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Cadmium Kinetics in Freshwater Clams. Uptake of Cadmium by the Excised Gill of *Anodonta anatina*

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There are several, and sometimes conflicting, reports on metal interaction during bioaccumulation from a mixture of heavy metals by marine or estuarine organisms. Concerning the influence of zinc on Cd uptake, it was found in a previous study with the freshwater clam *Anodonta cygnea* (Hemelraad et al. 1987) that, in accordance with most other investigations (Bryan and Hummerstone 1973; Eisler and Gardner 1973; Jackim et al. 1977; Ray et al. 1979), zinc retarded the accumulation of cadmium when present in a hundred-fold excess over the latter metal. Moreover, the presence of zinc also affected the distribution of cadmium between the separate organs, as well as the subcellular and molecular distribution. In the only in vitro investigation known, Carpene and George (1981) have shown that the uptake of cadmium by the excised gills of the sea mussel *Mytilus edulis* was not affected by co-exposure with other metal ions or by the presence of metabolic inhibitors. By contrast, bioaccumulation of cadmium in *M. edulis* was strongly reduced by co-exposure to zinc in a hundred-fold excess over cadmium (Jackim et al. 1977).

The clear effect of zinc on Cd accumulation in *A.cygnea* prompted us to investigate this phenomenon in an in vitro model. The primary aim was to detect whether the in vivo effect of zinc is caused by a direct influence on the gill epithelium or is sustained by a behavioral response of the animal. At the same time, the possible effect of some other exogenous factors on Cd uptake was examined. In addition, it was investigated whether the rate of in vitro uptake is a function of gill size.

MATERIALS AND METHODS

Specimens of the naiad clam, *Anodonta anatina* L., were acclimated to laboratory conditions for 3 to 5 wk, prior to use. The animals were kept in glass tanks, provided with a sandy sediment, in moderately streaming tap water (12°C ± 1°), and were not fed. Main water quality parameters were:

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Ca : 30-57 mg/L	Fe : 0.1 mg/L	SO_4	: 11-20 mg/L
Mg : 3-4 mg/L	HCO_3: 93-165 mg/L	pH	: 7.3-8.0
Na : 10-15 mg/L	Cl : 18-28 mg/L	diss.O_2:	5-12 mg/L

Table 1. Size-dependency of Cd accumulation in *A.cygnea* of three size classes (shell length 65 ± 10, 100 ± 15, 135 ± 20 mm), exposed to 50 µg/L Cd. Metal concentration was measured in three organs, after 3 and 13 wk. Values are the mean ± S.E. (n=4). After Holwerda and Van der Plas (unpublished).

Tissue	µg Cd/g dry wt		
	65 mm	100 mm	135 mm
Gills			
- 3 wk	151 ± 4	52 ± 6	34 ± 7
- 13 wk	218 ± 20	99 ± 8	67 ± 8
Midgut gland			
- 3 wk	18 ± 2	8 ± 2	9 ± 3
- 13 wk	59 ± 15	27 ± 6	23 ± 3
Kidney			
- 3 wk	67 ± 3	16 ± 4	14 ± 2
- 13 wk	142 ± 24	51 ± 5	34 ± 3

Except for the experiment on size-dependence of Cd uptake, shell length of experimental clams was taken between 7.0 and 10.0 cm. From each animal one pair of gill flaps (outer plus inner lamella), not bearing glochidia, was used for the background Cd level. This value was measured as 1.4 ± 0.5 µg Cd/g dry weight (mean ± S.D., *n*=40). The other gill pair, when free of glochidia, was excised carefully, to minimize the wound, and transferred into a polypropylene tube containing 40 mL of filtered (Millipore, pore size 0.22 µm) tap water, spiked with $CdCl_2$ (Titrisol 9960; E. Merck, Darmstadt, F.R. Germany) without or in combination with substances (analytical grade) potentially affecting the uptake of cadmium. The tubes were closed with "Parafilm" and incubated under gentle shaking at room temperature. Thereafter, the gill flaps were rinsed in double-distilled water for 1 min and blotted with a tissue paper, in order to remove adherent cadmium. Control and incubated flaps were lyophilized overnight.

