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Effect of Waterborne and Dietary Cadmium on Plasma Ions of the Teleost Oreochromis mossambicus in Relation to Water Calcium Levels


*Department of Zoology, University of Dar es Salaam, P. O. Box 35064, Dar es Salaam, Tanzania, and **Department of Zoology, University of Nijmegen, 6525 ED Nijmegen, The Netherlands

Abstract. The effects of cadmium administered via ambient water or food on plasma ions of the African freshwater cichlid Oreochromis mossambicus were studied for 2, 4, 14, and 35 days, in low calcium (0.2 mM) and high calcium (0.8 mM) water. In low calcium water, an environmentally relevant concentration of 10 μg/L water-borne cadmium induced a significant and dramatic hypocalcemia on days 2 and 4. Recovery of plasma calcium was observed on days 14 and 35. Hypermagnesemia was observed on day 2, but normal levels were already found on day 4. In high calcium water adapted fish, the extent of hypocalcemia and hypermagnesemia was less pronounced than in fish from low calcium water. Water-borne cadmium caused no significant changes in plasma phosphate, sodium, potassium, or osmolality. On days 2 and 4, dietary cadmium (averaging 10 μg Cd/fish/day) caused hypermagnesemia and hypocalcemia in low calcium water-adapted fish. Recovery was observed on days 4 and 14, respectively. In fish from high calcium water, dietary cadmium caused a significant reduction in plasma calcium on day 4 only; plasma magnesium was unaffected. Hyperphosphatemia was apparent on day 14, irrespective of the water calcium concentration. No changes in plasma sodium, potassium, or osmolality were found.

The results show that sublethal concentrations of cadmium, administered via the water as well as via the food, affect calcium and magnesium metabolism in tilapia. High water calcium ameliorates the effects of both water and dietary cadmium on plasma calcium and magnesium levels.

Among the various heavy metal pollutants, cadmium is frequently present in natural water bodies as a result of discharges from industrial processes or other anthropogenic contamination. The harmful effects of cadmium on mammals and other terrestrial animals have been widely studied and reviewed (Flick et al. 1971; Vallee and Ulmer 1972; Webb 1979; Korte 1983; Foulkes 1986). Aquatic vertebrates such as fish, live in very intimate contact with the environment through their gills. This makes them very susceptible to aquatic pollutants.

Since it is well established that freshwater fish take up most of the ions necessary for homeostasis from the water via the gills (Eddy 1982), cadmium-induced plasma ionic disturbances are apparently caused by impaired uptake and diffusional losses of ions via these organs (Larsson et al. 1981; Giles 1984). Ionic disturbances have also been reported after exposure of fish to sublethal concentrations of heavy metals. For example, changes in the plasma ionic composition have been observed in fish exposed to copper and zinc (Lewis and Lewis 1971; Spry and Wood 1985), mercury (Lock et al. 1981), and chromium (Van der Putte et al. 1983). With respect to cadmium, exposure of rainbow trout to sublethal levels induced hypocalcemia, with reduced plasma sodium, potassium, chloride and increased plasma magnesium (Giles 1984). In European flounder, cadmium-induced hypocalcemia and elevated levels of plasma phosphate, magnesium and potassium were observed (Larsson et al. 1981).

In addition to water, food could also be a source of cadmium for fish, since it accumulates in aquatic organisms through trophic transfers (Anonymous...
Water-Borne and Dietary Cd in Fish

1971; Williams and Giesy 1978; Coombs 1979). Indeed, Bryan (1976) concluded that food as a source of Zn, Mn, Co, and Fe for molluscs, crustaceans and fish was more important than water. From various studies on both water-borne and food-containing metals, reviewed by Dallinger et al. (1987), there is evidence that uptake of heavy metals such as Cd, Cu, Co, Pb, Hg, and Zn from food is also the predominant pathway in freshwater fish. Koyama and Itazawa (1977) reported significant hypocalcemia and elevated plasma phosphate levels in cadmium-fed carps. Similarly, plaice and thornback ray both accumulated more cadmium from food than from seawater (Pentreath 1977). In general, cadmium concentrations in natural waters are extremely low and a more important route of cadmium uptake by fish may be represented via the gut. Experiments with dietary cadmium may therefore yield more representative information for field situations.

