-26 o/oo and temperature from 18 to 20°C. Lobster gill segments were received frozen from the Milford Laboratory and lyophilized at Sandy Hook where they were stored at -10°C in a desiccator until the day of the enzyme assay. The freeze-dried gill segments were weighed and then homogenized in ice-cold deionized water in a motor driven Potter-Elvehjem all glass homogenizer.

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For the ATPase assay, as modified from the method of BONTING (1970), 1 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, and all of which contained Tris buffer, 0.9M, pH 7.5; sodium, 0.06M; potassium, 0.005M; magnesium, 0.004M; and ATP, 0.004M. The total incubation volume, including the gill homogenate was 5 mL and the component concentration values given above are for this total volume. Incubation was for 30 min at 25°C and the reaction was stopped by adding 1 mL of ice-cold 30% trichloroacetic acid (TCA). To a control set of mixtures, the TCA was added prior to enzyme addition and these samples were kept cold. All incubation and control samples were run in duplicate. After incubation and cooling, the samples were centrifuged to compact and clear protein precipitated by the TCA and 2 mL aliquots 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 read in a Pye-Unicam spectrophotometer at 700 nm. 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) (mostly mitrochondrial). Activity units are given in µmoles PO_4^{3-} liberated per hour per mg dry weight of gill tissue.

RESULTS AND DISCUSSION

Gill homogenates from lobsters exposed to cadmium (5.3×10^{-8}M Cd^{2+}) for 30 days showed an almost 25% increase in ouabain-insensitive ATPase activity. No difference in Na^+K^+ATPase, however, was apparent between control and cadmium-exposed animals. This latter enzyme is inhibited by ouabain and thus distinguishable in our assay system (BONTING 1970). Previous *in vitro* studies of ATPase activity in gill homogenates of the rock crab, *C. irroratus*, indicate that 10 ppm Cd (=8.9×10^{-5}M cadmium) when added to the assay incubation mixture, inhibited the Na^+K^+ATPase by almost 40% and also had a slight inhibitory effect on the remaining (ouabain-insensitive) ATPase activity (TUCKER & MATTE 1979).

TABLE 1

ATPase Activity in Gills of Lobsters Exposed, *in vivo*, to Cadmium.

ATPase Activity: µmoles PO_4^{3-}/hr/mg dry gill tissue		
	Controls (Non-Exposed) Animals)	6 ppb Cd Exposed Animals
Na^+K^+ATPase	38 ± 4 (5)*	35 ± 6 (6)
Mg^{2+}ATPase	345 ± 23 (5)	429 ± 30 ǂ(6)

* Mean ± S.E.M. (no. of samples)
ǂ Significantly different from control, $P < .05$

Although the in vitro cadmium concentration, 10 ppm, to which the rock crab enzymes were exposed, was much higher than the 6 ppb Cd in vivo exposure for the lobsters, the tissue metal levels were much less disparate because of the ability of the living animals to take up metal. Lobsters exposed for 30 days to 6 ppb cadmium accumulated from 1.3 to 3.4 ppm Cd in gill tissue (THURBERG et al. 1977). While the ouabain-insensitive ATPase activity in lobster gill tissue may result from several different enzymes, the largest part of the activity probably represents the mitochondrial Mg^{2+}ATPase involved in energy metabolism (PANEFSKY & WARNER 1965). THURBERG and his coworkers (1977) noted a statistically significant increase (approximately 15%) in gill tissue respiration in lobsters exposed to cadmium. One possibility, therefore, is that increase ATPase activity in the in vivo exposed lobster results indirectly from increased metabolic activity within gill tissue, rather than being a direct effect of cadmium on the enzyme.

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