INHIBITION OF NA^+ K^+ ATPase BY ZINC AND CADMIUM IN GILL MICROSOMAL PREPARATIONS OF THE GREEN SHORE CRAB *(CARCINUS MAENAS)*

Torstein Hølleland[1], Janet Wrestler[2] and David Towle[3]
[1]Zoological Laboratory, University of Bergen, 5007 Bergen, Norway
[2]University of Richmond, Richmond, VA 23173
[3]Department of Biology, Lake Forest College, Lake Forest, IL 60045

Na^+ K^+ ATPase as well as Na^+ transport in microsomal preparations of gills of the green shore crab have been shown to be inhibited by vanadate (Hølleland, Shetlar, McDonald, Alexander and Towle, Bull. MDIBL 27: 63-65, 1987-1988). Our current investigations show that other metals, i.e. zinc and cadmium, have a similar inhibitory effect on Na^+ K^+ ATPase activity.

Green shore crabs *(Carcinus maenas)* were caught at night while feeding on the mussel beds adjacent to Salsbury Cove, Maine. In the laboratory, the crabs were maintained at 10 ppt sea water and 13-18 C in recirculating aquaria for at least one week before experimentation. The crabs were fed mussels, *Mytilus edulis*, caught in the same area. The microsomal fraction was prepared from posterior gills as described earlier (Towle and Hølleland, Am. J. Physiol. 252: R479-R489, 1987, and Hølleland *et al.*, 1987-88). The final enzyme preparation had a protein content of about 2 mg protein/ml as determined by the coomassie blue method (Bradford, Anal. Biochem. 72: 248-254, 1976). Ten ul of the enzyme preparation in a total volume of 2.0 ml were used in the assay. The enzyme activity was determined according to Towle, Palmer and Harris (J. Exp. Zool. 196: 315-322, 1976) with the modification of using Tris buffer instead of imidazole and 1 mM ATP instead of 5 mM ATP (due to the strong inhibition at 5 mM ATP, Hølleland *et al.*, this volume). Aqueous stock solutions of $ZnSO_4$ and $CdCl_2$, ranging in concentrations from 2 uM to 20 mM, were kept refrigerated. All assays were done in triplicates.

Data analyses show that Zn and Cd inhibit the Na^+ K^+ ATPase (Fig. 1) and change the V_{max} but not the K_m (earlier described as non-competitive inhibition, but recently termed as mixed inhibition; Cornish-Bowden and Wharton, Enzyme Kinetics, in: In Focus, D. Rickwood, ed., Press Limited, 1988). The concentrations of Cd at which the Na^+ K^+ ATPase and the residual Mg^{++} ATPase is 50% inhibited (the I_{50} values) are 0.1 and 0.4 uM, and for zinc the I_{50} values are 0.2 and 20 uM respectively. Earlier reports on inhibition of Na^+ K^+ ATPase show similar results for cadmium (Kinne-Saffran, Schutz, Scholermann, Girard and Kinne, Bull. MDIBL 26: 15-17, 1986) and zinc (Donaldson, St-Pierre, Minnich and Barbeau, Can. J. Biochem. 71: 1217-1224, 1971), but no reports on such inhibition of the Mg^{++} ATPase have been found.

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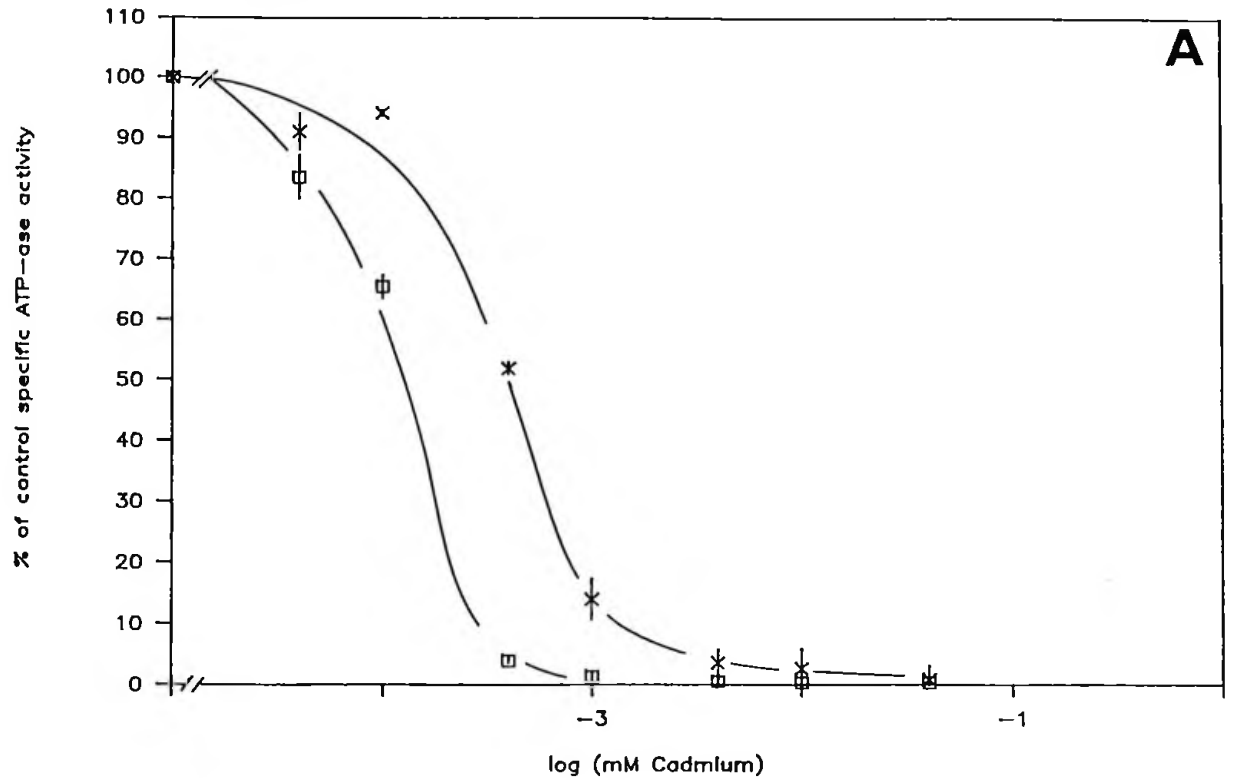

