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Cadmium Kinetics in Freshwater Clams. II. A Comparative Study of Cadmium Uptake and Cellular Distribution in the Unionidae *Anodonta cygnea*, *Anodonta anatina*, and *Unio pictorum*

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Abstract. A comparative study was made of Cd accumulation in three species of Unionidae. Up to eleven weeks of exposure, patterns of accumulation in whole animal were biphasic and didn't differ essentially between the species. After the eleventh week, Cd uptake increased strongly in *A. anatina*, whereas in *A. cygnea* metal concentration remained at a constant level. This dissimilarity is discussed in terms of differing ventilation activity. Large differences were observed between corresponding organs of the species with regard to the Cd concentration (on the basis of organ dry weight). However, when comparing the normalized burden values (on the basis of total dry weight) differences were found to be small. In conclusion, in the comparison of related species, normalized burden rather than concentration has to be considered as the more realistic parameter of Cd accumulation. Comparing mussels of different condition (summer and winter animals) of the same species, accumulation patterns diverged when the parameter of Cd concentration was used, but not with the parameter of Cd burden; the total body or organ mass factor holds elements of differing, seasonally dependent weight (*e.g.*, energy stores) that do not affect the extent of Cd uptake. Cd amounts and Cd concentrations (on a protein basis) were highest either in the nuclear fraction or in the cytosol in the two Anodonta species, depending on the organ. In gills of *A. anatina*, and in midgut gland of *A. cygnea*, 75% of total organ Cd was found in the nuclear fraction. A possible contribution of large membrane-limited vesicles and of calcium concretions to this fraction is discussed. Only in the kidney of either species was the amount of cytosolic Cd greater than that in the nuclear fraction. In the other cases, the ratio of Cd amounts in the nuclear and cytosolic fractions was about 3:2. Partition of Cd between the total particulate and the non-particulate fraction was similar at 5 and 16 weeks of exposure. After gel filtration, total cytosolic Cd was recovered in a high-molecular weight (HMW) fraction and in a protein fraction with apparent MW of 11 kD. In gills, mantle, and midgut gland, the contribution of the latter fraction increased with exposure time.

It is well documented that both marine and freshwater bivalve molluscs accumulate cadmium in high concentrations (Ravera 1984; Ray 1984), without any obvious distress. Rate and pattern of accumulation may vary considerably between the molluscan species. While marine bivalves accumulate Cd mainly in a linear mode (Ray 1984), freshwater clams accumulate it in a more complex way (Marquenie 1984; Hemelraad *et al.* 1985).

Even among related species, from the same environment, clearly different rates of Cd uptake occur. For instance, the oysters *Crassostrea gigas* and *Ostrea edulis* differ in rate of accumulation by a factor of two to three (Frazier and George 1983). Moreover, experimental exposure of the same species, but taken from an uncontaminated and a metal-contaminated environment, respectively, resulted in different rates of Cd uptake.

Although sparse, available data for freshwater molluscs tend to considerable differences in the rates of uptake of heavy metals (Zadory 1984). So far as it concerns laboratory experiments, with their controlled abiotic factors, the biotic factors like ventilation rate and concentration and synthesis of

metal-binding proteins (*e.g.*, thioneins) will determine the kinetics of uptake and internal transport of heavy metals.

In the previous paper (Hemelraad *et al.* 1985) the time-dependent Cd accumulation and organ distribution were studied in the freshwater clam *Anodonta cygnea*. In the present study a comparative investigation is presented relating to the Cd kinetics in three members of the family Unionidae: *A. cygnea*, *A. anatina* and *Unio pictorum*. Among other factors these species differ in their habitat (Ellis 1978), in filtration capacity (*ibid.*) and in the extent of tissue pigmentation. The latter factor has been connected with the tolerance to environmental pollution (Karnaukhov *et al.* 1977; Karnaukhov 1979).

Time-dependent uptake of Cd as well as the internal distribution were determined in order to obtain an insight into similarities and/or differences in three closely related species with respect to metal kinetics. This should be helpful in understanding the common principles of Cd accumulation in freshwater molluscs. In addition, the validity of the concentration term (μg Cd/g dry wt) as a commonly used and comparative measure for cadmium contents in whole animal and in separate tissues has been examined.

Data concerning the cellular fate of accumulated Cd in freshwater molluscs are not available in literature. Therefore, in the two Anodonta species, the subcellular and molecular distribution of Cd was also determined.

Materials and Methods

Animals

Anodonta cygnea zellensis Gmelin (swan mussel) were collected from ponds and ditches in the Maarsseveen-lake district near Utrecht in April 1984. *Anodonta anatina* L. (duck mussel) were collected from a pond near Leiden in October 1983. The animals were preserved under natural conditions in wire baskets placed in the moat of the botanical garden in Utrecht. *Unio pictorum* L. (painter's mussel) were collected in June 1983. These mussels were found at the same sites as *A. cygnea*. Species determination was essentially after Jansen and Vogel (1965). Prior to exposition all three species were treated as described earlier (Hemelraad *et al.* 1985). The mean shell lengths of *A. cygnea* and *A. anatina* were 11.9 cm ± 1.2 (6.1 to 14.3 cm) and 8.7 cm ± 0.2 (7.6 to 10.0 cm), respectively.

Exposure Systems and Metal Analysis

Anodonta species were exposed to approximately 25 μg Cd/L (ppb), as $CdCl_2$, at the same time and under identical conditions.

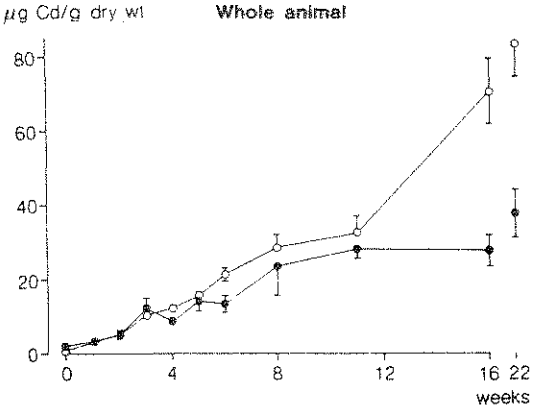


Fig. 1. Cd concentration *vs* exposure time in whole animals exposed to 25 ppb Cd; -○- *A. anatina*, -●- *A. cygnea*. Mean of 4 animals, ± SEM

Data concerning water quality and exposure system were described earlier (Hemelraad *et al.* 1985). The actual Cd concentration in the water, that was assayed weekly, amounted to 29 ppb ± 7 (SD). The exposure lasted from June until November 1984. *U. pictorum* and *A. cygnea* were exposed to 55 ppb Cd ± 5 at the same time and under identical conditions. The experiment was performed from November 1983 until February 1984. Dissection, tissue decomposition and determination of Cd were carried out as previously described (Hemelraad *et al.* 1985).

Subcellular and Molecular Fractionation

Gills, kidneys, midgut glands and mantles from four animals were pooled and gently homogenized in 25 mM Tris-HCl buffer of pH 8.0, using successively an Ultra-Turrax homogenizer and a Potter tube. The homogenates were centrifuged stepwise, with intermittent washing of the sediments, to obtain a nuclear (10 min at 500 g), a mitochondrial/lysosomal (30 min at 50,000 g), a microsomal (1 hr at 100,000 g) and a cytosolic (supernatant) fraction. The latter was chromatographed on a Sephadex G-75 column (Pharmacia), 0.9 × 50 cm, that was equilibrated and eluted (at 3.2 ml/hr) with 25 mM Tris-HCl buffer of pH 8.7. The fractionation procedure was carried out at 4°C.

Succinic dehydrogenase activity was determined by the Kmetec method (1966), acid phosphatase activity according to Bergmeyer (1974). Protein was determined by the Lowry method (Schacterle and Pollack 1973), using BSA as the reference protein. Cd concentration of the cytosolic and column fractions were measured without prior acid decomposition. The Cd contents of the organelle fractions were determined after acid decomposition.

Results

Comparison of Cd Accumulations in Three Species of Unionidae

In Figure 1, Cd accumulations are compared for *A. anatina* and *A. cygnea* that had been exposed at

Table 1. Comparison of mean dry weights of experimental animals during two periods, and after 22 weeks

	A. anatina g ± SD	*A. cygnea*
0 to 6 weeks	3.51 ± 0.42	3.61 ± 0.67
8 to 16 weeks	3.47 ± 0.51	3.46 ± 0.21
22nd week	2.19 ± 0.53	3.36 ± 0.28

the same time and under the same conditions. Two periods can be discerned. During the first eleven weeks metal concentrations in whole animals did not diverge strongly. Periods of high uptake alternated with apparent stagnation or decreased rate of Cd accumulation, as reported in the preceding paper (Hemelraad *et al.* 1985). By the end of the first period more or less saturation occurred in both species. Thereafter, metal concentration in *A. cygnea* increased only slightly, but in *A. anatina* accumulation started again, at a high rate. As illustrated by the data in Table 1, there was no significant decrease of mean animal weight over the time of exposure, except for the final group (22 weeks) of *A. anatina*.

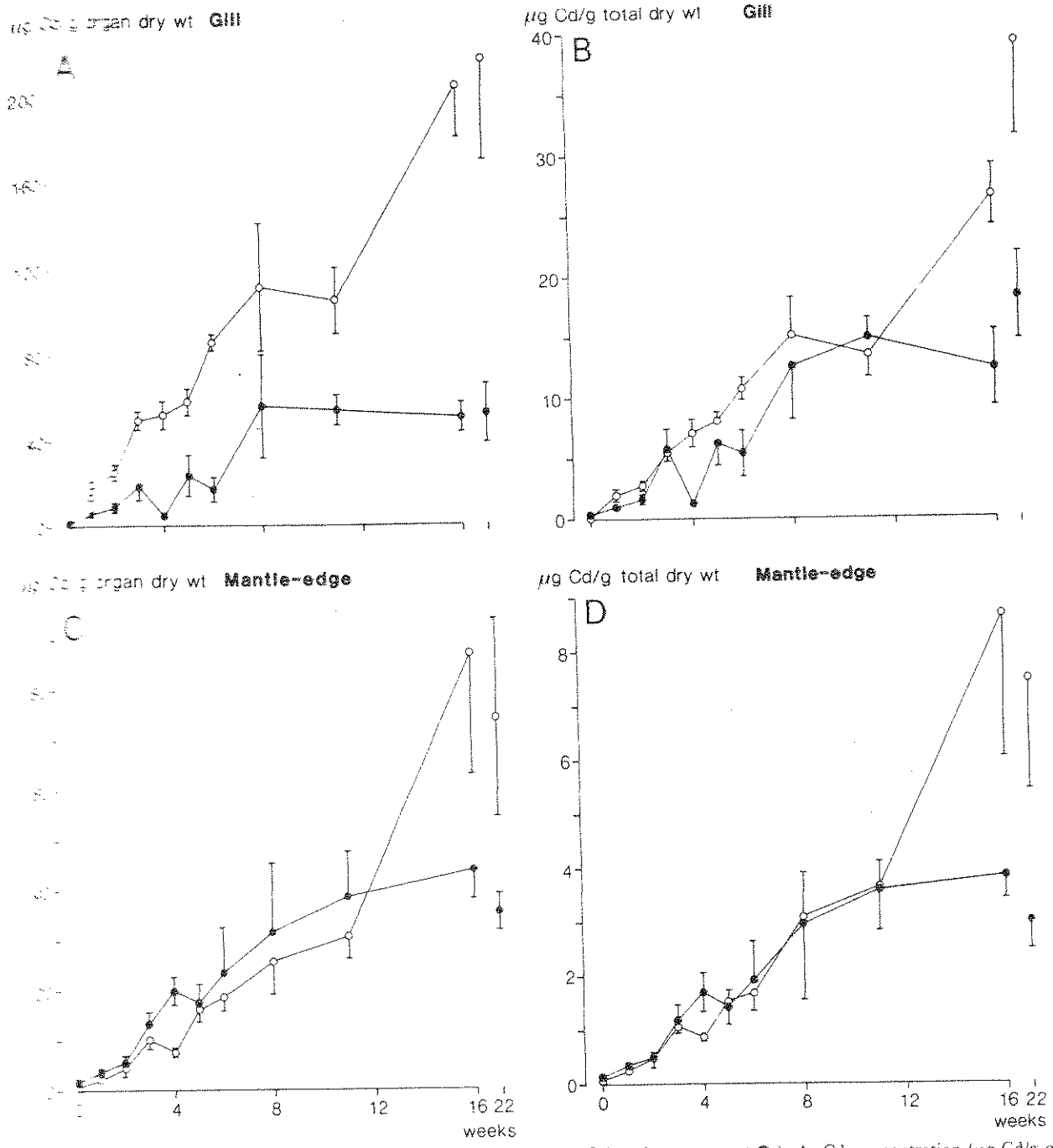
Of all organs examined, curves for gills, mantle-edge, midgut gland and kidney are given (Figures 2, A and C, Figures 3, A and C), as representative patterns of accumulation. Metal contents have been expressed as μg cadmium per g organ dry weight. The organ curves differ considerably from those of the whole animals (Figure 1). From the beginning, accumulations in gills and in kidney deviated between the species. Especially in *A. anatina*, time courses for these organs were multiphase. In the midgut gland deviation occurred not before the sixth week, but was very strong thereafter. In contrast to these three organs and also to whole animals, accumulation in mantle-edge of *A. anatina* was less than in *A. cygnea* over the whole period of eleven weeks.

The picture drawn alters when the parameter of "concentration" is replaced by that of normalized organ burden (*i.e.*, μg cadmium per g of *total* soft body weight), thus releasing any correlation with organ size. The curves are shown in Figures 2 and 3, B and D. Considering the first period of eleven weeks, it is evident that the differences between the species become smaller. For gills and mantle-edge the sets of curves strongly resemble that of whole animal, *i.e.*, the contributions of these organs to the total body burden (all values being normalized to 1 g of total dry weight, in order to correct for differences in animal weight) are the same for *A. anatina* and *A. cygnea*. For the kidney, also, the contributions were similar up to the point at which in *A. anatina* the second phase of accumulation started. The data for the midgut gland do not fit into this model. Whereas the concentrations in both species reached the same level at the end of the first phase (three to five weeks after the onset of exposure), this did not apply for the normalized burden. In this phase, the uptake into the organ was twice as high in *A. cygnea* as in *A. anatina*.

