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Cadmium Accumulation and Depuration in *Anodonta anatina* Exposed to Cadmium Chloride or Cadmium-EDTA Complex

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We have previously reported on the uptake and distribution of cadmium in unionids, experimentally exposed to cadmium chloride (Hemelraad et al.1986). The purpose of the present investigation was to study the effect of metal chelation on cadmium kinetics, including metal elimination in the post-exposure phase.

Generally, chelation of ionic metal by natural substances like humic acids or by synthetic compounds like EDTA decreases its environmental toxicity through a diminished rate of uptake, as compared with the free ion. For marine molluscs, reduced bioconcentration factors by chelation of cadmium with EDTA were found for barnacles (Rainbow et al.1980), oysters (Hung 1982), and for *Macoma balthica* (McLeese and Ray 1984). In *Mytilus edulis*, however, Cd uptake was enhanced in the presence of either humic and alginic acids, or EDTA (George and Coombs 1977). Furthermore, the influences of metal chelation on bioconcentration and on toxicity do not always run parallel; the presence of humic acid had no effect on the bioaccumulation of cadmium in *Daphnia magna*, whereas the toxicity was even increased (Winner 1984). To our knowledge, there are no data on the effect of chelation on metal kinetics in freshwater clams.

Data on rates of cadmium elimination from aquatic invertebrates are highly divergent, but Cd excretion is invariably found to be smaller than uptake. In *M.edulis* the loss of cadmium after a 2-day exposure to 0.1 µg/ml $CdCl_2$ was 18 times slower than Cd uptake (George and Coombs 1977). Compared with mercury and lead, cadmium had extremely long residence times in the oyster *Saccostrea echinata*, presumably due to its strong immobilization by metallothionein-like proteins (Denton and Burdon-Jones 1981). For *M.edulis* too, it has been concluded that while one part of cadmium is released, another part is transferred into fixed bonds (Borchardt 1983). This means that the organism has to be regarded as a multicompartment system. Recently, in an accumulation-depuration study with *Anodonta cygnea* (Salánki and V.-Balogh 1985), it was suggested that cadmium is released from the organs at different rates and that the metal is also redistributed in the depuration phase.

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MATERIALS AND METHODS

Specimens of the freshwater clam *Anodonta anatina* L. had been collected from a pond near Leiden (South Holland). Animals were held in glass aquaria with running tap water (unchlorinated, drinking-water standard) of ca.12°C, and were not fed. Main water quality parameters (minimum-maximum values) were:

Ca : 29-52 mg/L	Fe : max. 0.1 mg/L	hardness: 85-150 mg/L $CaCO_3$
Mg : 3-4 mg/L	Cl : 14-26 mg/L	pH : 7.4-8.4
Na : 8-15 mg/L	SO_4 : 9-18 mg/L	diss.O_2: 7-14 mg/L

Clams were exposed to cadmium chloride (Titrisol Art.9960, Merck AG, Darmstadt) or to the Cd-EDTA complex (molar ratio disodium ethylenediaminetetraacetic acid : cadmium chloride = 1:1), at 13 ± 1°C, in a static system that was refreshed weekly to the same level. Total Cd concentrations in the water were measured just before and after refreshment. Mean Cd concentrations over the entire exposure period were 16 and 21 µg/L for the Cd and Cd-EDTA groups, respectively. For the depuration phase the animals were transferred to uncontaminated aquaria (water volume: 120 L), with running tap water (15 L/h).

Prior to dissection, the animals were kept in unspiked tap water for 24 h, in order to eliminate absorbed cadmium. Cd concentration was measured by the use of atomic absorption spectrophotometry, as described earlier (Hemelraad et al.1986). Lyophilized tissues were decomposed in 65% (w/v) HNO_3 (analytical grade) for 1½ h at 80°C. Water samples were measured directly, after addition of HNO_3.

For the determination of the cytosolic Cd distribution the tissue supernatant fraction was chromatographed on a Sephadex G-75 column (Pharmacia, Sweden), 0.9 x 50 cm, that was equilibrated and eluted (at 3.2 ml/h) with 25 mM Tris-HCl buffer of pH 8.7. The fractionation procedure was carried out at 4°C. Eluate fractions were analyzed for UV absorbance (250 and 280 nm) and for Cd content.