In this investigation, we have compared the effects of a sublethal concentration of cadmium administered via the water or via the food in the African cichlid fish Oreochromis mossambicus (tilapia). Plasma ions and osmolality were determined. Cadmium was administered at sublethal concentrations, in the order of magnitude that may occur in natural waters (<10 μg Cd/L). In many studies aimed at evaluating the effects of cadmium on fishes, high concentrations (>1 mg Cd/L) of cadmium have been used. Hence severe physiological, behavioral and detrimental effects have been reported. Such high concentrations are rarely found in nature, except in cases of spillage or heavily polluted waters. The Working Group on Cadmium Toxicity (EIFAC 1977) has suggested that chronic exposure to low cadmium concentrations is more relevant to understanding the mechanisms involved in the intoxication process in teleost fish.

We further studied the influence of relatively low and high calcium concentration of the water on the toxic effects of cadmium. The effects of water hardness (mainly Ca²⁺ and Mg²⁺ ions) on heavy metal toxicity have been demonstrated in various species of teleosts (Pärt et al. 1985). Increased toxicity of cadmium to fish in soft water as compared to hard water has been demonstrated in catfish and guppies (Kinkade and Erdman 1975), goldfish (McCarty et al. 1978), striped bass (Palawski et al. 1985), brook trout (Carroll et al. 1979) and rainbow trout (Calamari et al. 1980; Pasco et al. 1986). Similar observations on teleosts exposed to zinc, copper and lead (Sinley et al. 1974; Zitko and Carson 1976; Judy and Davies 1979; Lauré and McDonald 1986) indicate a protective role of calcium against the toxic effects of heavy metals. It was also investigated whether the protective effect of the water-calcium concentration is limited to water-borne cadmium only, or also applies to dietary cadmium.

Materials and Methods

Freshwater acclimated laboratory stock of male Oreochromis mossambicus (tilapia) ranging from 12–14 cm in total length and a body weight of 16 to 25 g were used in the present study. Fishes were maintained in 100 L aquaria with continuous aeration and circulating filtered water, pH 7.4 (360 L/hr, Eheim pumps 1021) at 28°C on a daily 12 hr photoperiod.

Experimental Design

Fish were divided into six groups, three of which were kept in low calcium water (0.2 mM Ca²⁺), and three in high calcium water (0.8 mM Ca²⁺). Fish stock was kept at 0.8 mM Ca²⁺ in tapwater. Three weeks before the start of the experiments the fish were transferred to artificial freshwater of a similar composition as the tapwater, prepared according to Flik et al. (1985a). Adaptation of fish to water of 0.2 mM Ca²⁺ was performed by gradual reduction of the water calcium concentration during one week, followed by a period of two weeks in low calcium water before the start of the experiment. The concentrations of several electrolytes of low and high calcium water are shown in Table 1. The fish were exposed to either 10 μg Cd/L of ambient water or were fed Cd-containing food averaging 10 μg Cd/fish/day. The experimental set-up and cadmium exposure were as follows;

- a. group 1: low calcium water containing 10 μg Cd/L;
- b. group 2: high calcium water containing 10 μg Cd/L;
- c. group 3: low calcium water, 10 μg Cd/fish/day via the food;
- d. group 4: high calcium water, 10 μg Cd/fish/day via the food;
- e. group 5: low calcium control;
- f. group 6: high calcium control.

Cadmium was administered to water from a stock solution of 1,000 μg Cd/L (Cd(NO₃)₂; RCB, Bruxelles). The cadmium concentration in all tanks was monitored daily in a Video 11 Atomic Absorption Spectrophotometer (AAS Thermo Jarrell Ash USA) at 228.8 nm, fitted with a Furnace Aerosol Sampling Technique with Automatic Calibration (IL FASTAC II™) and Furnace Analyzer Model IL 655. The aquarium water was changed twice a week.

Food was prepared as a mixture containing Tetramin tropical fish food (5%), gelatin (5%) and agar (1%), dissolved in warm distilled water. Cadmium was blended into this mixture giving a final concentration of 10 mg Cd/kg of food for oral administration to groups 3 and 4. Samples from this food were digested in

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<th>Table 1. The concentration of several electrolytes in mM of normal and high calcium water; Pᵢ, inorganic phosphate</th>
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concentrated \( \text{HNO}_3 \) at 60°C, and the cadmium concentration determined. The results showed that the actual concentrations did not deviate more than 2% of the calculated cadmium concentration. Fish were daily fed at a ration of 5% of their total body weight. Per 20 g fish, this amounted to approximately 10 \( \mu \text{g} \) Cd/fish. Group 1, 2, 5, and 6 were fed similar cadmium-free food. Fish were fed twice daily and food was readily eaten. Care was taken to distribute the food evenly over the fish. After feeding, faeces were siphoned off from the bottom of the tank. In groups 3 and 4, the water cadmium concentrations averaged 0.27 ± 0.04 \( \mu \text{g} \) Cd/L, indicating minimum loss of cadmium from the food.

**Sampling and Analytical Procedure**

Fish from each tank were sampled after 2, 4, 14, and 35 days. The fish were slightly anaesthetized in 0.2% 2-phenoxyethanol, briefly blotted dry, and the body weight was recorded. The blood was collected from the caudal vessels in heparinized microhaematocrit capillaries. After centrifugation plasma was collected and the osmolality determined on a Vogel Osmometer. The plasma samples were stored at -20°C for further analysis of the plasma ions. Total plasma calcium was determined spectrophotometrically by a cresolphthalein complexone method (Sigma diagnostics). Plasma magnesium (285.21 nm) and phosphate (177.5 nm) were measured by Inductively Coupled Plasma Atomic Emission Spectrometer (Plasma IL200, Thermo Electron, USA). Plasma sodium and potassium were measured by flame photometer (Model IV Auto-analyzer, Technicon). Since no significant difference between the respective controls was observed, all data were pooled for statistical evaluation. The data for control and experimental groups were analyzed for statistical significance by analysis of variance (ANOVA) and Student's t-test at the 5% level.

**Results**

During the 35-day experimental period, symptoms of cadmium poisoning such as respiratory impairment, tetanic seizures and changes in swimming or feeding activity were not observed in tilapia. No mortality occurred during the experiment. Results on the effects of water-borne and dietary cadmium on plasma ions and osmolality are given in Table 2.

**Calcium**

Exposure of tilapia to cadmium via ambient water or food caused a significant decrease in the plasma calcium levels. The hypocalcemia was more prominent when cadmium was administered via the water than via the food. Following 2 and 4 days of exposure, the reduction of plasma calcium amounted to 40% and 31%, respectively, in fish adapted to low calcium water (group 1), and to 32% and 20% in high calcium water (group 2). Reduction of plasma calcium was also observed on days 2 (22%) and 4 (25%) in group 3, where cadmium was administered via the food in low calcium water acclimated fish, but not in fish in high calcium water (group 4). After 14 days, the plasma calcium levels of all fish showing hypocalcemia at days 2 and 4 (i.e. groups 1, 2 and 3), were back to normal. Thereafter, no changes occurred in plasma calcium levels.

**Magnesium**

Water-borne cadmium caused hypermagnesemia in tilapia adapted to low and high calcium water. After 2 days the plasma magnesium in group 1 increased by 96%, and in group 2 by 37%. Cadmium via the food caused a 52% increase in plasma magnesium in tilapia adapted to normal calcium water (group 3). On days 4, 14 and 35 the plasma magnesium levels were no longer significantly different from the controls. Cadmium fed tilapia in high calcium water (group 4) did not show changes in the plasma magnesium levels, neither during short-term (2 and 4 days) nor during long-term (14 and 35 days) exposure.

**Phosphate**

Exposure to water-borne cadmium had no effect on plasma phosphate. However, hypophosphatemia was observed in the cadmium-fed fish in both the low and high calcium adapted tilapia (Table 2).

**Sodium, Potassium and Osmolality**

Exposure to water-borne or dietary cadmium had no statistically significant effects on either plasma sodium and potassium levels or plasma osmolality in both the low calcium and high calcium adapted fish (Table 2).

**Discussion**

**Water-borne Cadmium**

In low calcium water acclimated fish, short-term exposure to 10 \( \mu \text{g} \) Cd/L in the water induced significant hypermagnesemia and hypocalcemia. Recovery was observed on day 4 for magnesium, and on day 14 for calcium, and no further disturbances...
of these ions were observed on further exposure for 35 days. No significant changes in other electrolytes or in plasma osmolality were found. It is therefore indicated that short-term exposure of tilapia in low calcium water to the relatively low concentration of 10 µg Cd/L, which has been reported to occur in polluted freshwater bodies, causes only a transient imbalance of the divalent cations. Little information on the effects of short-term exposure to sublethal concentrations of cadmium on the osmoionic regulation of teleosts is known to us. Reduced plasma calcium and increased plasma magnesium levels have been observed in European flounders after a four week exposure to water containing 5–500 µg Cd/L (Larsson et al. 1981) and in rainbow trout after exposure to 6.4 µg Cd/L for 3 days (Giles 1984). Recovery as observed for cadmium has also been reported for rainbow trout exposed for 331 days to copper, at a water concentration of 55 µg Cu/L (McKim et al. 1970). Similarly, Laurén and McDonald (1987) reported an apparent recovery of whole body calcium, sodium and potassium ions, 28 days after exposure to 55 µg Cu/L in rainbow trout (1 mM Ca²⁺).