The notable difference between the left and the right halves of Figures 2 and 3 is brought about by the denominator in the "concentration" parameter. In Table 2, the mean absolute and relative dry organ weights for all mussels of either species are given. The total soft body dry weights of *A. anatina* and *A. cygnea* were practically similar. By contrast, gills, kidney and midgut gland were much heavier in *A. cygnea* than in *A. anatina*. The reverse situation applies for mantle, mantle-edge and the guts/gonads complex.

Figure 4 shows the time courses of Cd accumulation in *A. cygnea* and *U. pictorum*. Both species, exposed to 55 ppb Cd at the same time, accumulated in a biphasic way. The first hyperbolic phase lasted for about six weeks and was followed by an increased accumulation rate. The zigzag course in the curve for *Unio* need not be interpreted in terms of a significant elimination of Cd after four weeks, but was most probably caused by the accidentally high mean animal weight of the 44-days sample (4.1 g, against 2.3, 2.1 and 2.4 g for the 12-, 28- and 78-days samples, respectively). Apparently, within the same species the rate of Cd uptake is weight-dependent; a small mussel would accumulate (relatively) faster than a larger one. Similarly, the 12-day value in the *Anodonta* curve might be "too high," because of the relatively low mean animal weight (3.1 g against 4.0, 3.4 and 3.9 g for the 28-, 44- and 78-days samples, respectively). Taking into account this weight factor, time courses for whole animal Cd accumulation are similar.

With regard to the individual organs, of which two typical examples are shown in Figure 5, similar features emerge as in the foregoing comparison of the two *Anodonta* species. The clear divergence of the "concentration" curves (Figures 5, A and C), especially by the end of the exposure time (78 days), is markedly reduced in the curves for the normalized organ loads (Figures 5, B and D), although, except at the end of the exposure time, the very small gills of *Unio* cannot fully compete with the much larger *Anodonta* gills as for the contribution of this organ to the whole body burden. Also, mantle and kidney relative organ dry weights differ even more between *A. cygnea* and *U. pictorum* than between the two *Anodonta* species (Table 3).



Time-dependent uptake of Cd into two organs of *A. anatina* (-○-) and *A. cygnea* (-●-). A. Cd concentration (µg Cd/g organ dry wt) in gill; B. Normalized burden (µg/g total dry wt) in gill; C. Cd concentration in mantle-edge; D. Normalized burden in mantle-edge. Values in A to D are the mean of 4 animals. ± SEM

Influence of Seasonal Condition

In Figure 6, time courses of Cd accumulation in *A.* [illegible] (from the present experiment) are compared with those reported earlier (Hemelraad *et al.* [illegible]). Both exposure experiments were conducted under comparable conditions, at the same temperature. In the former case, mussels were exposed in the summer, in the latter case in the winter. In the summer experiment, Cd concentrations were lower (Figure 6A), especially after six weeks. This resulted in a less pronounced biphasic character of the accumulation curve, compared to that in the winter experiment. From six weeks on, the ratio

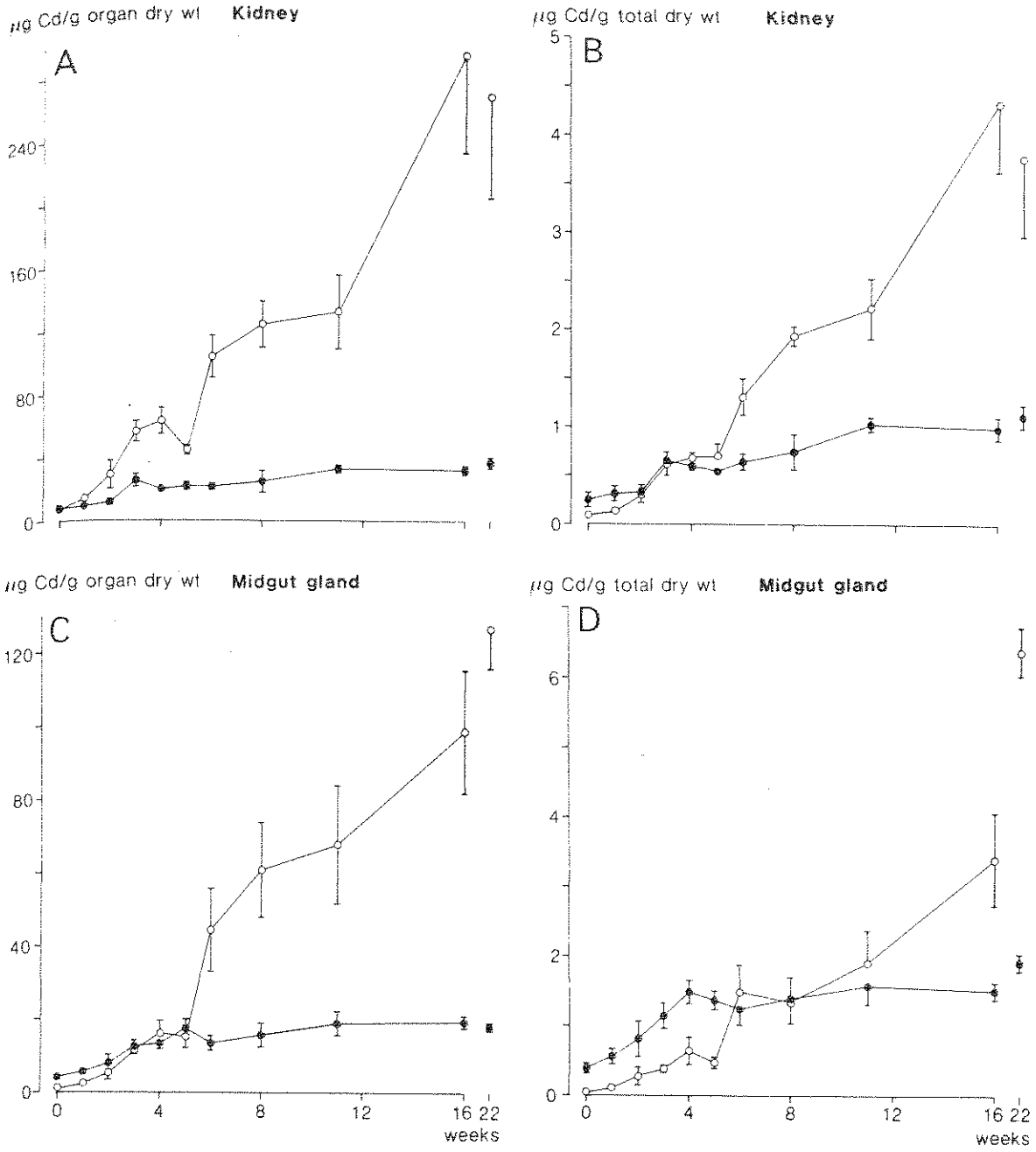


Fig. 3. Time-dependent uptake of Cd into two organs of *A. anatina* (-○-) and *A. cygnea* (-●-). A. Cd concentration (µg Cd/g organ dry wt) in kidney; B. Normalized burden (µg Cd/g total dry wt) in kidney; C. Cd concentration in midgut gland; D. Normalized burden in midgut gland. Values in A to D are the mean of 4 animals, ± SEM

was constant at a value of one and a half. However, practically the same value was found for the ratio of the total soft body weights of summer and winter animals (Table 2). When, therefore, Cd burden (*i.e.*, amount per animal) is taken in place of Cd concentration, a different picture arises (Figure 6B). Except for the samples at eight weeks, the curves overlap.

