Cadmium Kinetics in Freshwater Clams. IV. Histochemical Localization of Cadmium in Anodonta cygnea and Anodonta anatina, Exposed to Cadmium Chloride

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Abstract. By means of a histochemical staining method, the sulphide-silver technique, the localization of cadmium was studied in several tissues of freshwater mussels, Anodonta cygnea and Anodonta anatina, exposed to CdCl₂ (25 µg/L Cd) for periods up to 16 weeks. After three weeks of exposure, reaction products indicative of the presence of free or loosely bound Cd were observed in the pallial mantle epithelium. After six weeks, reaction products appeared in gill epithelia of both species, and in kidney epithelia of A. anatina. After nine weeks of exposure, reaction products were present also in kidney epithelia of A. cygnea. In most cases, reaction products were concentrated in distinct particles in the apical cell region. The initial appearance of reaction products in mantle and gill epithelia was followed by a stationary phase, in which the reaction pattern did not change significantly, despite a continued accumulation of the metal. After 16 weeks of exposure, a further increase in the amount of reaction products was noticed in midgut gland and kidney epithelia of A. cygnea, and in nearly all epithelia of A. anatina. In the latter species, reaction products were no longer restricted to distinct particles, but were distributed throughout the epithelial cells and the underlying connective tissue.

In the previous papers, the results on cadmium kinetics in Unionidae were presented that were based on data obtained by means of atomic absorption spectrophotometry (Hemelraad et al. 1986a, 1986b). In the present study, these data are supplemented by histological results, which are obtained with a metal-demonstrating histochemical technique.

The histochemical method was introduced by Timm (1958). It is based on the principle that metals, after being converted into their sulphide, catalyze the precipitation of metallic silver from a silver solution in the presence of a reducing agent. Since 1958, several modifications of the sulphide-silver technique (SST) have been described (Danscher 1981). A version has been used that is based mainly on the work of Danscher and colleagues (Danscher and Zimmer 1978; Danscher 1981).

A description is given of the localization of SST-positive reaction products in several organs of two species of Unionidae. Based on a comparison between control and Cd-exposed clams a semi-quantitative analysis is presented on the changes in the occurrence of reaction products.

Materials and Methods

Animals

Anodonta cygnea zellensis Gmelin (swan mussel) was collected from the Maarsseveen-lake district near Utrecht, and Anodonta anatina L. (duck mussel), was collected from a pond near Leiden. Prior to the experiment, the clams were kept in glass aquaria without substratum, in moderately streaming copper-free tapwater (12°C ± 1°) under an astronomical light regimen. No food was supplemented during this period. More details about the animals were given earlier (Hemelraad et al. 1986b).
Exposure System

After an acclimatization period of at least three weeks, one hundred and fifty specimens of Anodonta cygnea (mean shell lengths 11.9 ± 1.2 cm) and Anodonta anatina (mean shell lengths 8.7 ± 0.2) each were divided over two aquaria (250 L). In one aquarium, the mussels were exposed to approximately 25 µg Cd/L as CdCl₂ (Merck, no. 2011). The actual Cd concentration in the water, that was assayed weekly, amounted to 29 µg Cd/L ± 7 (SD). No food was added during the experiment. Additional data concerning water quality, exposure system, and Cd measurement were given earlier (Hemelraad et al. 1986a). This exposure was performed simultaneously with that of the biochemical study on the accumulation and distribution of Cd in Anodonta sp. (Hemelraad et al. 1986b).

Sampling and Histochemical Procedure

Four control and four Cd-exposed specimens of both species were sacrificed at 3, 6, 9 and 16 weeks of exposure. Representative samples of mantle, gill, midgut gland, kidney, and posterior adductor muscle were rapidly dissected and submersed in sulphide solution (0.1% Na₂S in 0.1 M phosphate buffer, pH 7.4) for 10 min at room temperature. The tissue was fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 4 hr. Samples were rinsed twice for 15 min in phosphate buffer, dehydrated in ethanol, and embedded in paraffin. Temperature was held below 50°C, in order to minimize oxidation of metal sulphides (Brunk and Sköld 1967; Danscher and Zimmer 1978). Sections were cut at 7 µm and collected on glass slides. After deparaffinization and rehydration, all sections were developed in a freshly prepared silver nitrate/hydroquinone solution for 1 hr, in complete darkness, at 21°C. This solution consisted of gum arabic 20% (Merck), 6.5 mM silver nitrate (Merck), 77 mM hydroquinone (Merck) and 120 mM citric acid (Merck). Finally, the sections were rinsed in distilled water (three times for 5 min), dehydrated and mounted. Selected samples were also developed directly in the silver nitrate/hydroquinone solution, without prior sulphidation.

To improve contrast, part of the sections was stained with Alcian Blue (pH 3.5). The method of Von Kossa (Pearse 1985) was used for the detection of calcium carbonate and phosphate deposits. For localization of iron, the Turnbull’s Blue method (Pearse 1985) was applied.

Results

Mantle

Histological sections of the central mantle region showed that the mantle is lined by a pallial epithelium, facing the mantle cavity, and an extrapallial epithelium facing the cavity between mantle and shell (Figures 1–4). The linings are one cell layer thick and consist of prismatic cells. The pallial epithelial cells bear cilia and are interspaced with mucous cells. The main part of the mantle is occupied by glycogen-containing cells, separated by strains of connective tissue. As a consequence of the fixation used for the SST, glycogen has largely disappeared. Fields of glycogen ghost cells may be interspersed by lymph vessels, muscular tissue or nerve bundles. The subepithelial connective tissue contains a variable number of Von Kossa-positive calcium concretions and amoebocytic cells. In addition, a unique cell type was observed. Electron-microscopic observations revealed that the cytoplasm of this cell type is completely filled with large electron-dense granules. For that reason, this cell type is referred to as ‘granulocytes.’ In general, the mantle of A. cygnea contained more calcium concretions and granulocytes than that of A. anatina. After application of the Turnbull’s Blue method, large amounts of reaction products, indicating the presence of iron, were found mainly in the calcium concretions of both species.

In mantle samples of unexposed A. cygnea treated with the sulphide-silver technique, conspicuous reddish-brown or black reaction products were present in the granulocytes and in the calcium concretions. All other tissue components were generally free of silver deposits (Figure 1).

After 3 weeks of Cd exposure, small concentrations of reaction products were observed in the apical region of the pallial mantle epithelium (Figure 2). Extension of the exposure (6, 9, or 16 weeks) did not cause a notable further increase in the amount of silver deposits, either in the pallial epithelium or in any of the other mantle components.

Mantle samples of unexposed A. anatina were largely free of SST positive reaction products. Small amounts of silver deposits could be seen only in the periphery of nerve bundles (Figure 3).

After exposure of A. anatina to Cd for 3, 6, or 9 weeks, small amounts of reaction products were observed throughout the pallial epithelium. Exposure to Cd for 16 weeks, however, caused a different reaction pattern. The pallial epithelium was clearly visible as a reddish brown band, indicating that large numbers of silver grains are distributed at random throughout the cells (Figure 4). Under these exposure conditions, diffusely distributed reaction products were observed also in the extrapallial epithelium and in some places in the connective tissue, underlying the epithelium.

Table 1 shows the distribution of SST-positive reaction products in the mantle and other tissues at the various sampling times.

Gills

Gill samples collected from the ventral free edge of a gill lamella, sectioned in dorsal-ventral direction,