Similar to tilapia from low calcium water, fish from high calcium water showed significant hypocalcemia and hypermagnesemia after short-term exposure to 10 µg Cd/L in the water. The magnitude of this disturbance was less in the high calcium water acclimated fish, than in the fish from low calcium water. The protective effect of calcium to toxic metals in the water has been described before for various metals. For example, when rainbow trout were exposed to cadmium in water containing 0.125, 0.5 and 8.0 mM Ca²⁺, respectively, cadmium toxicity (expressed as 48 hr LC50) decreased with
increasing hardness (Calamari et al. 1980). Cadmium toxicity was about seven-fold lower in the hardwater acclimated fish (8.0 mM Ca\(^{2+}\)) than in fish from water containing 0.5 mM Ca\(^{2+}\). Increased mortality was observed in the larvae and juveniles of striped bass exposed to cadmium in soft water, whereas in hardwater cadmium toxicity and uptake was reduced (Palawski et al. 1985; Wright et al. 1985). Similar effects of water hardness on rainbow trout exposed to copper (Laurén and McDonald 1986) and zinc (Bradley and Sprague 1985) have been reported. In addition to cadmium-induced hypocalcemia, hypermagnesemia was greater in the low Ca water tilapia than in those from high calcium water. Increased plasma magnesium was also found to occur in cadmium exposed rainbow trout (Roch and Maly 1979) and European flounder (Larsson et al. 1981). These findings and those mentioned above clearly demonstrate the protective effect of calcium in metal toxicity.

Notable changes in the concentrations of plasma phosphate, sodium, potassium and osmolality were not observed. High Cd concentrations are known to increase the ionic permeability of the plasma membrane of the blood cells (Plischker 1984; Sørensen et al. 1985). For plasma sodium, conflicting results on the effects of cadmium have been reported for different freshwater and marine teleosts. Larsson et al. (1976) showed increased plasma sodium levels in flounder exposed to cadmium for 15 days, while in a following study on the same species no changes were observed in plasma sodium or osmolality after 4 and 9 weeks of exposure (Larsson et al. 1981). In brook trout, exposure to cadmium significantly increased plasma chloride levels but had no effect on plasma sodium (Christensen et al. 1977), whereas reduced plasma sodium was reported for cadmium-exposed goldfish (McCarty and Houston 1976). The reported differences in the effects of water-borne cadmium on the monovalent plasma ions among teleost could be attributed to species differences in sensitivity, and to differences in the concentration of cadmium used. Our data show that the divalent cations are affected at lower cadmium concentrations than the monovalent ions.

Dietary Cadmium

Oral administration of about 10 \(\mu\)g Cd/fish/day to tilapia in low calcium water reduced plasma calcium. In carp and mammals, oral administration of cadmium was also found to cause a decrease in plasma calcium (Kennedy 1966; Koyama and Itazawa 1977). We further observed increased plasma magnesium levels. Thus, in tilapia, dietary and water-borne cadmium have the same effects on plasma calcium and magnesium levels. We suggest that dietary cadmium circulates through the blood and affects the gills and other osmoregulatory organs in a similar way as cadmium present in the water. This is consistent with observations of Rowe and Massaro (1974), who showed that after an intragastric dose of radioactive cadmium to white catfish cadmium was also present in the skin and gills. It is well established that gills, gut and kidneys are involved in calcium and magnesium handling in fish (Mayer-Gostan et al. 1983; Björnsson and Nilsson 1985; Flik et al. 1985a).

When the same amount of cadmium as administered to fish from low calcium water was fed to fish in high calcium water, only slight hypocalcemia and hypermagnesemia was observed. Thus, not only the effects of water-borne, but also those of dietary cadmium are ameliorated by the calcium concentration of the water.

