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Occurrence of calcium concretions in various tissues of freshwater mussels, and their capacity for cadmium sequestration

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The freshwater unionids *Anodonta anatina, Anodonta cygnea* and *Unio pictorum* were examined with respect to the distribution of calcium concretions among the various organs, and to a possible role of concretions in heavy metal detoxification. Gills of all three species contain large amounts of concretions, up to 55% of the tissue dry weight in *A. cygnea*. Smaller amounts are present in the mantle and the midgut gland, and also in granulocytes. The other organs, including the kidney, are practically devoid of concretion material. By electron-microprobe analysis, the concretions appear to be of the phosphatic type, and also contain manganese, iron and zinc. When animals were exposed to $CdCl_2$, cadmium was bound to the concretion fraction of the various organs. But, in almost all cases, Cd concentrations in the concretion material were lower than those in the whole organ. Furthermore, the contribution of the concretion fractions in Cd accumulation decreased over the course of exposure. It is concluded that calcium (pyro)phosphate concretions do not function as preferential sequestration sites for cadmium.

Key words: Mussel, freshwater; *Anodonta*; *Unio*; Calcium concretion; Cadmium; Detoxification

INTRODUCTION

Organisms are capable of forming a diverse array of minerals, which fulfil important biological functions such as support (skeleton, cuticle), protection (shell), sensing (otoliths, auditory ossicles), grinding of the food (teeth, grinding apparatuses), and storage and detoxification (granules). Mineral deposits can be formed intra- and extracellularly (Lowenstam, 1981).

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TABLE I

Occurrence of inorganic calcium concretions in bivalve molluscs.

Species	Tissue	Composition	Reference
MARINE			
Argopecten irradians	kidney	Ba,Cd,Cr,Cu,Hg,Ni,Pb,Sr,Zn	Doyle et al., 1978
Mercenaria mercenaria	kidney		
Argopecten irradians	kidney	Ca,Cd,Cr,Cu,Fe,Mg,Mn,P,Zn	Carmichael et al., 1979
A. gibbus	kidney		
Pecten maximus	kidney	Ca,Cd,Cu,Fe,K,Mg,Mn,P,S,Zn	George et al., 1980
FRESHWATER			
Ligumia subrostrata	gills	Ca,PO_4,Fe,HCO_3,Mn	Silverman et al., 1983
Velesunio angasi	whole body	Ba,Ca,Mg,Ra-226	Jeffree and Simpson, 1984
Anodonta grandis	gills	Ca,PO_4, organic matrix	Steffens et al., 1985
Ligumia subrostrata	gills		
Ellipsio crassidens	gills		
Magaritifera hembeli	gills		

Calcium concretions are found in many aquatic invertebrate groups: bivalved (see Table I) and gastropod molluscs (Haley and Gibson, 1971; Sminia et al., 1977; Mason and Nott, 1981), crustaceans (Guary and Négrel, 1981; Becker et al., 1974; Hopkin and Nott, 1979), cirripedes (Walker et al., 1975), and polychaetes (Pirie et al., 1985).

Calcium carbonate and phosphate are the dominating inorganic ions in concretions. In addition, Fe, K, Mg, Mn, S and Zn are found, as well as small amounts of Cd, Cr, Cu and Pb. Easily soluble calcium carbonate granules often appear in connective tissues. The more insoluble type, consisting of variable mixtures of Ca, Mg, phosphate, and trace elements, is found in midgut gland and renal cells, and has been considered a potential trap of toxic metal ions (Simkiss, 1981a).

Calcium deposits in aquatic invertebrates bind heavy metal cations with differing affinity (Simkiss, 1981b), also depending on the granule composition (Mason and Nott, 1981). Detoxifying, intracellular granules in gastropods are situated in the digestive gland (Simkiss, 1981a). In marine bivalves, in a short-term exposure to a high Cd concentration, the metal has been found to bind to the concretions in the kidney (Carmichael and Fowler, 1981). Cadmium, among other heavy metals, has also been detected in kidney concretions of unexposed animals (Doyle et al., 1978; Carmichael et al., 1979). Metal-containing tertiary lysosomes appear in kidney cells of *Mytilus edulis* (George and Pirie, 1979; George, 1983).

Recently, a study was published on the kinetics of cadmium in three species of Unionidae (Hemelraad et al., 1986a,b). As the unionid *Anodonta grandis* has been

found to contain considerable amounts of calcium concretions (Steffens et al., 1985), it appeared interesting to investigate their possible role in cadmium detoxification. In this study, we report on the occurrence and nature of calcium granules in diverse tissues of freshwater mussels, and on the contribution of granules in the accumulation of cadmium.

MATERIALS AND METHODS

Animals

Anodonta anatina L., *Anodonta cygnea zellensis* Gmelin and *Unio pictorum* L. were collected from ponds and ditches in the Maarsseveen lake district near Utrecht in autumn 1985. The animals were kept in aquaria without substratum, in streaming tapwater for 3 wk prior to analysis and onset of exposure. During acclimation and under experimental conditions the animals were not fed.

Isolation of calcium concretions

Calcium concretions were isolated from the separate organs, essentially after Silverman et al. (1983). Lyophilized tissues were homogenized in a small amount of distilled water with an Ultra-Turrax homogenizer. The homogenate was heated for 2 min at 100°C. An equal volume of 1 N NaOH was added, and the mixture incubated at 60–70°C for 1 h. Concretion material was separated by centrifugation (10 min at 5000 *g*). The sediment was resuspended and washed three times in 1 N NaOH. Final washing was with distilled water. The pellet was lyophilized to constant dry weight.

Chemical analysis of the concretions

Isolated fractions of concretions were analyzed for calcium, cadmium, copper, iron, manganese and zinc by means of atomic absorption spectrophotometry (AAS). The protein content of the isolated concretions was determined with the Coomassie Brilliant Blue method (Bradford, 1976). Carbohydrate and lipid content was measured with the anthrone and vanillin methods (Holwerda et al., 1977), respectively.

Scanning electron microscopy and electron-microprobe analysis

Isolated concretion material was wetted with a distilled water/alcohol mixture, transferred onto an aluminium stub, dispersed by sonication and air-dried. Samples were sputter-coated with gold-palladium and observed by scanning electron microscopy (Stereoscan S 150) for purity control and examination of the surface structure. Elemental composition of carbon-sputtered samples was determined with the Stereoscan equipped with a Link energy dispersive X-ray microanalysis system. The microscope was operated at 20 kV and a spectrum collection time of 100 s.

Histochemistry

Small samples of several organs were collected and immediately frozen in liquid nitrogen or immersed in neutral formalin. The frozen samples were allowed to warm to −20°C and then sectioned at 10 μm thickness. The formalin-fixed samples were dehydrated, embedded in paraffin, and sectioned at 5 μm.

Cryostat sections were stained with the Alizarin red S procedure for calcium (Pearse, 1960). Paraffin sections were treated by the Von Kossa method for calcium carbonate and phosphate deposits (Pearse, 1960), and counterstained with Alcian blue. The latter method was also applied to organ pieces that had previously been decalcified by immersion in 5% HNO_3 for 10 min, followed by rinsing in distilled water.

Exposure system

Mussels were exposed to $CdCl_2$ at a nominal Cd concentration of 40 μg/l in glass aquaria. To the flow-through system, tapwater and metal solution were supplied with pumps at rates of 7 l/h and 50 ml/h, respectively. Water quality parameters are given in Table II. The experiment was started with about 50 animals of each species in 150 l water of 11 to 12°C. Exposure lasted from September until November 1985. Cd concentration in the water was measured twice a week by AAS. Actual concentrations amounted to 36.4 μg/l ± 1.2 (SD).

Metal analysis

Exposed animals were examined individually for cadmium in gills (*A. anatina* and *U. pictorum*), and in gills, mantle, and midgut gland (*A. cygnea*). Both the whole tissue and the isolated concretion fraction were analyzed. Prior to dissection, the animals had been kept in unspiked tapwater for 24 h, in order to eliminate adherent cadmium.

TABLE II

Water quality parameters for the exposure of animals.

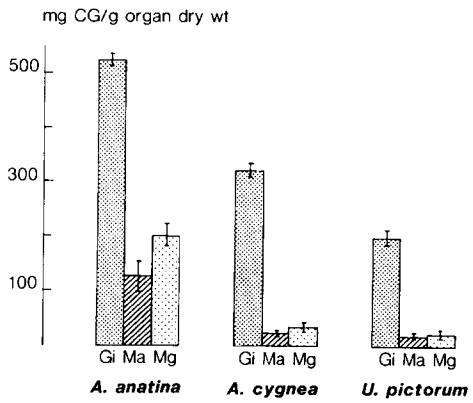
Parameter	Dimension	Minimum	Maximum
Total hardness	mmol/l	0.84	1.45
Chloride	mg/l	14	26
Bicarbonate	mg/l	95	115
Phosphate	mg/l	0.07	0.25
Calcium	mg/l	29	52
Iron	mg/l	0.1	0.1
Magnesium	mg/l	3	4
Zinc	μg/l	10	10
Cadmium	μg/l	0.1	0.1
pH		7.4	8.4
DO	mg/l	7	14

Gills and mantle from one side of each animal were used for the isolation of concretions, and from the opposite side for assay of Cd in tissue. From gills that contained glochidia, only the inner demibranches, free of glochidia, were used. The midgut gland was randomly cut into two parts. The excised organs were lyophilized for 48 h and weighed. The concretion fraction was isolated as described above. Cd was measured by AAS after decomposition of dry tissue and concretion fraction with nitric acid, according to Hemelraad et al. (1986a). Other metals in concretion fractions of unexposed animals were assayed similarly. For the determination of calcium, $LaCl_3$ (0.125%, w/v) was added in order to suppress chemical interferences. Water samples were assayed without prior decomposition. All samples were assayed in tenfold (RSD < 5%).

RESULTS

Occurrence of calcium concretions in various organs

Gills of all three unionid species contain large amounts of calcium concretions (Fig. 1). In *A. anatina* gills, inorganic concretions make up even more than 50% of the total dry weight. Considerably less concretion material is present in the mantle and the midgut gland. The values of Fig. 1 indicate, however, no more than this particular situation; especially in the *Anodonta* species, concretion amounts varied strongly. For example, weight portions of between 3 and 30% and of between 2 and 12% (of total organ dry weight) were measured for the concretion fraction in midgut gland and mantle, respectively, of *A. cygnea* collected in different seasons



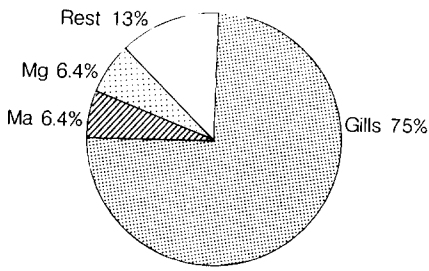


Fig. 1. Contents of calcified granules (CG) in gills (Gi), mantle (Ma), and midgut gland (Mg) of three unionid mussel species. Mean of 4 animals, ± SEM.

Fig. 2. Distribution of calcium concretions as % of total body content among organs and the remainder (Rest) of *A. cygnea*. Ma = mantle, Mg = midgut gland.

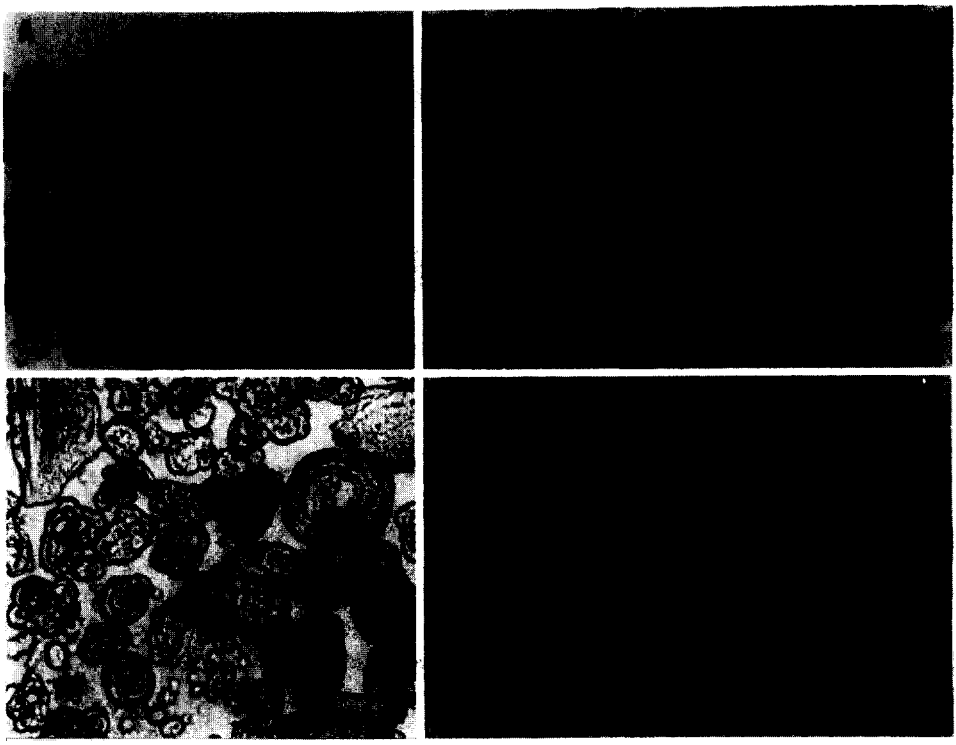


Fig. 3. (A) Rostral to caudal section across a gill demibranch of *A. anatina* stained with the Von Kossa method, counterstained with Alcian blue. Paired chitinous rods (cr) are located in the gill bars (gb). In the connective tissue underneath the bars calcium granules (gr) occur in big clumps. Bar = 50 μm. (B) Lightmicroscopical photograph of concretion material from *A. cygnea* gills. In the upper left corner part of the chitinous rod (cr) is seen. Bar = 8 μm. (C) Section from the midgut gland of *A. cygnea* showing large amounts of calcium concretions between the tubules (acini, a). As a result of staining with the Von Kossa method the granules appear black in the Alcian blue counterstained tissue. Bar = 50 μm. (D) As under (C), but pretreated with 5% HNO_3 prior to the staining procedures. Bar = 50 μm.

and at different sites. Concretion content of the gills proved less dependent on external factors. Fig. 2 shows, for *A. cygnea*, how the amount of concretion material, making up about 8% of the total soft part dry weight, is distributed among the organs. Gills contain three-quarters of it, and the mantle and the midgut gland 6% each. The rest of the concretions are situated in the edge of the labial palps and in the visceral complex.

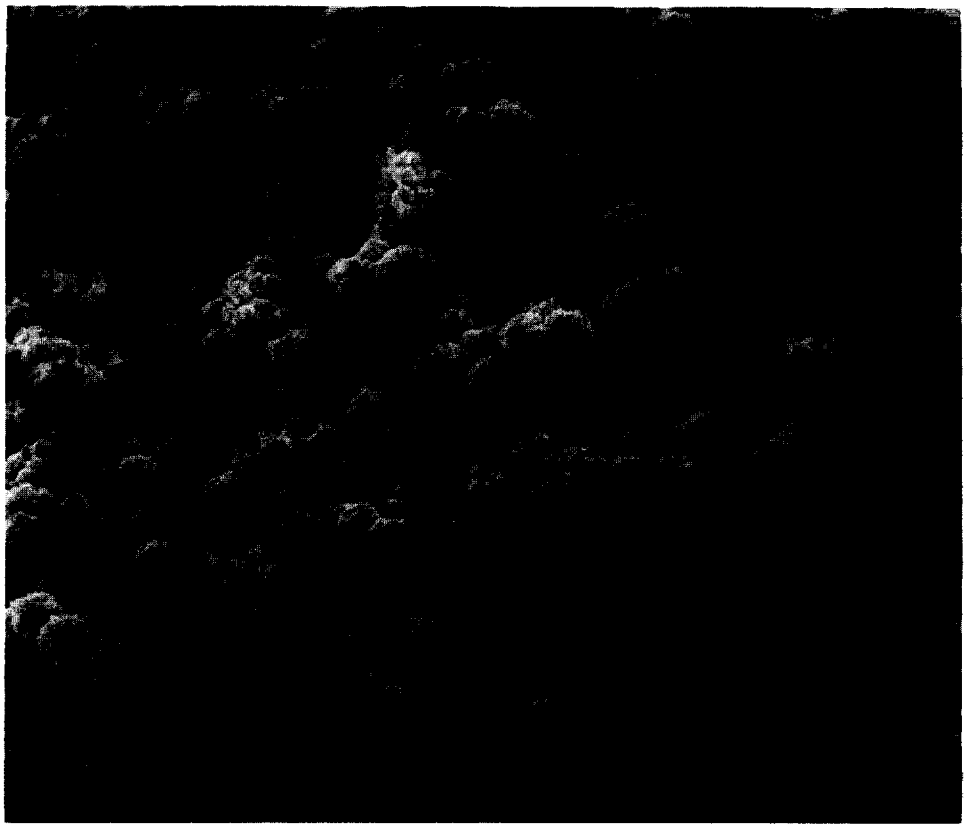
Location of the granules in the organs, their appearance and size

Calcified concretions in the gills of all three species are located in both loose and dense connective tissue, where they fill a noticeable part of the organ volume (Fig. 3A). The gill bars get their rigidity from paired chitinous rods that consist of

Fig. 4. Scanning electron micrographs of calcified concretion fractions. (A) = isolated from *A. cygnea* gills; (B) = isolated from the midgut gland of *A. cygnea*.

smaller units with a length of 200–300 μm, as described earlier for *A. grandis* (Steffens et al., 1985; Silverman et al., 1985). The calcium concretion material isolated from the gills is pure white in colour. Granular concretion dimensions vary from about 1 to 7 μm, the larger spherules clearly showing concentrical layers (Fig. 3B). Beside single granules also clusters of granules occur with a diameter of 10–15 μm (Fig. 4A).

In the midgut gland, concretions appear as single granules (Fig. 4B) that are more homogeneous in size and structure than those of gills. The isolated granule fraction is usually dark brown, and the colour remains after several washes in NaOH. Midgut gland concretions can be found in the intertubular spaces in large amounts (Fig. 3C). When granules are scarce, they are found in granulocytes forming narrow strings around the glycogen containing cells. Most of the concretion material is solubilized in weak acid (Fig. 3D), but a small amount seems to resist acid treatment. In the cells of digestive gland tubules (acini) no granules were detected by Al-



cian red S or Von Kossa staining.

Mantle granules are located near the epithelial tissue and are more numerous in the mantle edge than in the mantle itself. The size of the concretion varies between 1–15 μm. Small, single granules dominate. The edges of labial palps also contain many granules. In the kidney, sometimes a few granules of small diameter (< 0.5 μm) were observed in the connective tissue underlying the epithelium. Muscles (foot, adductors) are practically devoid of concretion material.

Granule composition

As revealed by electron-microprobe analysis, the major components of the inorganic concretions are calcium and phosphorus (Fig. 5), the latter element supposedly occurring in the form of (pyro)phosphate (Silverman et al., 1983). On a weight basis calcium makes up about one third of the concretion material (Table III). The X-ray spectrum also revealed detectable amounts of manganese, iron, and zinc. Both by electron-microprobe analysis (Fig. 5B) and by atomic absorption spectrophotometry (Table III), the amount of iron in granules of midgut

TABLE III

Composition of the calcified concretion fractions isolated from unexposed *A. cygnea* gills and midgut gland. Values represent the mean (± SEM) of five individuals for the metals, and the mean of four individuals for the organic components.

Component	Gill	Midgut gland
	Dry concretion (μg/g)	
Calcium	318 300 ± 24 500	365 000 ± 45 600
Manganese	31 400 ± 1 437	36 900 ± 3 260
Iron	10 090 ± 2 055	28 900 ± 3 800
Zinc	2 800 ± 270	3 150 ± 580
Copper	8.3 ± 1.0	30.0 ± 3.2
Cadmium	2.7 ± 0.3	7.0
	Dry concretion (g/100 g)	
Proteins	2.2	3.4
Carbohydrates	1.0	1.8
Lipids	0.3	0.1

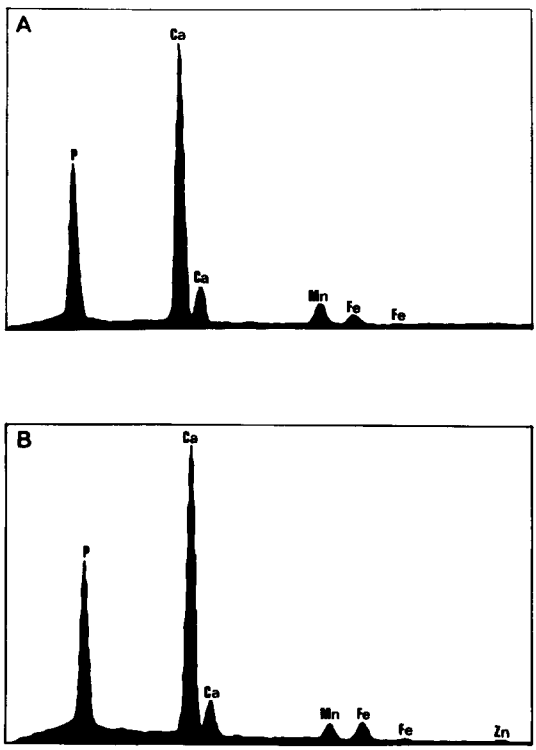


Fig. 5. Elemental X-ray spectrum of isolated calcium concretions from gills (A) and midgut gland (B) of *A. cygnea*. The gill spectrum shown is for the granular type of concretion occurring in the connective tissue. Chitinous rod material gave an identical spectrum.

gland was found to exceed that in gills. This was also the case for the trace metals cadmium and copper. The organic components (Table III) were analyzed primarily as a purity check of the isolated concretion fractions. Carbohydrate and lipid contents were low, and the protein amount probably should be considered (Steffens et al., 1985) as a component of the organic matrix.

Cadmium accumulation in total tissue and concretion fractions

Cadmium accumulated in total gill tissue of *A. anatina* and *U. pictorum* at comparable rates, and nearly linearly during 6 wk (Figs. 6A, F). In *A. cygnea*, accumulation in total gill was much higher during the first 3 wk, but ceased thereafter (Fig. 6D). A plateau in the accumulation curve, especially in that for the gill, has been observed earlier (Hemelraad et al., 1986a). Over the first 3 wk, the Cd accumulation rate (on the basis of dry weight) in the gill concretion fraction of all three species

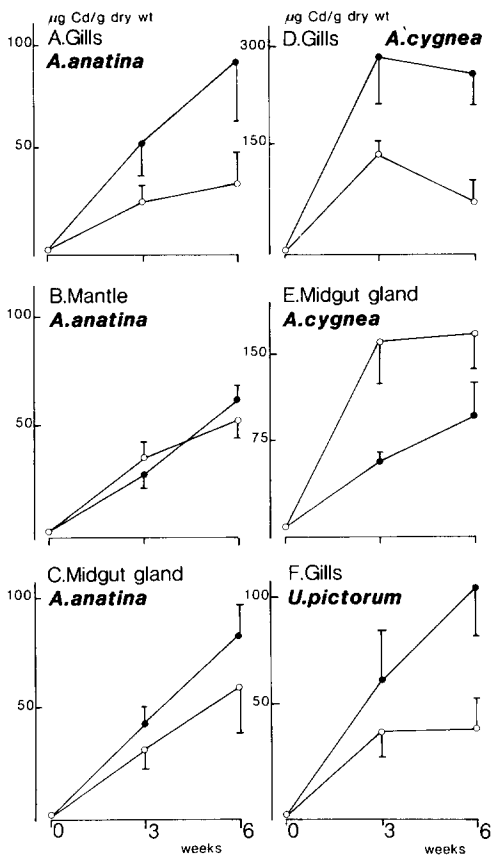


Fig. 6. Cd accumulation in total organs (●—●) and in isolated concretion fractions (○—○) of *A. anatina*, *A. cygnea*, and *U. pictorum* exposed to 40 µg/l Cd. Mean of 8 animals, ± SEM.

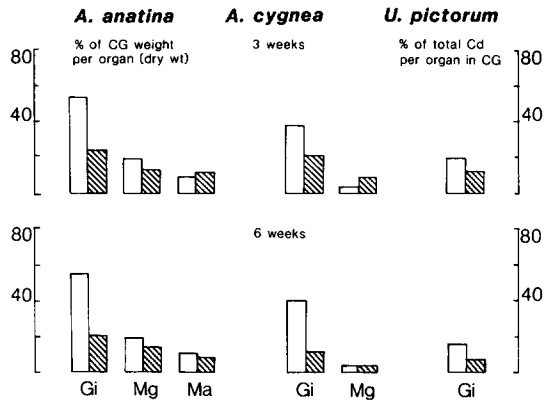


Fig. 7. Open bars: relative amounts as % of total organ dry weight of calcium concretions in the organs (Gi = gills, Mg = midgut gland, Ma = mantle); Hatched bars: % of total organ Cd load found in calcium concretions after 3 to 6 wk of exposure to 40 μg/L Cd. Values are the mean of 8 individual animals, (±SEM).

was substantially lower than in total gill tissue. In addition, after 6 wk of exposure, the contribution of the concretions in Cd accumulation had decreased. Fig. 7A shows that in gills of all species, weight proportions of the concretions are higher than the corresponding Cd proportions. For example, whereas in *A. anatina* gills and in *A. cygnea* gills, concretions make up 55 and 40% of the total organ weights, respectively, no more than around 20% of total Cd was found in this compartment. After 6 wk relatively less Cd was associated with the concretions, especially in *A. cygnea* and *U. pictorum* (Fig. 7B). The latter phenomenon cannot be ascribed to a disappearance of calcium granules, e.g. by a solubilization or excretion process; from a comparison of Figs 7A, B (corresponding left bars) it is clear that the weight portions of the concretion fraction were constant. In mantle and midgut gland of *A. anatina*, a roughly linear accumulation of cadmium in total organs was found over the whole period (Fig. 6C). After three weeks, Cd concentrations in total tissues and in concretion fractions differed only little, but, as in gills, the participation of concretions in sequestering cadmium decreased upon exposure for a further period of three weeks.

The midgut gland of *A. cygnea* shows a different picture (Fig. 6E), in that the Cd concentration of the concretion fraction clearly exceeded that of total tissue. So, this fraction accommodated cadmium more than proportionally: the contribution of the concretions to Cd accumulation was nearly 10% (Fig. 7A). Again, after 6 wk, this value had decreased to less than 5% (Fig. 7B).

From Fig. 7A it appears that, at that time of exposure, an inverse relationship exists between the tissue amount of calcium concretions and the contribution of the concretion fractions to the sequestration of accumulated cadmium. This relationship, although less clear, still applies to the situation after 6 wk of exposure.

DISCUSSION

Occurrence of calcified concretions in various tissues

All freshwater mussels studied so far contain remarkably high amounts of calcium concretions in the gills. The content in *A. cygnea*, about 55% by weight, equals that of *A. grandis* (Silverman et al., 1985). The lowest amount was measured in *Unio pictorum*, namely about 20%. This value was also reported for the unionid *Ligumia subrostrata* (Silverman et al., 1985). By contrast, gills of marine bivalves have not been found to contain inorganic calcium granules.

As yet, no data were available on the occurrence of calcified concretions in the midgut gland of freshwater mussels. In this study, the presence of concretions in the midgut gland was shown in all three species studied. For the *Anodonta* species we found that the concretion content varied strongly in the midgut gland, as well as in the mantle. The variation may be governed both by a fluctuating rate of shell growth and by extrinsic factors, such as water composition and diet.

In some marine bivalve species calcified concretions have been demonstrated in the kidney (see Table I). We have no indications that such particles also occur in the kidney of freshwater mussels. In the *Anodonta* kidney (heavy) metal-containing structures are present (Herwig, personal communication) like those described for the kidney of *M. edulis* (George and Pirie, 1979). However, these 'granules', also called tertiary lysosomes, are membrane-limited, and typically consist for the major part of organic material (George et al., 1980).

Role of concretions in Cd sequestration

Among the various functions that have been ascribed to the calcified concretions, a possible role in heavy metal detoxification has received special attention. In this study, we found that a substantial portion — up to 20% — of the cadmium which had accumulated after 3 wk of exposure was associated with the concretion fraction. However, in most cases, Cd concentration of concretions was lower than in total (dry) tissue. It is, therefore, clear that the organic matrix of the cell, including organelles, membranes and soluble proteins, accommodates the greater part of cadmium, both in an absolute and relative sense. Moreover, the contribution of the concretion fractions to the sequestration of cadmium had diminished after the second period of 3 wk. These observations confirm the conclusion of Simkiss (1981a) that cadmium is not preferentially bound to the phosphorite granules, but shows a greater affinity to the intracellular thiol groups.

During the first phase of exposure, however, Cd accumulation can be expected to exceed the capacity of the cell to sequester a major part of the metal into metallothioneins (MT). In earlier reports on the Cd kinetics in freshwater mussels (Hemelraad et al., 1986a,b) it was indicated that the first plateau in Cd accumulation, occurring in the period between 4 and 6 wk after the onset of exposure (to 25 μg/l Cd), is related to the induction of MT synthesis. The present data con-

firm this view; after 6 wk the cellular concentration of MT-thiol groups has increased and this leads to a decreased participation of calcium granules in the binding of cadmium. The outlined Cd dynamics is most strongly illustrated in *A. cygnea*. In the gills of these animals, in contrast to the two other species, the first plateau of Cd accumulation was already reached after about 3 wk of exposure. In the second period the total Cd concentration remained constant, but cadmium in the granule fraction decreased considerably (Fig. 6D). Also in the midgut gland of *A. cygnea* the percentage of cadmium in the calcium granules was reduced to half in the second exposure period (Fig. 7). In conclusion, the role of the calcified concretions as a Cd sink probably is a temporary one. Initially, for lack of sufficient other ligands, part of the heavy metal is bound to the granules. But at prolonged exposure, a more efficient Cd sink becomes available in the form of induced MTs.

In marine molluscs, kidney concretions have been assigned a function in heavy metal elimination through granule excretion (Fowler et al., 1985). We think that this mechanism does not operate in freshwater bivalves, as the kidney did not contain calcium granules, either in unexposed or in exposed animals.

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REFERENCES

Becker, G.L., Chung-ho Chen, J.W. Greenwalt and A.L. Lehninger, 1974. Calcium phosphate granules in the hepatopancreas of the blue crab *Callinectes sapidus*. J. Cell. Biol. 61, 316–326.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Carmichael, N.G. and B.A. Fowler, 1981. Cadmium accumulation and toxicity in the kidney of the bay scallop *Argopecten irradians*. Mar. Biol. 65, 35–43.

Carmichael, N.G., K.S. Squibb and B.A. Fowler, 1979. Metals in the molluscan kidney: comparison of two closely related bivalve species (*Argopecten*), using X-ray microanalysis and atomic absorption spectroscopy. J. Fish. Res. Board Can. 36, 1149–1155.

Doyle, L.J., N.J. Blake, C.C. Woo and P. Yevich, 1978. Recent biogenic phosphorite: concretions in mollusk kidneys. Science 199, 1431–1433.

