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Zinc Treatment of the Digestive Gland of the Slug *Arion ater* L. 1. Cellular Distribution of Zinc and Calcium

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Extensive literature has been published on the molluscan capability for metal bioaccumulation. As a consequence, a variety of species have been proposed for environmental pollution biomonitoring. Mostly bivalves have been preferred when assessing marine pollution (Phillips 1977; