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Uptake and Elimination of Cadmium by Japanese Eel, *Anguilla japonica,* at Various Temperatures

H.-N. Yang, H.-C. Chen

Institute of Zoology, National Taiwan University, Taipei, 107, Taiwan, Republic of China

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There is no evidence that cadmium is biologically essential, but its toxicity to organisms is well known. The so-called Itai-Itai disease in Japan, characterized by osteomalacia and renal tubular malfunction, has been attributed to cadmium poisoning in irrigation water (Yamagata and Shigmatsu 1970). The degree of contamination in aquatic environments is frequently assessed by comparing contaminant concentrations in associated biota. Bioaccumulation, however, is influenced by environmental factors other than the degree of contamination, environmental factors such as salinity (Phillips 1976),temperature (Unlu and Fowler 1979) and pH (Heath 1987).

The use of cadmium in the electroplating industry in Taiwan is intensive, but the wastewater used in this industry is seldom treated and has been a serious problem (Chen 1993). The Japanese eel (*Anguilla japonica*) is an important freshwater aquacultural fish in Taiwan; thus, it is important to know the accumulation and elimination of cadmium in the Japanese eel due to cadmium-polluted water at various temperatures in order to protect eel resources as well as human heath.

MATERIALS AND METHODS

Japanese eels, *A. japonica*, were collected from the Lukan area of Taiwan. The fish were acclimatized at 15, 25 and 30°C in laboratory tanks for at least two weeks. Eels weighing 8-11 g were selected for the experiment. Fifteen fish were transferred to experimental tanks (50L) containing 30 L dechlorinated tap water. The concentrations of cadmium studied were 10, 50 and 100 µg/L. Triplicate cultures were established for each test concentration, and triplicate untreated cultures served as controls. The test water was renewed every three days, and the fish were fed with *Tubifex* sp. The experiment was designed first to expose the fish to the cadmium solution for 28 days and then to transfer them into clean water for another 14 days. The fish were removed weekly for analysis of tissue metal concentration; i.e., fish were sampled at 7, 14, 21 and 28 days after exposure and 7 and 14 days after depuration. Two fish were removed from each tank for metals analysis (a total of 6 fish were removed from each test concentr-

Correspondence to: H.-C. Chen

ation and control). Organs or tissues (gill, liver, kidney, intestine, muscle and bone) were removed from anaesthetized fish and samples were prepared for metal analysis. Cadmium, copper and zinc were determined by atomic absorption spectroscopy (A.A.S., Hitachi Z-6100). The standard reference materials were Reference Materials MA-A-2 IAEA/Monaco (fish fresh homogenate). The recoveries were 91.2±2.6% and the level of detection was 0.005 µg/g for cadmium. The accumulation factor (A.F.) was calculated according to the formula of Holwerda (1991). The results of combined effects of temperature and the concentration of metals were subjected to multiple analysis of variance using 'Statgraf' (Statistical Graphics Corporation, U.S.A.). Statistical difference at a significant level ($p<0.05$) was employed to compare the effects.

RESULTS AND DISCUSSION

The tissue level of cadmium in *Anguilla japonica* exposed to 50 µg Cd/L at different temperatures is shown in Fig. 1. The order of cadmium accumulation in organs was kidney > liver > gill > intestine > muscle and bone. Generally speaking, the uptake of metals in aquatic organisms can occur by two major routes. These involve gills, in the case of dissolved forms, and digestive organs in the case of metals associated with ingested material such as food or sediments (Leland and Kuwabara 1985). The distribution of cadmium in the body depends on the form in which it reaches the blood. Cadmium uptake has been concluded to be the result of passive diffusion through calcium channels in gills (Verbost et al. 1989). Scott and Bradwell (1983) reported that cadmium was first transferred into blood, then into serum albumin and then transported to the storage organs. Inorganic cadmium tends to accumulate first in the liver, but cadmium administered as thiol complex can be more readily taken up by the kidneys (Hammond and Foulkes 1986). Our results showed that greater than 95 % of the total body burden of cadmium was in the kidney, liver and gill. Quantitatively, these organs are the most important in sequestration of cadmium (Thomas et al. 1985). Therefore, kidney and liver were the critical organs for cadmium accumulation and could be used as indicators of cadmium pollution. For kidney, liver, gill and intestine, the accumulation of cadmium paralleled the overload of this metal in the water. In our study, temperature also had an affect on accumulation. For kidney, liver and gill, the increase in temperature increased the accumulation, but this was not so for intestine. Temperature coefficients (Q10) for accumulation frequently exceed two, but excretory processes are undoubtedly temperature- dependent as well, thus, when body burden is measured, one is actually seeing the results of these opposite forces (Heath 1987).