Sodium, potassium, and osmolality of the blood plasma were not significantly affected by dietary cadmium. Similar to water-borne cadmium, dietary cadmium specifically affects the divalent cations and not the monovalent cations at the low cadmium concentrations used.

However, a substantial difference between the effects of water-borne and dietary cadmium was observed: plasma phosphate levels, which were unaffected in fish exposed to cadmium in the water, significantly increased in fish receiving cadmium via the food. Since it is difficult to imagine that the presence of cadmium in the food stimulates intestinal phosphate uptake, such an increase is possibly caused by mineral mobilization from the bone. No concurrent increase of plasma phosphate was observed when cadmium was administered via the water. In contrast to calcium, magnesium, sodium or potassium entering the body via both the gills and gut of tilapia, phosphate is absorbed exclusively via the gut (Flik et al. 1985b). In mammals, calcium and phosphate homeostasis of the extracellular fluid is highly dependent on the skeletal tissue for the exchange of calcium and phosphate (Martin et al. 1985). It has long been questionable whether minerals can be mobilized from the acellular type of bone that occurs in most teleost fish, including tilapia (Weiss and Watabe 1979). However, it has been shown in our laboratory that phosphate is released from bone in female tilapia put on a phosphate-free diet (Urasa et al. 1985). Skeletal anomalies and changes in the calcium and phosphate composition in fish exposed to heavy metals and other
pollutants have been well documented (Bengtsson et al. 1975; Muramoto 1981; Moreau et al. 1983). It is possible, therefore, that cadmium, directly or indirectly, stimulates bone demineralization, e.g., as a response to the severe hypocalcemia following cadmium exposure, with hyperphosphatemia as a secondary effect. In the present study, analysis of calcium and phosphate concentrations and of the Ca/P ratio of the operculum, scales and caudal finrays showed no difference between the cadmium-exposed and control fishes (data not shown), which points against bone demineralization. However, the amount of phosphate required to produce hyperphosphatemia is small and may not lead to noticeable changes in bone mineral density.

Cadmium and Calcium

Whereas high concentrations of cadmium result in losses of calcium as well as monovalent ions in fish (Giles 1984), the present experiments show that low cadmium concentrations specifically and dramatically reduce plasma calcium levels. Reduction of plasma electrolytes caused by exposure to waterborne heavy metals or other pollutants has been attributed to diffusional losses caused by increased permeability of the gill epithelium to water and ions (Giles 1984). The specific reduction of plasma calcium reported in the present study is unlikely caused by increased branchial permeability, since only calcium is affected. We suggest that the marked hypocalcemia induced by cadmium is caused by disturbance of the active Ca\(^{2+}\)-mechanisms in the gills. Calcium and cadmium share similarity in ionic radius and valency and they may specifically interact and compete in a way different from other ions (Gardiner 1976; Webb 1979). Recently, in our laboratory Verbost et al. (1987a) have shown that the branchial high-affinity Ca\(^{2+}\)-ATPase activity, accounting for most of the calcium uptake of the fish, is extremely sensitive to cadmium. They further demonstrated that this sensitivity is shared with similar calcium-transport mechanisms in rat enterocytes (Verbost et al. 1987b). The specific interaction of cadmium with calcium may explain why plasma sodium, potassium, and osmolality were not affected by the low cadmium concentrations used in the present study, whereas a severe hypocalcemia developed. The ameliorative effects of high water Ca\(^{2+}\) levels on the effects of cadmium on plasma calcium and magnesium may be due to the competition of Ca\(^{2+}\) with cadmium for gill surface sites, as suggested by Pagenkopf (1983).

It is possible that the effects of cadmium on magnesium are secondary to the effects on calcium. In fish from high calcium water, both hypocalcemia and hypermagnesemia were less pronounced compared to fish from low calcium water. Experimental changes in plasma calcium levels in mammals are often associated with changes in magnesium (Ebel and Günther 1980). This has also been observed in fish. For example, in tilapia prolactin-induced hypercalcemia was accompanied by hypomagnesemia (Wendelaar Bonga et al. 1983). It is furthermore possible that hypermagnesemia is caused by renal damage. According to Ebel and Günther (1980), renal dysfunction in mammals is the cause of hypermagnesemia in several kinds of disorders. Also, Giles (1984) observed a significant rise in plasma magnesium, concomitant with a decline in urinary magnesium levels, in rainbow trout exposed to 6.4 \(\mu\)g Cd/L in water.

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