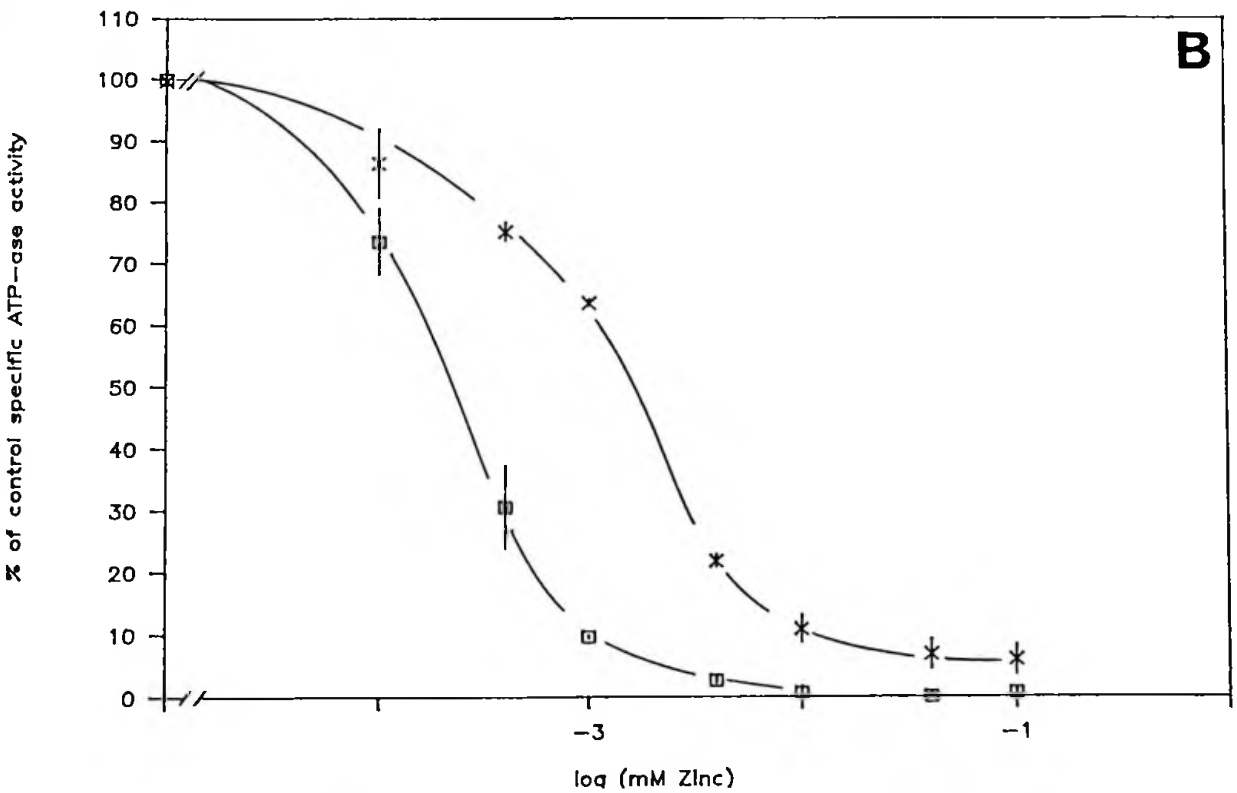

Figure 1. The specific activities of the Na^+ K^+ ATPase (squares) and the Mg^{++} ATPase ("X"es) are plotted as percent of control (no inhibitor) against the logarithm of concentration (mM) of inhibitor (cadmium in A and zinc in B). Bars represent standard errors of mean values, each determined in triplicate. Representative data from three separate experiments are presented.