Cd concentrations were determined by the use of atomic absorption spectrophotometry, as described before (Hemelraad et al. 1986a). Briefly, the lyophilized tissue was decomposed in 65% (w/v) HNO_3 for 1½ h at 80°C. Samples of the medium before and after incubation were acidified with HNO_3 and measured directly. The mean of the values before and after incubation was used as the actual metal concentration. Accumulation values were defined as the difference between the Cd content of the incubated pair of flaps and of the control pair from the same animal, corrected for the small variations of incubation time and Cd concentration of the medium.

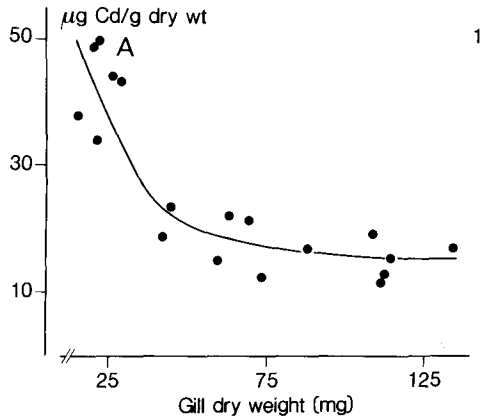

Figure 1. Weight-dependence of Cd uptake in the excised gill. Incubation: 60 min at 5 µM Cd. Weights are for one pair of flaps.

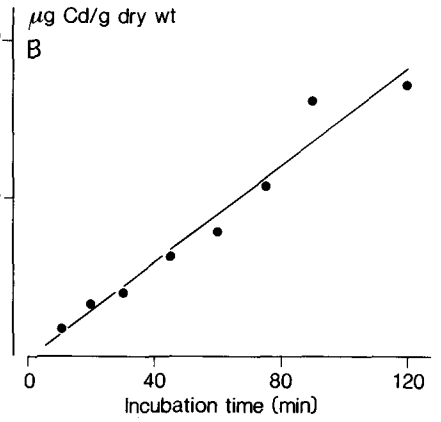

Figure 2. Time progress curve of Cd uptake. Gills incubated at 10 µM Cd (up to 45 min) or 5 µM Cd (beyond 45 min).

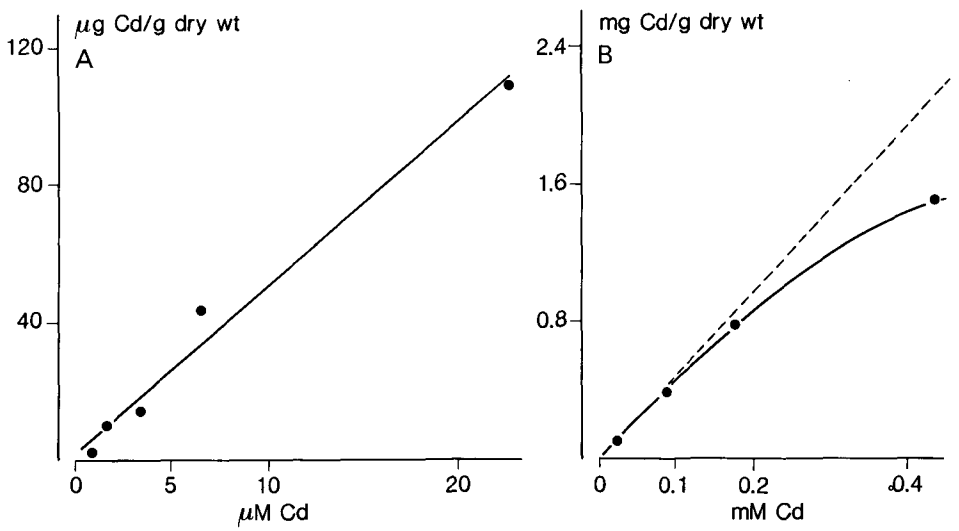

Figure 3. Relation between Cd uptake in the excised gill and the Cd concentration in the medium. Left-lower Cd range (1-22 µM), incubation time 60 min (r^2 = .98, after correction for gill weight variation). Right-higher Cd range (.02-.44 mM), incubation time 30 min; values recalculated to 60 min of incubation.

