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Cadmium Accumulation in the Mummichog, *Fundulus heteroclitus,* Adapted to Various Salinities

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It is commonly known that salinity affects the toxicity of cadmium to aquatic biota. This toxicity has been shown to decrease with salinity in a variety of estuarine organisms (Verriopoulos and Moraitou-Apostolopoulou 1981; Voyer et al. 1982). The effect of salinity on cadmium toxicity may be primarily due to greater complexation of free cadmium ions by chloride ions with increasing salinity (Engel and Fowler 1979). A high concentration of cadmium is found in the liver, kidney, intestine, and gills of fish after cadmium exposure (Kuroshima 1987). The kidney, intestine, and gills are important organs for osmotic regulation of fish and each function of these organs is altered by salinity of the medium in which fish live. This alteration in the kidney, intestine, and gills may influence cadmium accumulation. This study was conducted to examine cadmium accumulation in tissues of mummichog, Fundulus heteroclitus, adapted to various salinities and to evaluate the effects of salinity on cadmium accumulation from a physiological viewpoint.

MATERIALS AND METHODS

Adult males (7.8-14.1 g, N=45) and juveniles (0.4-1.2 g, N=45) of mummichog, Fundulus heteroclitus, reared at our facility were used. The fish were kept in adaptation media of freshwater, 5%, 10%, 15%, 20%, 25%, 50%, 75%, and 100% seawater for at least three weeks before the experiments. Groups of five each were exposed to 1 mgCd/l for 24 hrs at 21.8℃ in 20-l glass aquaria with aeration. The concentration and time for exposure were determined by results from preparatory tests. Fish were not fed for 24 hrs

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prior to testing nor during testing. Each test salinity was prepared by dilution of seawater from Sagami Bay (salinity: 32‰, pH: 7.7, Ca: 390 mg/l, Mg: 1000 mg/l), filtered through activated carbon, with tap water (pH: 7.7, Ca: 13 mg/l, Mg: 3 mg/l) dechlorinated by aeration. At the end of the 24-h exposure, the fish from each experimental aquarium were removed and rinsed with cadmium-free water and 10 mM EDTA. The hepatopancreas, kidney, intestine, and gills were dissected from adult fish. Samples for cadmium determination were stored at -80℃ before use.

Data from animal experiments demonstrate that cadmium is bound to albumin and proteins with higher molecular weights in blood initially after exposure and mainly taken up by the liver, while cadmium bound to metallothionein synthesized in the liver is taken up by the kidney. Therefore, single administration of metallothionein-bound cadmium as well as long-term administration of cadmium gives rise to an uptake of cadmium in the kidney. In the present study, the relationship between the accumulation of metallothionein-bound cadmium and the kidney function for osmotic regulation was examined. Adult males (5.9-12.7 g, N=21) of mummichog were divided into five test groups. After acclimatization to freshwater, and 25%, 50%, 75%, and 100% seawater, they were anesthetized with MS 222 and administered purified rabbit liver metallothionein (Sigma) containing 75.2 mg cadmium per mg solid. The administration was done by a single intraperitoneal injection of 0.5 mgCd/kg BW, dissolved in distilled water. Ninety-six hrs later, the hepatopancreas and kidney were dissected and stored at -80℃.

Each tissue sample for cadmium analysis was freeze-dried and digested with a 1:1 mixture of HNO_3 and $HClO_4$. Cadmium analysis was conducted by atomic absorption spectrophotometry (Hitachi Z-8000 with Zeeman modulation) using reference standards from $CdCl_2$ prepared in 1 N HCl. The detection limit was 0.01 ngCd/l. Dunnett's multiple range test was used to determine significance.

RESULTS AND DISCUSSION

Results from cadmium exposure of mummichog at various salinities are shown in Table 1. Cadmium concentrations in the whole body of juveniles, and in the hepatopancreas, kidney, and gills of adult fish disproportionately decreased with increasing salinity, ranging from freshwater to 25% seawater and showed no further change at higher salinity. Depression of cadmium accumulation was most remarkable in the gills.

Freshwater fish take up most of the ions necessary for homeostasis from water via the gills (Eddy 1982). Verbost et al. (1987) reported that Cd^{2+} readily enters branchial epithelial cells of freshwater fish, as does also Ca^{2+} via La^{3+}-sensitive apical Ca^{2+} channels. Pärt et al. (1985) proposed that gill permeability for cadmium decreases with increase in the hardness of water. The hardness of freshwater and 25% seawater used in this study was approximately 45 mg/l and 250 mg/l as $CaCO_3$, respectively. The rapid decrease in cadmium accumulation in the gill, hepatopancreas, and kidney of mummichog exposed in freshwater to 25% seawater was, thus, probably due to decrease in the active uptake of cadmium at the gills. The cadmium concentration in the intestine increased

Table 1. Cadmium accumulation in whole body samples of juveniles and in individual tissues of adult mummichog exposed to 1 mgCd/l for 24 hrs at various salinities.