Fowler, B.A., D.W. Engel and E. Gould, 1985. Ultrastructural and biochemical effects of prolonged cadmium and copper exposure on kidneys of the scallop *Placopecten magellanicus*. Proc. Third Int. Symp. on Responses of Marine Organisms to Pollutants, Plymouth.

George, S.G., 1983. Heavy metal detoxification in the mussel *Mytilus edulis* — composition of Cd-containing kidney granules (tertiary lysosomes). Comp. Biochem. Physiol. 76C, 53–57.

George, S.G. and B.J.S. Pirie, 1979. The occurrence of cadmium in subcellular particles in the kidney of the marine mussel, *Mytilus edulis*, exposed to cadmium. Biochim. Biophys. Acta. 580, 234-244.

George, S.G., B.S.J. Pirie and T.L. Coombs, 1980. Isolation and elemental analysis of metal-rich granules from the kidney of the scallop, *Pecten maximus* (L.). J. Exp. Mar. Biol. Ecol. 42, 143-156.

Guary, J.C. and R. Négrel, 1981. Calcium phosphate granules: a trap for transuranics and iron in crab hepatopancreas. Comp. Biochem. Physiol. 68A, 423-427.

Haley, G.F. and M.A. Gibson, 1971. Calcium storage in the soft tissues of freshwater gastropods. The influence of environmental calcium concentrations. Can. J. Zool. 49, 1001-1004.

Hemelraad, J., D.A. Holwerda and D.I. Zandee, 1986a. Cadmium kinetics in freshwater clams. I. The pattern of cadmium accumulation in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. 15, 1-7.

Hemelraad, J., D.A. Holwerda, K.J. Teerds, H.J. Herwig and D.I. Zandee, 1986b. 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*. Arch. Environ. Contam. Toxicol. 15, 9-21.

Holwerda, D.A., J. van Doorn and A.M.Th. Beenakkers, 1977. Characterization of the adipokinetic and hyperglycemic substances from the locust corpus cardiacum. Insect Biochem. 7, 151-157.

Hopkin, S.P. and J.A. Nott, 1979. Some observations on concentrically structured intracellular granules in the hepatopancreas of the shore crab, *Carcinus maenas* (L.). J. Mar. Biol. Assoc. U.K. 59, 867-877.

Jeffree, R.A. and R.D. Simpson, 1984. Radium-226 is accumulated in calcium granules in the tissues of the fresh-water mussel, *Velesunio angasi*: support for a metabolic analogue hypothesis? Comp. Biochem. Physiol. 79A, 61-72.

Lowenstam, H.A., 1981. Minerals formed by organisms. Science 211, 1126-1130.

Mason, A.Z. and J.A. Nott, 1981. The role of intracellular biomineralized granules in the regulation and detoxification of metals in gastropods with special reference to the marine prosobranch *Littorina littorea*. Aquat. Toxicol. 1, 239-256.

Pearse, A.G.E., 1960. Histochemistry. Theoretical and applied. J. & A. Churchill, London, 998 pp.

Pirie, B., Liu Fayi and S. George, 1985. Ultrastructural localisation of copper and zinc in the polychaete, *Nereis diversicolor*, from a highly contaminated estuary. Proc. Third Int. Symp. on Responses of Marine Organisms to Pollutants, Plymouth.

Silverman, H., W.L. Steffens and T.H. Dietz, 1983. Calcium concretions in the gills of freshwater mussel serve as a calcium reservoir during periods of hypoxia. J. Exp. Zool. 227, 177-189.

Silverman, H., W.L. Steffens and T.H. Dietz, 1985. Calcium from extracellular concretions in the gills of freshwater bivalves is mobilized during reproduction. J. Exp. Zool. 236, 137-147.

Simkiss, K., 1981a. Calcium, pyrophosphate and cellular pollution. Trends Biochem. Sci. 6, 3-5.

Simkiss, K., 1981b. Cellular discrimination processes in metal accumulating cells. J. Exp. Biol. 94, 317-327.

Sminia, T., N.D. de With, J.L. Bos, M.E. Nieuwmegen, M.P. Witter and J. Wondergem, 1977. Structure and function of the calcium cells of the freshwater pulmonate snail *Lymnea stagnalis*. Neth. J. Zool. 27, 195-206.

Steffens, W.L., H. Silverman and W.L. Dietz, 1985. Localisation and distribution of antigens related to calcium-rich deposits in the cells of several freshwater bivalves. Can. J. Zool. 63, 348-354.

Walker, G., P.S. Rainbow, P. Foster and D.J. Grisp, 1975. Barnacles: possible indicators of zinc pollution. Mar. Biol. 30, 57-65.