The data on tissue content of cadmium have been expressed as the amount of metal per unit (dry) weight, as is common use in literature. It has already been observed (Zaroogian 1979, Hemelraad et al.1986) that the "concentration" parameter will be misleading in case of variability of the weight term. This may happen when experiments are conducted in different seasons or extend over several months, under starving conditions. The main factors determining the variability of soft body weight and organ weights will be the seasonally dependent nutritive status and, in non-feeding experimental animals, utilization of energy reserves. The problem has been recognized, especially with respect to the use of mussels as biomonitors. The solution has been sought in the application of a correction factor for weight variability (Cain and Luoma 1986) or by the use of shell weight as a less variable parameter (Fischer 1983). With respect to Cd kinetics during exposition and in depuration, it was observed (Zaroogian 1979) that elimination of cadmium in *Crassostrea virginica* did not occur

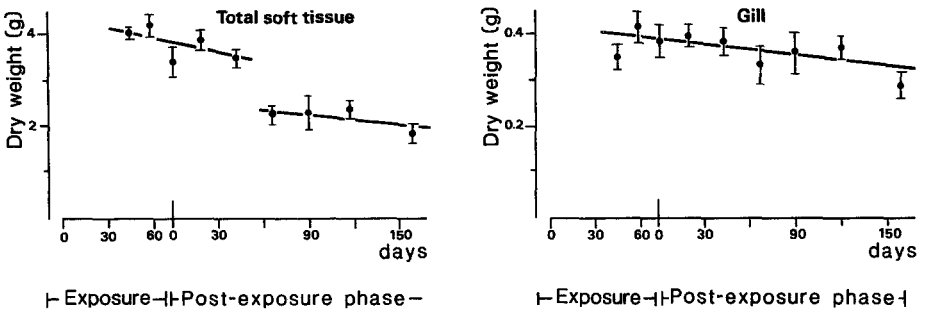

Figure 1. Time dependent change of total soft body weight and gill dry weight. For each sample point, weights in the Cd group (n=4) and in the Cd-EDTA group (n=4) were combined (→n=8; mean ± SE). Weights before the 44th day of exposure were not included because of the presence of glochidia in the gills.

when metal concentration was considered; however, when the relationship of metal content and body weight with time was included, a loss of cadmium was indicated.

For the current experiment, total soft body weights and gill weights from eight individuals at the various sampling times are depicted in Figure 1. The mean gill dry weight tends to decrease slowly over the exposure and post-exposure phases. By contrast, total body weight, in either exposure condition, shows a sharp decrease between day 42 and 66 of the depuration phase. Loss of weight was most pronounced in those tissues showing the highest glycogen content, namely mantle and visceral mass. On the other hand, gill weight is much more constant, because glycogen content is low, and the amount of inorganic calcium concretions considerable, up to 50% of the dry weight (Pynnönen et al.1987). Therefore, while neglecting the very moderate and gradual decreases of the dry weight of gills during the whole period of uptake and elimination, as well as those of the other organs and the total soft body weight within the two discernable periods (i.e., up to the 42d day, and beyond the 66th day of post-exposure), we have corrected for the sudden loss of weight between these periods. Individual organ and total dry weights for the four last sampling times were related to the respective gill dry weight, as by:

$$W_{xi} = W_{gi} \quad \overline{W_{xc}/W_{gc}}$$

where W_{xi} = corrected total or organ dry weight for any individual clam of the sample points from day 66 of post-exposure; W_{gi} = corresponding, measured gill dry weight; $\overline{W_{xc}/W_{gc}}$ = mean of ratios of total or organ dry weight and gill dry weight, through day 42 of post-exposure.

The Mann-Whitney *U*-test was applied in the statistical treatment of data.

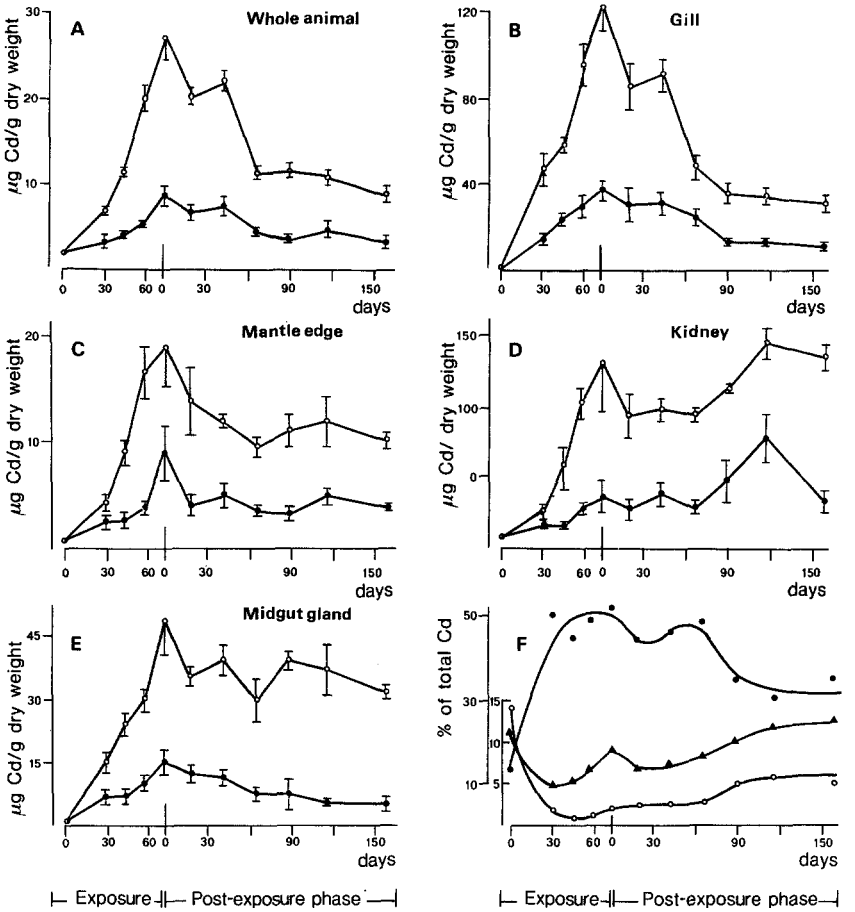

Figure 2. A-E: Cd concentrations in total soft body and in some organs during exposition to $CdCl_2$ (o-o) or CdEDTA (●-●) and depuration. Values are the mean (± SEM) of four animals. F: Cd burden (as percentage of total body cadmium) in gills (●-●, larger scale), mantle edge (▲-▲, smaller scale) and kidney (o-o, smaller scale). Values are recalculated from those of Figure 2A-E ($CdCl_2$ group).

RESULTS AND DISCUSSION

Figure 2A-E shows the time courses of Cd accumulation and elimination for total soft parts (as sum of the organs) and for gills, mantle edge, kidney and midgut gland. Over the entire period of exposure and post-exposure, Cd concentrations were reduced by a factor of three when the metal was dosed as the EDTA complex. This result compares well with a reduction of 70% found (Hung 1982) for oyster (40 d, 50 ppb Cd as $CdCl_2$ and CdEDTA), and the same value (Rainbow et al. 1980) for barnacle (30 d, 100 ppb). For *M.balthica* (14 d, 45 ppb), a reduction of Cd uptake by EDTA chelation up to 55% was found (McLeese and Ray 1984). It seems certain that firmly complexed Cd is less available for uptake than the free ion, which is the predominant form in relatively pure fresh water or than the

weak chloro-complexes in sea water.

However, reduction factors appear to be smaller than would be predicted on the basis of calculated ambient Cd^{++} activity, in the presence of a chelator. Using perfused trout gills, Pärt and Wikmark (1984) concluded that uptake of cadmium in the presence of complexing agents is not solely a function of free Cd^{++} concentration; the complexed metal is also available to the gills. It is tempting to speculate that the Cd complex is bound to the gill epithelium, whereupon dissociation of cadmium from the binding complex and subsequent uptake of the metal ion may occur. This would explain why weaker complexes, e.g., humate are more available than stronger ones, like EDTA (Hung 1982).

The ratio of Cd concentrations in whole body and separate organs of the Cd-exposed and the CdEDTA-exposed groups is remarkably constant over the exposure and post-exposure periods. For whole body and gills, this is illustrated in Figure 3,where Cd concentrations in the CdEDTA-dosed animals were multiplied by a factor of three. In both cases the curves largely overlap. Apparently, the extent of Cd uptake neither influences the time courses of accumulation and elimination, nor the tissue distribution of the metal. This result has been taken as the basis for statistical treatment of the data; at each sample point, Cd concentrations derived from the Cd treatment were combined with those from the CdEDTA treatment, by multiplying the latter values by a factor of three.

During the exposure phase, Cd concentration in total soft parts and in separate tissues increase (Figure 2A-E). By the end of this phase, the order, for either exposure condition, is: kidney = gills >> midgut gland > total soft parts > mantle(edge) > muscles. This is in accordance with previous data (Hemelraad et al.1986).

Metal elimination from whole animal (Figure 2A) after dosing was discontinued appears to proceed in three well discernable stages. Within 3 wk a first fraction is lost. The decrease is significant (Table 1) for total soft parts (0→19d) and for gills (0→42d). This fraction must represent labile metal that could in part be extracellular or, otherwise, not yet bound to cellular ligands. Chou et al. (1978) reported that about half of accumulated cadmium in oyster tissue is unbound or free.

Between 19 and 42 d of post-exposure, elimination of metal ceases. Thereafter, in the next 3 to 4 wk, a second, considerable portion is lost from total parts and gills. At the same time, there is a notable decrease of organ weights, especially of glycogen containing tissues. Therefore, elimination of this metal fraction is thought to occur via an energy-dependent mechanism. This second compartment may comprise the cadmium fraction that is reversibly bound to intracellular ligands. It was found earlier (Hemelraad et al.1986) that a large portion (> 80%) of cadmium in the gills is bound to the particulate fraction. In addition, a relatively large portion of cytosolic cadmium in the gills is associated (Figure 4A) with high-molecular weight proteins (34 and 24% for the Cd-

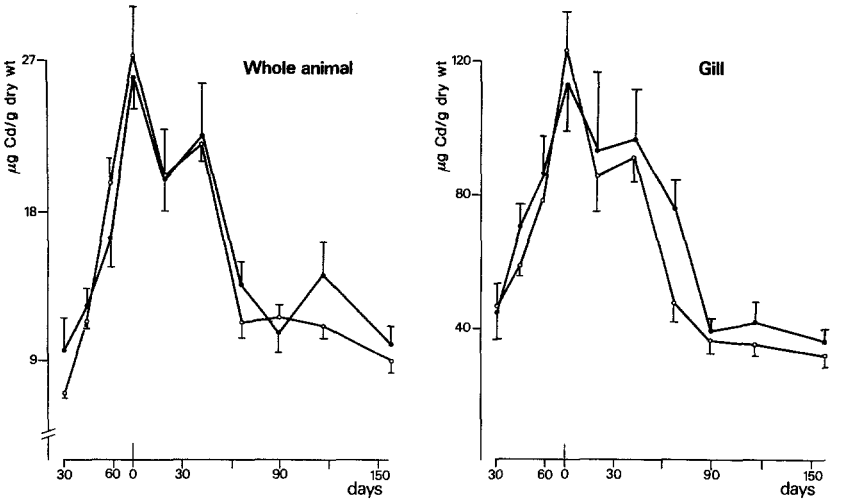

Figure 3. Comparison of the Cd- and CdEDTA time courses of accumulation and depuration, in whole animal and gill: data from the latter exposure condition (●-●) multiplied by three.