The same is found for the separate organs, when the burden—but not the normalized burden!—is taken to compare the summer and winter animals. For example, Cd amounts per gill are much more similar (Figure 6D) than Cd concentrations (Figure 6C). The difference between the latter curves is "corrected" by the difference of organ weights. Whereas for many organs the relative weight varied

Table 2. Mean dry weight of whole animal and separate organs for *A. anatina* (n = 40) and two groups of *A. cygnea* (in summer, n = 40; in winter, n = 36)

	A. anatina[a] (summer)		*A. cygnea*[b] (summer)		*A. cygnea*[c] (winter)	
	mg	%	mg	%	mg	%
Whole animal	3500	—	3537	—	2297[d]	—
Foot/visceral complex	1329	38	867	25	476	21
Gills	450	13	1003	28	525[d]	23
Mantle edge	397	11	289	8.2	221	9.6
Kidney	377	11	285	8.5	308	13
Liver	314	9.0	302	8.5	208	9.1
Adductor muscle	259	7.4	227	6.4	185	8.1
Mantle	182	5.2	106	3.0	90	3.9
Midgut gland	124	3.5	326	9.2	180	7.8
Rectum	44	1.3	105	3.0	83	3.6
Labial palps	24	0.7	27	0.8	21	0.9

[a] Animals from 0 to 16 weeks
[b] Animals from 0 to 22 weeks
[c] Values derived from Hemelraad *et al.* 1985
[d] Corrected for the presence of glochidia

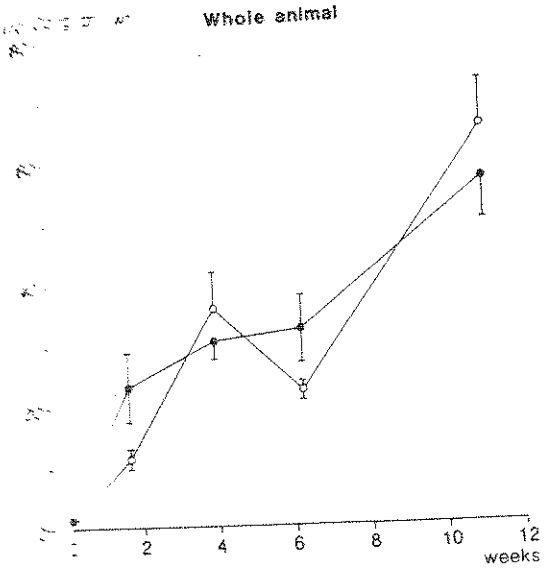


Fig. 4. Cd concentration *vs* exposure time in whole animal exposed to 55 ppb Cd; -○- *U. pictorum*, -●- *A. cygnea*. Mean of 4 animals ± SEM

Depending on the species considered, winter and summer animals of one and the same species showed rather constant relative organ weights.

Subcellular Cd Distribution

Two examples, gills and kidney, of the subcellular distribution of Cd and the marker enzymes succinic dehydrogenase (SDH) and acid phosphatase (AP), after a 16-week exposure period, are given in Figures 7, A and B. In all organs examined, Cd concentration (on a protein basis) was highest either in the nuclear or in the cytoplasmic fraction and lowest in the mitochondrial/lysosomal fraction. SDH activity was divided, with varying ratio, between the mitochondrial and the nuclear fractions. The bulk of AP activity was also found in these fractions, with sometimes little activity present in the two other fractions (Figures 8, A and B).

In Figures 9, A to D, Cd distributions are shown for *A. cygnea* (5 and 14 weeks) and *A. anatina* (5 and 16 weeks), in the four organs. No time-dependency was observed for the partition of Cd between the particulate and the non-particulate (cytoplasmic) fractions. However, species- as well as organ-specific differences occurred. Only in the kidney, of either species, the cytoplasmic Cd amount exceeded that of the particulate fraction. In the mantle also, there was no difference between the species, but in this organ only about one third of the Cd amount was present in the cytoplasm. For gills and midgut gland striking differences were observed between the species. In the gills of *A. cygnea* the cytoplasmic contribution amounted to 37%, about the same value as for mantle tissue. In *A. anatina* the cytoplasm contained only 18% of the total amount in gill. The reverse situation is seen in Figure 9C for the midgut gland. An explanation for the differences between the organs on the one hand and between the species (for two out of four organs) on the other could be the varying quality of subcellular fractionation. However, a relation between the obvious differences in distribution of Cd and the grade of organelle separation was not visible.

Molecular Distribution of Cytoplasmic Cd

The Sephadex G-75 elution pattern of a cytoplasmic fraction (from *A. cygnea* kidney, after six weeks) is shown in Figure 10. Cadmium was separated into two protein containing peaks, while no metal was detectable in the low-molecular weight fraction. The first Cd peak (P_1) was concomitant with the void volume peak, the second (P_2) was located at the switch of the absorbance ratio A_{280}/A_{250}, showing an apparent molecular weight of 11 kD (Figure 11). Therefore, the P_2 peak is provisionally interpreted as containing metallothionein (MT)-bound Cd.

For gills and kidney the percentages of MT-bound (P_2) Cd are shown in Figures 12, A and B, independent of the exposure time. In *A. anatina* gill the percentage of Cd in P_2 increased from 20% to a level of 60% that was constant from the ninth week. In

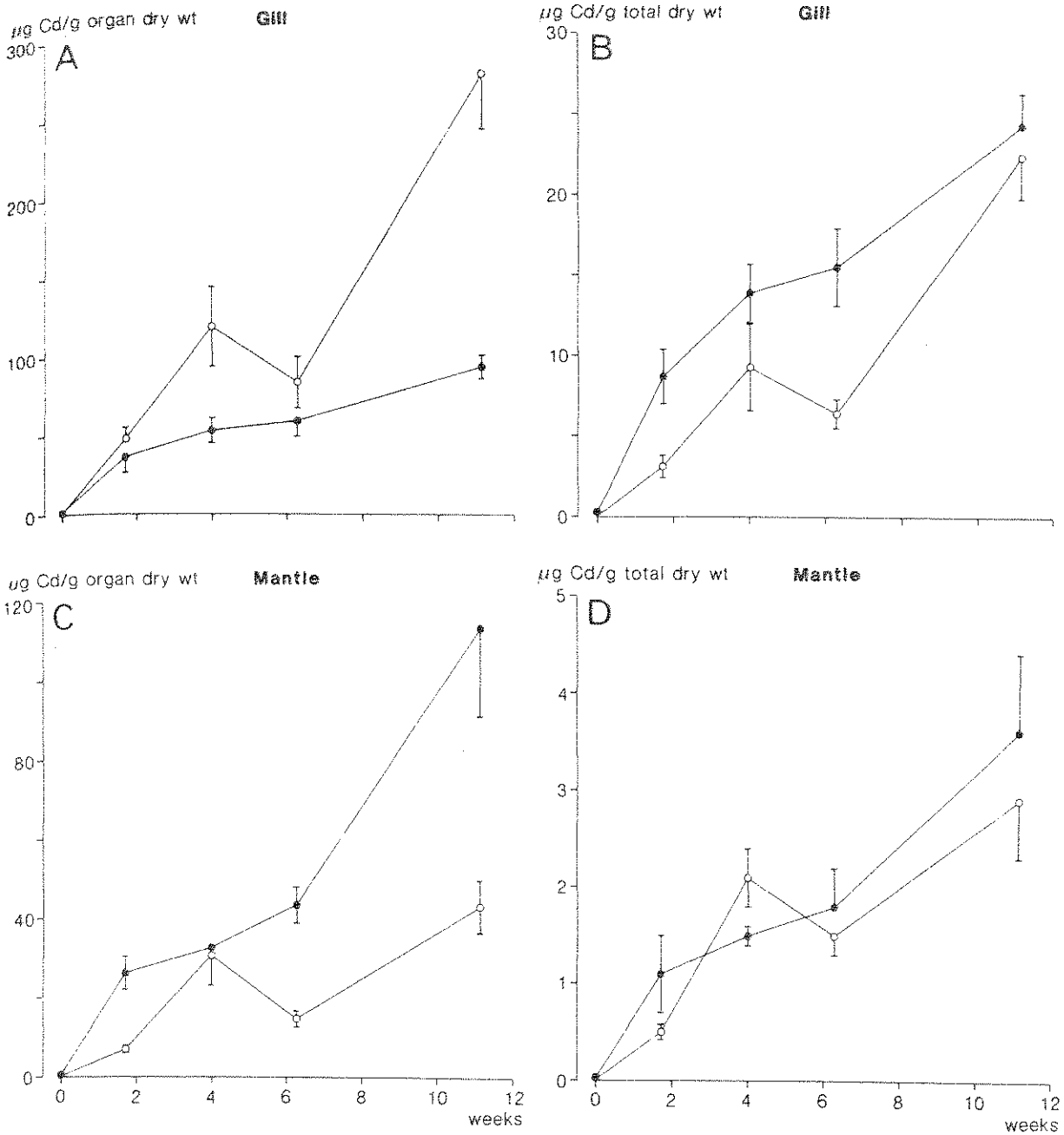


Fig. 5. Time-dependent uptake of Cd into two organs of *U. pictorum* (-○-) and *A. cygnea* (-●-). A. Cd concentration (µg Cd/g organ dry wt) in gill; B. Normalized burden (µg Cd/g total dry wt) in gill; C. Cd concentration in mantle; D. Normalized burden in mantle. Values in A to D are the mean of 4 animals. ± SEM

A. cygnea the P_2 portion also started at about 20%, but increased more slowly. By the end of the exposure (25 weeks) also in *A. cygnea* gill 60% of the cytoplasmic Cd was in the P_2 fraction. A similar pattern, namely an increasing P_2 to P_1 ratio with time, was observed for mantle and midgut gland of both species (not shown). In the kidney, no clear time-dependency was found. In *A. cygnea* the percentage of Cd in P_2 fluctuated around a mean value of 73. In *A. anatina* the course was more irregular, at a clearly lower mean level of 53%.

Discussion

In the preceding paper, it was demonstrated that *A. cygnea* accumulates Cd, in the whole animal as well as in most of the organs, in a non-linear way,

Table 3. Mean weight (as percentage of total soft body weight) of some organs of *A. cygnea* and *U. pictorum*. For both species, n = 37

	A. cygnea %	*U. pictorum* %
Gills	26	7.5
Gut-gonad complex	22	39
Mantle	12	6.8
Midgut gland	4.0	8.4
Kidney	1.9	1.0

The present data confirm these accumulation characteristics for three species of Unionidae. The general pattern is most pronounced in the time course of the separate organs, *e.g.*, Figures 2A and 3A. Two or more phases can be discerned each consisting of a period of elevated uptake and terminating in stagnation of accumulation. The overall course, therefore, resembles that of two or three superimposed hyperbolas. This phenomenon has been connected (Hemelraad *et al.* 1985) with a fluctuating animal activity with regard to ventilation. Response of this factor to short-term Cd exposure in the case of *A. cygnea* has been reported (V.-Balogh and Salánki 1984).

In comparative studies, with *A. anatina* and *Anodonta* on the one hand and *A. cygnea* and *Unio pictorum* on the other, generally small differences were encountered in whole body Cd content (per unit dry weight) through the first eleven weeks of exposure. Occasionally larger differences, especially in the latter comparison, may arise from unequal animal weight distribution among the sample pools. Our data tend to a negative correlation between Cd uptake and body size. The same has been reported for many marine molluscs, but also a positive correlation and absence of body size dependence occurs (for a survey, see Ray 1984).

A much larger divergence between species was found when considering Cd concentrations of individual tissues, *e.g.*, Figures 2A and 5A. Therefore, although right species seem to differ with respect to the internal Cd distribution. However, also large differences exist between the relative organ weights, especially for gills, mantle, kidney and the gut-gonads complex. When these two sets of differences are brought together, to compare the amounts of tissue-bound Cd per unit *total* soft body weight, the divergence of the time courses diminishes strongly. This means that the contributions of the individual organs to the total Cd load were more similar in the three species than were the organ concentrations. Only the small gills of *Unio* cannot match the much heavier gills of *A. cygnea* (Table 3) with respect to the contribution of this organ to the body load. As a typical example, mantles of *U. pictorum* and *A. cygnea* accumulate about the same amount of Cd (per g total soft body weight), although this organ of the latter species has only one-half the weight of that of *Unio*.

These findings raise the question as to the reliability of the concentration parameter. In accumulation studies, it is common practice to express heavy metal contents as μg per unit dry or wet weight; also, when different species are considered (Greig 1979; Salánki *et al.* 1982). The conclusion is that the term "dry weight" comprises elements that make its use, in comparative studies, doubtful. One could think of elements of a variable mass factor that do not have the capacity for uptake and storage of heavy metals; for example, the amount of metabolic stores (glycogen, lipids) or the presence of so-called calcium granules which may constitute a considerable part of the total organ weight in freshwater mussels (Steffens *et al.* 1985).

Not all organs obey the rule of constant organ contribution to the total body burden. The example of diverging contributions of the gills in *Unio* and *A. cygnea* was already mentioned. In the comparison of the two *Anodonta* species (Figure 3B), the contributions of the kidneys diverge when the second phase of accumulation is initiated, namely, after five weeks. For the midgut gland, contributions diverge from the very beginning of exposure (Figure 3D) for a period of six weeks. For the next period of five weeks, the normalized burden values were similar, whereas Cd concentrations diverged strongly (Figure 3C). Generally, in comparative studies, the mode of expressing the extent of accumulation has to be considered very carefully in order to avoid premature inferences with regard to similarities or differences between species.

Drastic divergence between the *Anodonta* species, regardless of the mode of expressing the extent of Cd accumulation, occurred after eleven weeks of exposure. Whereas in *A. cygnea* accumulation increased only slightly, uptake in *A. anatina* started again at a very high rate. In both species, mortality was negligible during the first 16 weeks (two and three animals out of 142, at the start of the exposure), but increased drastically between 16 and 22 weeks (31 and 27 animals out of 40 remaining after 16 weeks for *A. anatina* and *A. cygnea*, respectively). Therefore, over 90% of the total mortality during 22 weeks occurred in the last period. Concomitant investigations of ventilation activity and metabolic status will be necessary for explaining the strong difference in Cd accumulation after eleven weeks and the simultaneous appearance of mortality. It is tempting to speculate about an essential role of the pigments. In contrast to *A. anatina*,

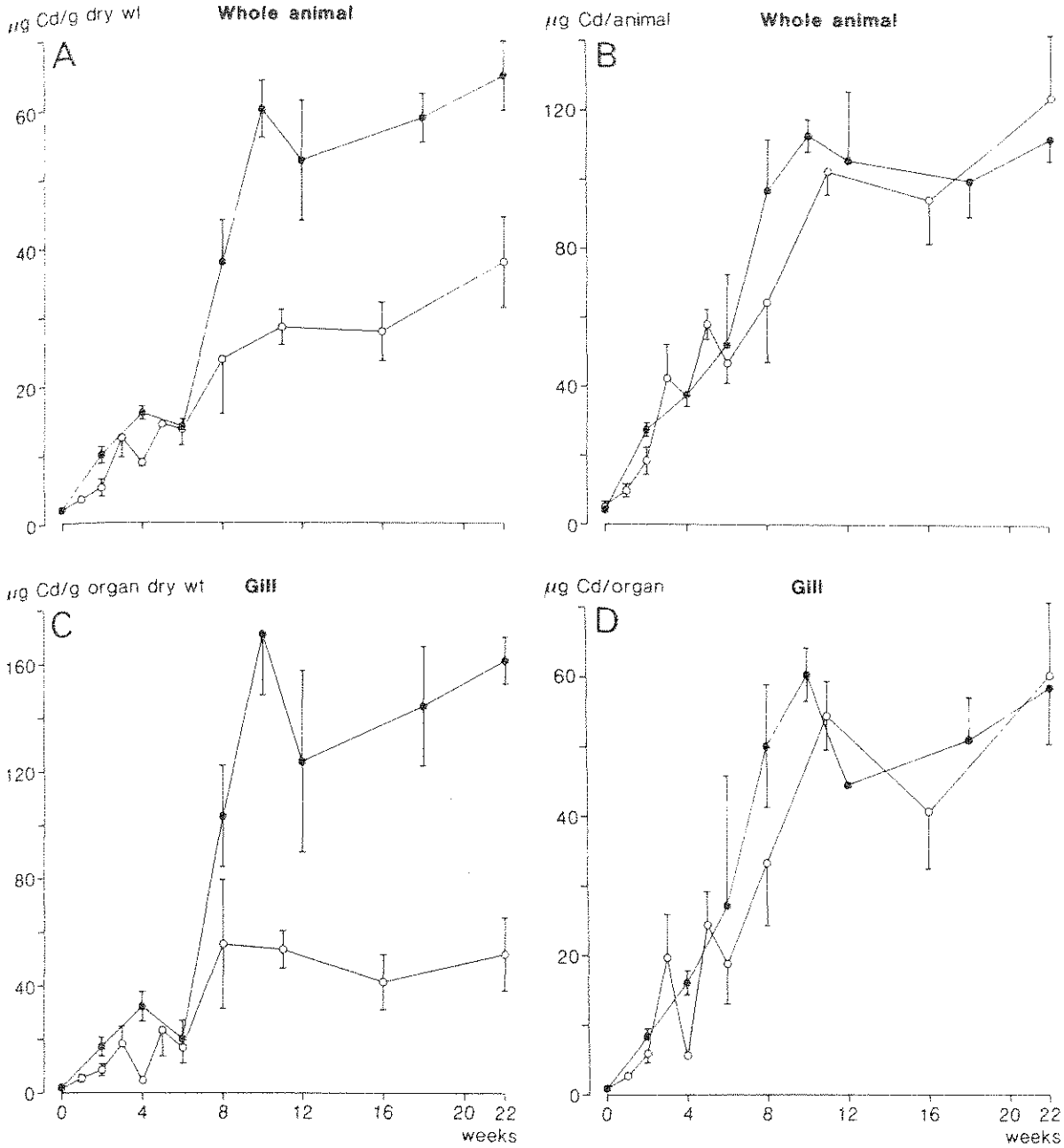


Fig. 6. Comparison of Cd uptake into *A. cygnea* during exposition to 25 ppb Cd in summer (-○-) and winter (-●-). A. Cd concentration (µg Cd/g dry wt) in whole animals; B. Cd burden (µg Cd per animal) in whole animals; C. Cd concentration (µg Cd/g organ dry wt) in gill; D. Cd burden (µg Cd per organ) in gill. Values in A to D are the mean of 4 animals, ± SEM

A. cygnea is strongly pigmented. Pigmentation has been related to the tolerance of anoxia (Zs.-Nagy 1977; Karnaukhov 1979). Moreover, for marine molluscs a correlation has been found between the tolerance of pollution and the degree of pigmentation (Karnaukhov 1979). If indeed the extent of pigmentation and the tolerance of anoxia and pollution are related phenomena, then the divergence of accumulation was caused by a differential behavioral response. *A. cygnea* as the more adapted species would reduce the ventilation rate in order to prevent itself against a further increase of noxious metal accumulation but eventually collapse through anoxic stress. *A. anatina* being less able to withstand anoxia and, therefore, to reduce the ventilation rate for longer periods, would accumulate Cd again up to a lethal level.

When the present data of Cd uptake into *A.*

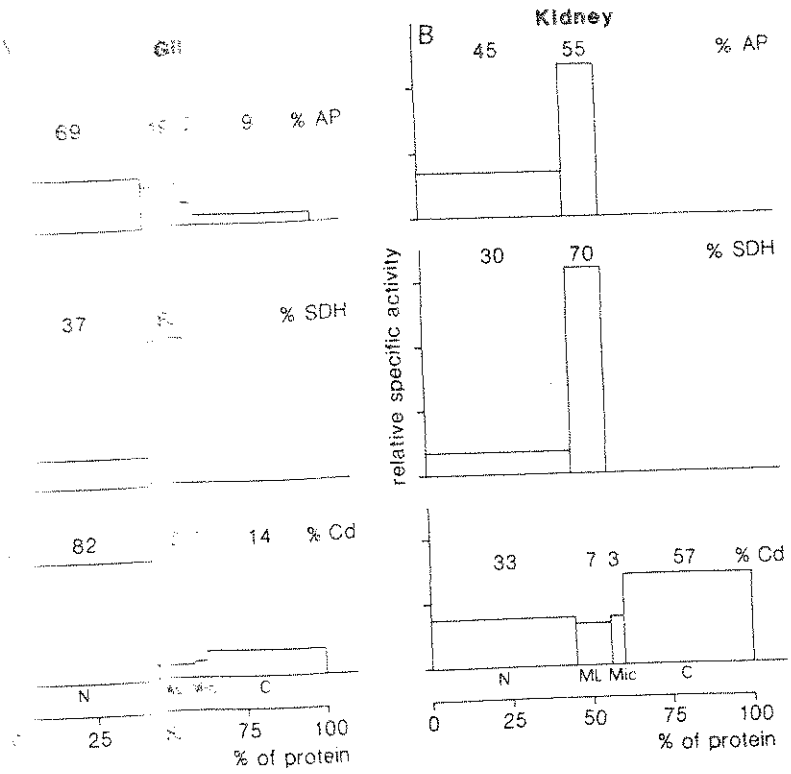


Fig. 7. Subcellular distribution of Cd and the marker enzymes acid phosphatase (AP) and succinic dehydrogenase (SDH) in gill (A) and kidney (B) of *A. anatina*, exposed to 25 ppb Cd for 16 weeks, among the nuclear (N)-, mitochondrial/lysosomal (M/L)-, microsomal (Mic)- and cytosolic (C) fractions

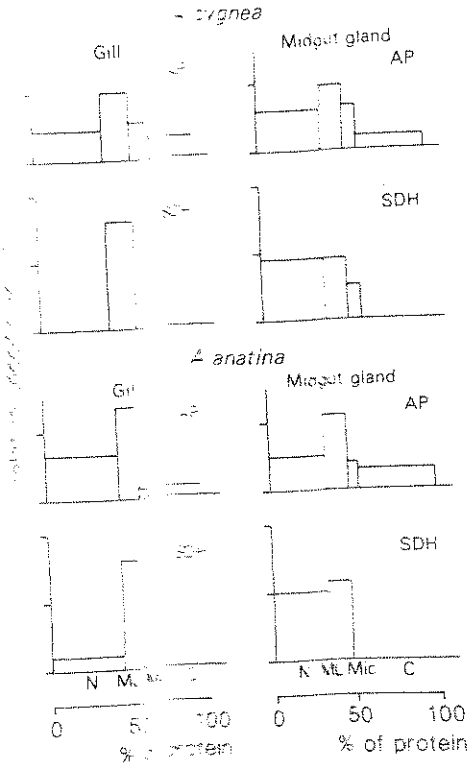


Fig. 8. Subcellular distribution of the marker enzymes AP and SDH in gill and midgut gland of *A. cygnea* and *A. anatina*, exposed to 25 ppb Cd for 6 weeks

cygnea are compared with those previously reported (Hemelraad *et al.* 1985), it must be concluded that usage of the concentration parameter may be misleading. Concentrations of Cd in whole animal as well as in the separate organs differed by a factor one and a half (values in winter being higher), as did the total body dry weights (Table 2; animals of summer condition being heavier). In this case, replacement of the concentration parameter for normalized burden, that was applied above, makes no sense, since the relative organ weights were similar. Instead, usage of the absolute burden term makes clear that Cd uptake into whole animal and separate tissues was similar in either case, independent of the condition of the animals. Especially in long-lasting investigations, as for instance in biomonitoring work, care must be taken to select proper criteria as to the measure of heavy metal contents.

The subcellular and molecular distribution of Cd was investigated, using three variables, exposure time, organ- and animal specificity. With respect to exposure time, a striking constancy of subcellular distribution was found per organ, per species (Figures 9, A–D). This is the more notable as the sample points of five and 14/16 weeks, respectively, fell into separate phases of accumulation. Especially in *A. anatina*, the rate of uptake increased strongly after 11 weeks. Apparently, irrespective of the rate of uptake, the cells partition the Cd supply

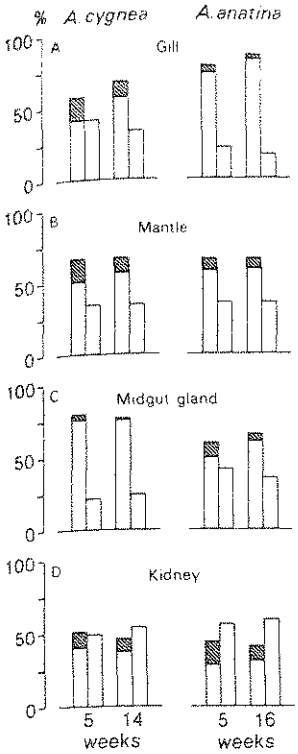


Fig. 9. Relative distribution of Cd in gill (A), mantle (B), midgut gland (C) and kidney (D) of *A. cygnea* and *A. anatina*, exposed to 25 ppb Cd for 5 and 16 weeks. Left bars—particulate fractions (upper part: M/L- plus Mic-fraction, lower part: N-fraction); right bars—cytosolic fraction

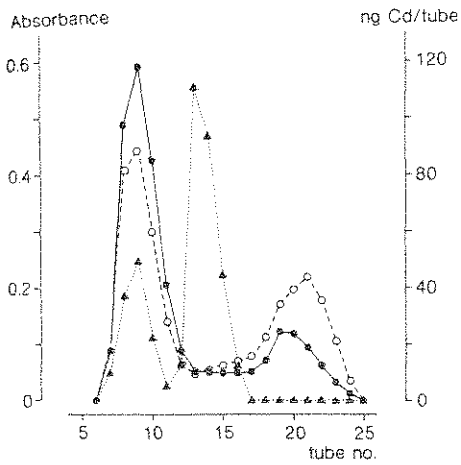


Fig. 10. Sephadex G-75 elution profile of kidney cytosolic fraction of *A. cygnea*, exposed to 25 ppb Cd for 6 weeks: -○- A_{250}, -●- A_{280}, -▲- Cd

among the organelles in a constant manner. It was demonstrated earlier that in *A. cygnea*, exposed to 25 ppb Cd, a steady state of organ distribution occurred after five to six weeks of exposure (Hemelraad *et al.* 1985). Thus, starting from this time, the distribution of Cd among the organs and within the cells is constant. It is possible that a pre-steady

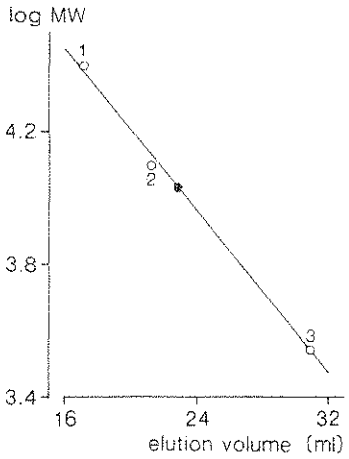


Fig. 11. Calibration of the Sephadex G-75 column with (1) chymotrypsinogen, (2) cytochrome C and (3) insuline chain B; -○- markers, -●- P_2