In this study the longer the exposure period, the higher the level of the cadmium accumulation in kidney (Fig.2). Suzuki et al. (1990) found that there seemed to be no homeostatic regulation processes for all the non-essential metal, and that cadmium was taken up by liver and kidney without any regulation. We also found that at the higher test concentrations, depuration for 14 days did not eliminate the cadmium content, but it continued to accumulate (Fig.2). This may

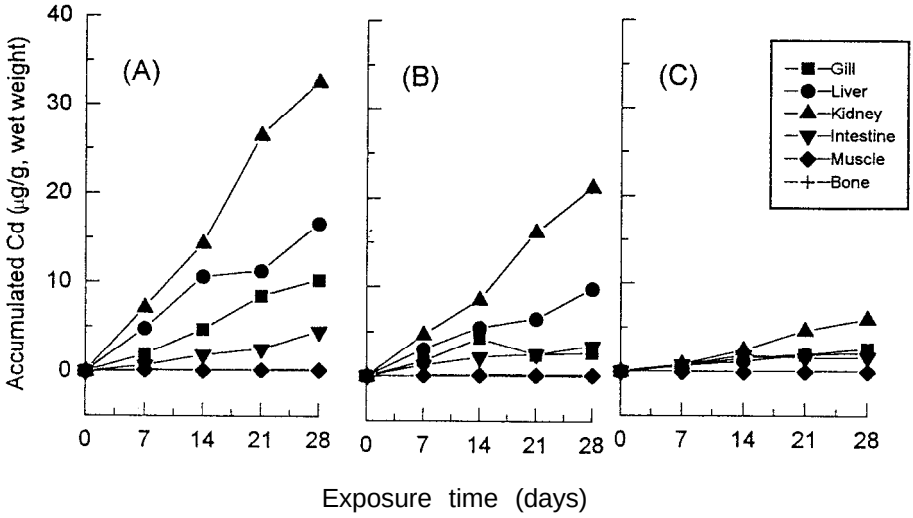

Figure 1. Cd-accumulation in organs *of Anguilla japonica* when exposed to 50 μg Cd/L at (A)30°C(B)25°C(C)15°C for 28 days.

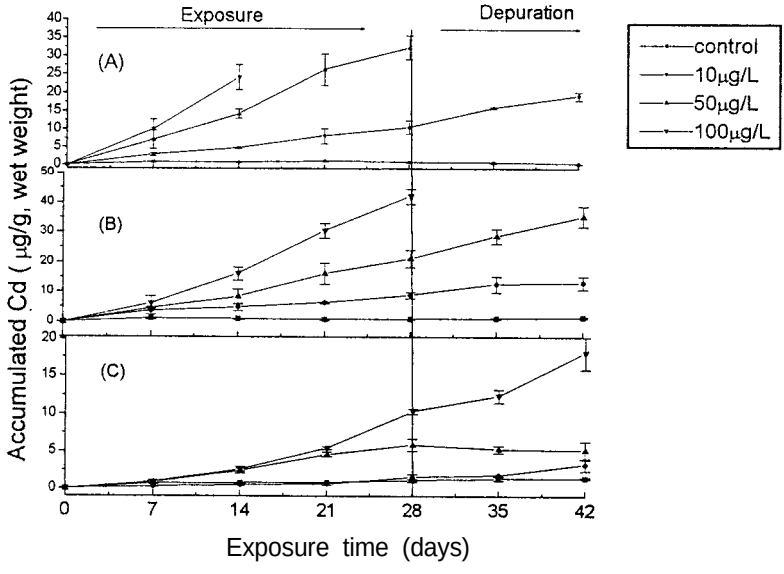

Figure 2. Cd-accumulation *in* kidney of *Anguilla japonica* at (A) 30°C (B) 25°C(C) 15°C for 28 days, followed by transfer into clean water.

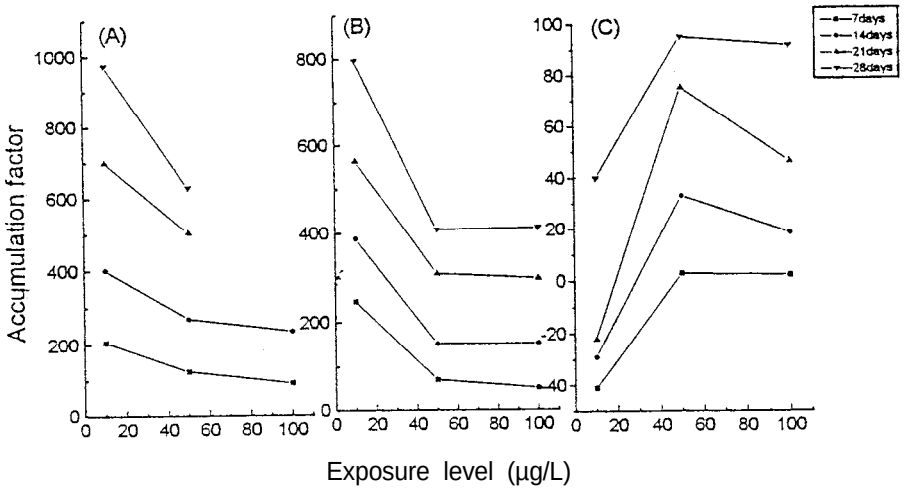

Figure 3. Accumulation factor in kidney of *Anguilla japonica* at (A) 30°C (B) 25°C(C) 15°C for 28 days.