RESULTS AND DISCUSSION

Hemelraad et al. (1986b) have suggested that small clams accumulate cadmium at a higher rate than larger ones. For example, on exposure of *A.cygnea* of varying size classes to cadmium chloride, it was observed (D.A. Holwerda and B. van der Plas, unpublished experiments) that very small (young) animals accumulated cadmium about 4 times

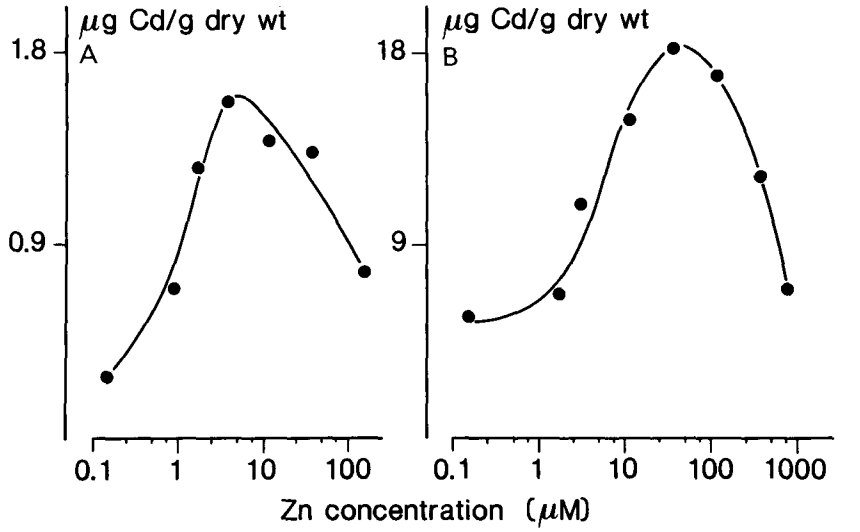

Figure 4. Effect of Zn (as $ZnCl_2$) on Cd uptake by the excised gill. Left-Zn range 0.15-150 µM, incubation for 60 min at 0.5 µM Cd. Right-Zn range 0.15-750 µM, 60 min at 2 µM Cd.

faster than the animals from the largest size class (Table 1). Two mechanisms are conceivable that could underly the size-dependence of the rate of uptake. First, young animals could have a higher ventilation rate than older ones, which would result in a larger mean Cd^{++} gradient over the gill membrane. Second, qualitative differences could exist between small and large gills, the simplest of these being a differing ratio of resorbing surface and weight. In the present study, it was found (Figure 1) that the same phenomenon is shown by the excised gill; very small gills accumulated up to 3 times more cadmium (on a dry weight basis) than the largest ones. It is, therefore probable that the high metal uptake by young animals is not caused by a relatively higher ventilation rate, but is a feature of the resorbing tissue itself. The curve of Figure 1 was used to correct uptake data from the other experiments for variability of the gill weights, that ranged from 40 to 135 mg.

Figure 2 shows that there was a linear uptake of cadmium with time, extending beyond the incubation times of 30 or 60 min taken in the further experiments. In Figure 3, the relation between Cd uptake and the Cd concentration in the medium is shown. The last point in the lower Cd range (Figure 3A) is identical to the first in the higher Cd range (Figure 3B), and the dotted line in the right panel has been given the same direction as the solid line in the left panel. It appears that Cd uptake proceeded linearly (r^2 = .98 and .96, for the weight-corrected and uncorrected data, respectively), up to about 0.1 mM. At higher exposure concentrations the rate of uptake declined progressively. It is, therefore, possible that part of the cadmium is transported via a saturable system. However, other factors might also cause retardation of metal uptake. First, the difference between total Cd concentration and the activity of the free ion, as the supposed metal species taken up,

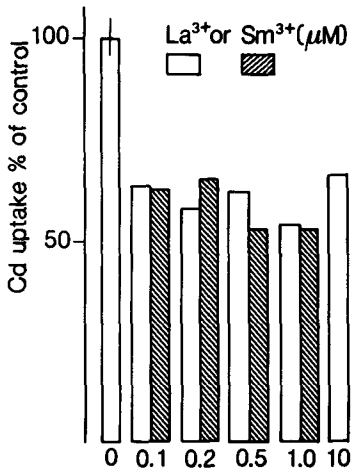

Figure 5. Effect of lanthanum and samarium on cadmium uptake. Conditions: 10 μM Cd, 60 min; control: mean ± S.E. (n=4).