Table 1. Statistical evaluation of differences (Mann-Whitney *U*-test (two-tailed, at $\alpha = .05$). Numbers are days of post-exposure. T = total soft parts, G = gills, K = kidney; * = significant, ↓ = decrease, ↑ = increase, o = not significant

T	0	19	42	66	G	0	19	42	66	K	0	19	42	66
19	*↓				19	o				19	o			
42		o			42	*↓	o			42		o		
66			*↓		66			*↓		66			o	
116				o	116				o	116				*↑

exposed and the CdEDTA-exposed group, respectively). By contrast, in the kidney the particulate Cd fraction is smaller (40%), and cytosolic cadmium (Figure 4B) mainly bound to MT-like proteins (88 and 95%, respectively). The hypothesis of an energy-dependent elimination of metal has been noted by Bias and Karbe (1985) who concluded from their Cd accumulation-elimination study with *Dreissena polymorpha*, and from results with *M.edulis* (Borchardt 1983) that an effective elimination of cadmium depends on enhanced metabolic processes, as under conditions of full food supply.

Beyond the 9th week further Cd loss from whole body and from gills is very slow. In conclusion, three compartments of Cd storage are discerned. The first one is a labile fraction that is not truly incorporated into the tissues, and that is released quickly. The second fraction constitutes reversibly bound cadmium, elimination of which may be energy-dependent. The last compartment consists of firmly bound metal, the greater part of which is connected to strong ligands, such as MT-like and other, presumably -SH group containing proteins. Such a multi-compartment system for the stor-

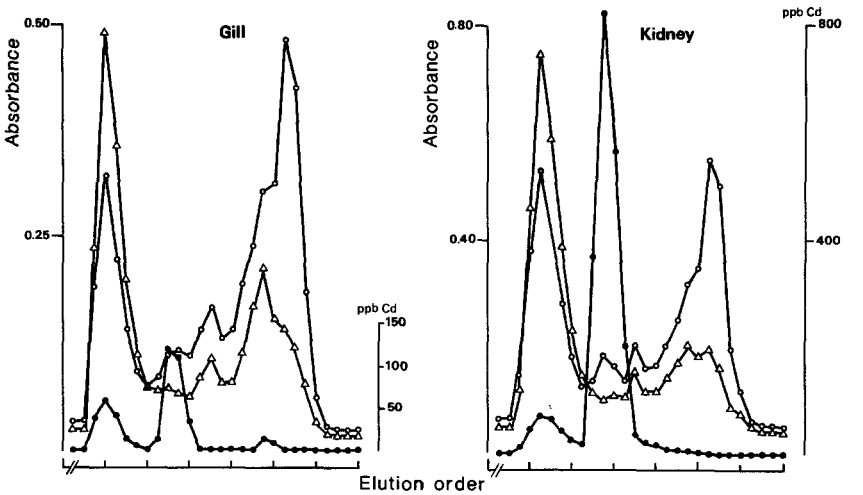

Figure 4. Sephadex G-75 chromatography of the cytosolic fraction of gill and kidney of $CdCl_2$-exposed clams, at day 130 of post-exposure; ●-● cadmium, o-o A_{250}, Δ-Δ A_{280}.

age of cadmium, from which the metal is released at different rates, is well documented (Borchardt 1983).

At the same time as part of the cadmium is released, another part may be exchanged between organs. In our experiments, the latter process becomes apparent during the third phase of depuration when labile and reversibly bound metal have disappeared. In this phase, Cd concentration in the kidney increases significantly. Midgut gland and mantle edge also provide bonds for Cd storage, at least in that the 42→66-d release is not significant in these organs. Figure 2F shows that Cd portions (as % of total burden) increase in these organs, whereas that of the gills decreases to about 30%. Similar, though less elaborated results were reported by Salánki and V.-Balogh (1985) in a study on Cd accumulation and depuration in *Anodonta cygnea*. Part of the metal rapidly released from gills, muscle and viscera. In the second phase, a much slower release was observed, whereas, after a lag period, cadmium increased in the kidney. Cd redistribution was also noted during the depuration phase in *M.edulis* (Theede and ter Jung 1988). It should, however, be stressed that the mechanism underlying redistribution, namely internal transport in the direction of fixed bonds, will not be restricted to the depuration phase, but will operate from the beginning of uptake of metal.

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