have been caused by the increase of cadmium concentration in the liver during the clearance period. Kuroshima (1987) found that the cadmium level in the liver of *Girella punctata* still increased during the clearance period in cadmium-free seawater after the end of exposure, and he suggested that cadmium once taken up in a body is hardly excreted but is redistributed among tissues. Turnover of cadmium is very slow, and this results in body burdens that can be cumulative over an individual's lifetime (Roesijadi 1992). Cadmium elimination from the tissue is biophasic. It is known that aquatic organisms have legends of various binding strengths by which metal transport across the membranes may be facilitated. Therefore, the biophasic elimination of cadmium may be composed of the rapid elimination of cadmium bound weakly to legends and the slow elimination of cadmium bound strongly to legends (Kuroshima et al. 1993). However, we found that at the lower concentration, the accumulation was not much different between the exposure and depuration period. Temperature may influence the rate of reaction of the organism to the poison, especially at the survival time (Abel 1989). This was observed here as the fish exposed to 100 μg Cd/L for 14 days and 50 μg Cd/L for 28 days at 30°C, 100 μg Cd/L for 28 days at 25°C were all dead, but not in any of the treatments at 15°C (Fig.2).

The calculated accumulation factor (AF) has two major purposes: first, to measure how much cadmium is accumulated with respect to aqueous exposure concentration; second, to find the finite limit in the ability of fish to accumulate metals (Sorensen 1991). In our study, the longer the exposure period, the higher the accumulation factor (Fig.3). Theoretically, the most stable AF values would be those obtained at equilibrium time, when the concentration of cadmium in the body (or tissue) is stable with respect to the aqueous concentration.

Accumulation factor values are probably more representative of chronic than acute cadmium exposures (Sorensen 1991). In the present study with cadmium, we also found that the higher the concentration of cadmium, the lower the accumulation factor at 30 and 25°C(Fig.3). Temperature also had a very marked influence on the accumulation of cadmium: the higher the temperature, the higher the accumulation factor. This coincides very well with higher cadmium toxicity with higher temperature. Similarly, the lower toxicity of cadmium to eel may have been due to a decrease in the ability to accumulate cadmium at 15°C. Accumulation was not much different between 50 and 100 μg Cd/L which indicates that the toxic effects of cadmium were similar between these two treatments.

In fish exposed to a sublethal level of cadmium, the majority of the intracellular cadmium is eventually distributed to the cytosol. Gel filtration chromatography of cytosol reveals that a large portion the cadmium elutes together with

Table 1. The ratio of *Cu* and *Zn* in kidney of *Anguilla japonica* exposed to cadmium at three different temperatures for 28 days, followed by transfer into clean water for 14 days. There were no significant differences among the treatments ($p<0.05$).

Ratio of Cu and Zn

Temp. (°C)	Cadmium(Cd^{+2}) concentration (μg/L)	Exposure period (days) 7	14	21	28	Depuration period (days) 7	14
30	0	0.18±0.02	0.18±0.02	0.23±0.08	0.23±0.05	0.24±0.00	0.18±0.01
	10	0.18±0.03	0.26±0.06	0.27±0.04	0.36±0.03	0.36±0.03	0.35±0.02
	50	0.17±0.03	0.18±0.02	0.22±0.06	0.17±0.08	———	———
	100	0.21±0.07	0.15±0.05	———	———	———	———
25	0	0.10±0.02	0.17±0.01	0.20±0.08	0.13±0.01	0.16±0.03	0.11±0.02
	10	0.34±0.05	0.37±0.09	0.39±0.09	0.34±0.06	0.33±0.02	0.27±0.01
	50	0.21±0.09	0.43±0.05	0.28±0.09	0.28±0.08	0.28±0.06	0.28±0.03
	100	0.22±0.03	0.22±0.09	0.18±0.05	0.15±0.07	———	———
15	0	0.15±0.04	0.26±0.08	0.18±0.10	0.18±0.00	0.22±0.06	0.26±0.05
	10	0.24±0.09	0.21±0.11	0.25±0.08	0.32±0.12	0.23±0.02	0.24±0.04
	50	0.35±0.09	0.41±0.12	0.45±0.18	0.52±0.15	0.27±0.05	0.33±0.08
	100	0.23±0.06	0.27±0.06	0.27±0.03	0.28±0.03	0.27±0.05	0.26±0.04

metallothionein (MT) (Hogstrand and Haux 1991). Metallothionein has a central role in regulation of essential metals, such as zinc and copper, and in the detoxification of these and non-essential metals, such as cadmium and mercury (Roesijadi 1992). In our study the ratios of copper and zinc were not significantly different among the treatments (Table1). It would appear that cadmium is unable to displace zinc (copper) from endogenous MT, except in vitro (Thomas et al. 1985). Clearly, more information is required on the regulation of zinc and copper by MT when fish are exposed to cadmium pollution.

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