EFFECT OF HEAVY METALS ON RECTAL GLAND VOLUME, MORPHOLOGY AND CYTOSKELETON OF SHARK (SQUALUS ACANTHIAS) RECTAL GLAND

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Cadmium is a toxic metal with an ubiquitous distribution in the environment. The mechanism by which cadmium exerts a toxic effect is not known. However, there are several possible sites of action. One is that the metal inhibits the Na-pump. In fact, 10^{-5} M cadmium completely inhibits Na,K,-ATPase activity in membrane vesicles prepared from shark rectal gland (Kinne-Saffran et al., Bull. Mt. Desert Isl. Biol. Lab. 26:15-17, 1986). In this study sidedness was not determined, i.e., the cadmium was on both sides of the membrane vesicle. This has important implications for the potential toxicity of cadmium in the marine envirnoment since, if inhibition occurred only after penetration to the cytosolic surface, then acute exposures may not be detrimental to gland function. To investigate this we exposed slices of rectal gland to cadmium for one and three hours. At the end of this time period cellular ions and volume were determined as described previously (Kleinzeller and Goldstein, J. Comp. Physiol. B154:561-571, 1984). The results are shown in Table 1:

Table 1
Effect of cadmium on intracellular ion concentration and water content

	H_2O_i (kg/kg D.W)	[Na] (mM)	[K] (mM)
Control (N=4)	2.43 ± .10	83 ± 10	112 ± 5
1mM Cd - 1h (N=3)	2.36 ± .03	80 ± 5	101 ± 3
1mM Cd - 3h (N=4)	2.45 ± .14	86 ± 7	99 ± 5

Cadmium had no effect on the concentration of Na and K, indicating that at 1mM this metal does not inhibit the Na-pump after a three hour exposure. Thus we conclude that the inhibition of the Na^+,K^+-ATPase in vesicles is due to an action on the cytosolic side of the membrane and that in intact cells of the rectal gland cadmium does not readily reach this site.

Mercurials are well known environmental toxins. Previously it has been demonstrated that in the rectal gland exposure to the organic mercurial PCMBS leads to a loss of K^+ and a gain of Na^+, with little cell swelling, in the first two hours, followed by a massive swelling in the third hour of exposure (Kleinzeller et al., Bull. Mt. Desert Biol. Lab. 163-164). This indicates that PCMBS could be acting at two sites, one that affects maintenance of normal ion gradients (Na-pump) and, another that may be involved in the volume control mechanism. Organic mercurials are known to disrupt the actin cytoskeleton in red blood cells (Kunimoto et al., Biochim. Biophys. Acta 905:257-267). Thus one site of action of PCMBS in the rectal gland may be the actin filament system.We analyzed the effect of PCMBS on cell morphology and the distribution of actin in rectal gland slices at early time points and after prolonged exposure to the mercurial. We found that at one hour the cell structure, as determined in the light microscope, was similar to controls whereas at three hours the tubular cells were clearly swollen. The swelling was characterized by the appearance of "lakes" or enlargements of the basal cell compartment. The apical pole of the cell was not significantly altered and the tubular lumens were patent. Analysis of the distribution of F-actin (Mills et al. Bull. Mt. Desert Isl. Biol. Lab 26:13-14) showed that the apical microvillar localization was retained throughout the exposure to PCMBS. At one hour there was a reduction in the actin associated with the basolateral memebrane. However this varied between tubules and even within cells from the same tubule. At three hours the association of actin with the basolateral membrane was lost. Exposure to 1 mM N-ethylmaleimide for up to five hours had no effect on cell

structure or actin distribution. These results indicate that one potential site of action of PCMBS in the shark rectal gland is the actin filament system and that disruption of the cytoskeleton may play a part in the massive swelling seen after prolonged exposure to this mercurial. Whether cytoskeletal changes occur at mercurial levels where binding saturates, 0.01 mM (Booz, et al., Bull. Mt. Desert Isl. Biol. Lab. 27: 1-3)), or the effects are reversible, 0.1 mM (F. Ziyadeh, personal commun.) remains to be determined.

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