Table 2. Effect of metabolic inhibitors on Cd uptake by the excised gill. Incubation conditions: 2 μM Cd, 30 min. Mean ± S.E.(n=3).

Addition	Uptake (μg Cd/g dry wt)
None	4.77 ± 0.17
Antimycin A (0.5 μM)	4.75 ± 0.44
Rotenone (0.2 μM)	4.15 ± 1.18
m-Cl-CCP[a] (1.0 μM)	4.58 ± 0.90
Iodoacetate (1.0 mM)	4.46 ± 0.68

[a] Carbonyl cyanide, m-chlorophenyl-hydrazone.

increases with increasing total Cd. Secondly, the structural integrity of the gill may be affected, which leads to inhibition of metal uptake; it was observed that, at high metal concentrations in the medium, the tissue turned white.

Metal interaction with respect to bioaccumulation has been well-documented (Ray 1984). For *A.cygnea* it has been found that a 100-fold excess of zinc inhibited the uptake of cadmium, and altered the distribution of the latter among the organs (Hemelraad et al. 1987). Figure 4 shows that zinc also affected the in vitro uptake of cadmium, in a biphasic way. At increasing external Zn the uptake of cadmium was stimulated. Maximum stimulation occurred at a Zn concentration that depended on the Cd concentration (Figure 4A versus 4B). At higher Zn, the rate of Cd uptake decreased again. In view of the short incubation time, it is not expected that the effect of zinc is mediated by intracellular interactions. Rather, zinc in the external medium will affect membrane permeability. This metal has an important role in membrane function (Chvapil 1973). Zinc deficiency causes a loss of plasma membrane integrity, which will lead to an altered function of permeability channels (Bettger and O'Dell 1981). Apparently, increasing Zn in the medium maximized the membrane function of the excised gill with respect to Cd uptake. Supersaturating Zn concentrations were inhibitive, either through a gross interference with membrane organization or through a more specific, competitive interaction with the cadmium transporting mechanism.

The present data with *Anodonta* gills do not confirm the results obtained with *M.edulis*: when the isolated gill of the sea mussel was incubated for 6 min in 1 μM Cd, there was no effect of a 75-fold (on a molar base) excess of zinc (Carpene and George 1981). In

vivo uptake of cadmium also has been found to respond biphasically to the presence of zinc (Elliott et al. 1986). In a 10-d exposure of *M.edulis planulatus* to 10 µg/L Cd, uptake of this metal was increased when zinc was present at 100 µg/L (compared to the control of 11 µg/L), but decreased at 200 µg/L. These effects of zinc were however, rather small.

Lanthanum (La^{3+}) is a specific antagonist of Ca^{2+} (Weiss 1974). The metal has been applied as a calcium channel blocker. Figure 5 shows that the ion, as well as Sm^{3+}, inhibited the uptake of cadmium by the isolated gill. Crystal ionic radii of these metals and those of Ca^{2+} and Cd^{2+} differ by only a few percent. Maximum inhibition, already reached at 0.1 µM, was no more than 40%. It appears, therefore, that calcium channels are involved in the uptake of cadmium, but only to an extent of about half the total amount. The other half must be taken up through other mechanisms. It has been argued that cadmium may also be transported by endocytosis (George and Viarengo 1984; Hemelraad and Herwig 1988). Also, indications have been given that metal complexation by the plasma membrane may play a role in the transport of the metal (Simkiss 1983; Viarengo 1985; Holwerda et al. 1987). It is, therefore, supposed that Cd uptake proceeds via diverse mechanisms, involving mediated transport through calcium channels and at least one of other ways, that include membrane-specific ligands, and endocytosis. One or more of these ways will be affected by the presence of zinc. In accordance with Carpene and George (1981), no indication was found (Table 2) that transport of cadmium is an energy-dependent process.

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