	FW	5 % SW	10 % SW	15 % SW	20 % SW	25 % SW	50 % SW	75 % SW	100 % SW
Whole body	1.39 ± 0.38	0.57 ± 0.14 ***	0.52 ± 0.10 ***	0.77 ± 0.37 ***	0.42 ± 0.12 ***	0.26 ± 0.09 ***	0.21 ± 0.07 ***	0.21 ± 0.02 ***	0.22 ± 0.06 ***
Hepato-pancreas	3.33 ± 1.99	1.12 ± 0.66 ***	0.79 ± 0.41 ***	0.58 ± 0.27 ***	0.27 ± 0.04 ***	0.19 ± 0.12 ***	0.18 ± 0.12 ***	0.14 ± 0.07 ***	0.29 ± 0.25 ***
Kidney	9.44 ± 5.10	2.05 ± 0.57 ***	1.92 ± 0.67 ***	1.31 ± 0.56 ***	0.71 ± 0.22 ***	0.54 ± 0.19 ***	0.46 ± 0.15 ***	0.42 ± 0.24 ***	0.48 ± 0.12 ***
Intestine	2.34 ± 0.45	4.28 ± 5.37	2.06 ± 1.35	3.80 ± 2.69	3.59 ± 3.55	6.97 ± 2.40	6.15 ± 1.36	6.99 ± 1.99	9.90 ± 3.33 ***
Gill	7.34 ± 2.25	1.83 ± 0.27 ***	1.08 ± 0.27 ***	0.80 ± 0.24 ***	0.52 ± 0.12 ***	0.54 ± 0.17 ***	0.27 ± 0.05 ***	0.35 ± 0.11 ***	0.55 ± 0.14 ***

Values are means ± SD for five individual fish.
unit : μg/g of wet weight
*** : $p<0.001$
FW : freshwater, SW : seawater

with salinity from 2.34±0.45 μg/g (wet weight) in freshwater to 9.90±3.33 μg/g in 100% seawater. Marine fish swallow seawater and absorb water together with monovalent ions from the intestine to compensate for water loss from the body. The higher concentration of cadmium in the intestine of mummichog adapted to the higher salinities may possibly have been due to the ingestion of water containing cadmium. Since water is actively, but cadmium poorly, absorbed from the intestine, cadmium dissolved in the water can be concentrated in the intestinal tract. In mammals, cadmium orally administered induces histopathological changes (Phillpots 1986), the inhibition of enzyme activity (Kobayashi and Kimura 1985) and transport of nutrients (Iturri and Peña 1986) in the small intestine. The intestinal function of seawater fish may possibly be damaged during exposure to cadmium.

Data from mammal experiments generally demonstrate cadmium bound to metallothionein to be less effectively trapped by the liver and mostly taken up by the kidney (Suzuki 1984). The results from the administration of metallothionein by an intraperitoneal injection are given in Table 2. The cadmium concentration in the kidney decreased with increasing salinity and at salinities of 75% and 100% seawater were significantly lower than those in freshwater. The cadmium concentration in the hepatopancreas showed a tendency to decrease with

Table 2. Cadmium accumulation in the kidney and hepatopancreas of mummichog 96 hrs after intraperitoneal injection of metallothionein.

	FW	25%SW	50%SW	75%SW	100%SW
Kidney	7.17 ± 1.45	5.32 ± 4.25	4.62 ± 1.51	2.92 ± 1.66 *	2.31 ± 0.94 **
Hepato-pancreas	6.46 ± 3.12	9.00 ± 3.27	12.75 ± 5.61	12.98 ± 7.03	12.94 ± 3.16
Ratio	0.8583 ± 0.3798	0.7694 ± 0.7526	0.4241 ± 0.2282	0.2420 ± 0.0789	0.1952 ± 0.1123

Values are means ± SD for four individual fish except for five in FW.
unit : μg/g of wet weight
* : $p<0.05$, ** : $p<0.01$
FW : freshwater, SW : seawater

salinity. The ratio of cadmium concentration in the kidney to that in the hepatopancreas in each fish decreased with salinity. The function of the kidney to maintain the proper osmotic conditions in the body may possibly be related to the accumulation of cadmium bound to metallothionein in the kidney. Kuroshima (1992) reported cadmium accumulation to be high in the liver and kidney of red sea bream but only in the kidney of carp after intraperitoneal injection of metallothionein. Little cadmium was found in the intestine and gills. In the kidney of freshwater fish, essential electrolytes, glucose, and vitamins are selectively reabsorbed from urine at the proximal tubules, while the main function of the kidney of marine fish is excretion of divalent cations. Cadmium bound to metallothionein can be freely filtered through the glomeruli and reabsorbed at the proximal tubules with other electrolytes (Foulkes 1982). The low cadmium concentration in the kidney of mummichog adapted to high salinity may, thus, possibly be due to the lack of active reabsorption at the proximal tubules. That the glomerular filtration rate in marine fish is generally lower than in freshwater may also be a contributing factor.

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