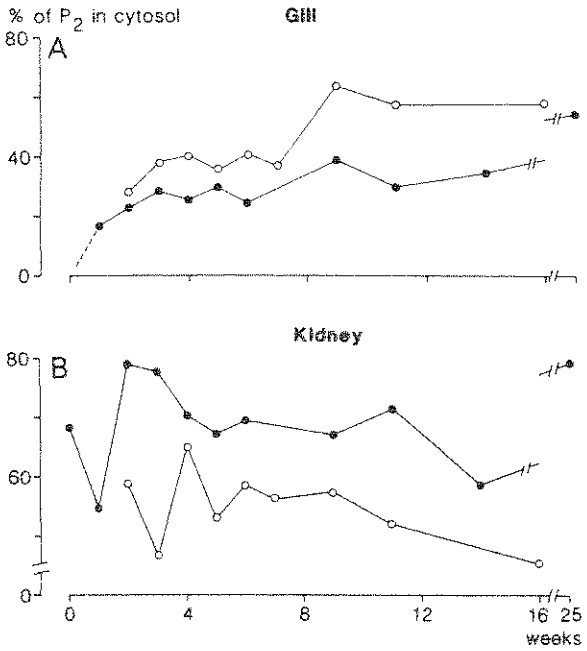


Fig. 12. Contribution (in %) of P_2-Cd to total cytosolic Cd in gill (A) and kidney (B), in dependence of the exposure time; -○- *A. anatina*, -●- *A. cygnea*

state of subcellular distribution would have been observed within the initial phase up to four weeks.

Clear differences appeared between the separate organs. Generally, Cd concentrations of the mitochondrial/lysosomal and the microsomal fractions were smaller than those of the nuclear and cytosolic ones. Whereas the amounts of nuclear and cytosolic protein were nearly equal (40.5% ± 1.8 -SEM- and 39.7% ± 2.2 -SEM-, respectively), Cd amounts, and hence Cd concentrations (on a protein base) differed in these fractions. The largest difference was found in gills of *A. anatina* and in midgut gland of *A. cygnea:* about 75% of total organ Cd was as-

sociated with the nuclear fraction and only 20% was in the cytosol.

The question arises as to the high nuclear Cd concentration in some cases as well as to the species-dependent differences. Data concerning the subcellular Cd distribution in freshwater invertebrates are lacking in the literature. In marine animals, strongly diverging distributions have been observed. In unexposed oysters 40–50% of total Cd was found in the nuclear or particulate fraction (Coombs 1979; Julshamn and Andersen 1983a; Sharma 1983). In *Mytilus edulis*, after short-term exposure, a considerable amount of Cd was found associated with the particulate fraction (Nolan and Duke 1983). By contrast, very low contributions of the nuclear fraction were observed in unexposed *M. edulis* (Coombs *loc.cit.*; Julshamn and Andersen 1983b) and in *Modiolus modiolus* (Julshamn and Andersen 1983c).

It is possible that part of the Cd in the 500 g sediment is bound to DNA. It has been reported (Waalkes and Poirier 1984) that, *in vitro*, double stranded DNA has high-affinity binding sites for Cd. On the other hand, the differing share of the nuclear fraction in the cellular Cd distribution points to a variable composition of the 500 g pellet. Unbroken cells and cell debris will sediment at low centrifugal force. In our experiments, the nuclear fraction was always contaminated with lysosomes (AP activity) and mitochondria (SDH activity). The latter cell organelles contribute to a small extent in view of the low Cd amount in the mitochondrial/lysosomal fraction; this does not necessarily hold for the lysosomes. These organelles may strongly vary in size. Electron microscopical studies of *Anodonta* kidney show that part of the lysosomal population, especially the residual bodies or tertiary lysosomes, have dimensions comparable to those of nuclei. If Cd is accumulated in such "membrane-limited vesicles", as reported for *M. edulis* (George and Pirie 1979; Janssen and Scholz 1979), then the Cd amount will reach a high level in the 500 g sediment.

Yet, another factor must be mentioned that may influence the composition of the first sediment. In gastropods (Abolins-Krogis 1958) and in the freshwater bivalve *Ligumia subrostrata* (Silverman *et al.* 1983) extra- and intracellular inorganic concretions, also called calcium granules, occur. These particles function as storage sites of calcium and other essential minerals and play a role in calcium homeostasis. In addition, the granules could function in cellular metal detoxification (Simkiss 1981). In *Anodonta*, also, considerable amounts of calcium granules are found in the gills (Steffens *et al.* 1985). The contribution of the granules to the storage of Cd is under investigation.

Marginal differences between the *Anodonta* species were found in the subcellular Cd distribution in mantle and in kidney tissue. The highest contribution of the cytosol was seen in the latter organ, namely 50 to 60% of total organ Cd. This may be related to the storage function of the kidney; in unexposed animals by far the highest concentration of Cd was observed in this organ. At the onset of exposition, the kidney was already provided with a pool of cytoplasmic Cd-binding proteins, or synthesis of these could be readily inducible.

Based on the results of the subcellular distribution, the organs examined can be grouped into three classes. The first group is characterized by a distribution ratio of 65:35 for particulate and nonparticulate Cd (mantle and gills of *A. cygnea*, mantle and midgut gland of *A. anatina*). The second group (midgut gland of *A. cygnea* and gills of *A. anatina*) shows a more extreme ratio (about 80:20), with Cd predominantly associated with the particulate fraction. Here, a stronger involvement of membrane-limited vesicles (tertiary lysosomes) and/or calcium granules in Cd accumulation could be assumed. The third group consists of the kidneys in which more than half of the total Cd amount is present in the cytosol. This organ serves a special role in Cd storage when ambient metal concentrations are well below the experimental one. No obvious correlation has been found between cellular distribution and organ accumulation.

Total cytosolic Cd was present in two peaks after gel filtration: the void-volume peak (P_1) and a peak (P_2) with an apparent molecular mass of 11 kD. No Cd was found in the low-molecular weight (LMW) range. This pattern of Cd partition was independent of exposure time, type of organ and species. Very small amounts of Cd in the LMW fraction are commonly observed, for instance in exposed sea mussel (Noël-Lambot 1976), in crab and shrimp (Olafson *et al.* 1979) and in exposed *Ostrea lutaria* (Sharma 1983). In cases of a substantial Cd amount in the LMW region, *e.g.* in shrimp (Olafson *et al. loc.cit.*) and the oyster *Ostrea edulis* (Julshamn and Andersen 1983a), animals had not been exposed.

The second Cd peak was eluted at a V_e/V_0 ratio, which is consistently found in the Sephadex G-75 chromatography of metallothioneins (MT), indicating an apparent molecular mass of about 11 kD. This corresponds to a real molecular mass in the range of 5–6 kD (Pulido *et al.* 1966; Olafson *et al.* 1979). It is, therefore, likely that in *Anodonta* MT's are synthesized, resembling those of vertebrates.

With respect to the P_2 contribution to total cytosolic Cd, organ-specific and species-dependent differences were observed. In gills (Figure 12A) and also in mantle and midgut gland (not shown) the percentage of Cd in P_2 increased with exposure time, especially during the first four weeks. Appar-

ently, MT-synthesis must be induced in these organs. By contrast, for kidney of either species the percentage of Cd in P_2 did not change systematically in the course of the exposition (Figure 12B). Most probably, the basis for this observation as well as for the relatively large participation of the cytosolic compartment in sequestering Cd has to be sought in the role of the kidney as the primary site of accumulation when environmental Cd is low.

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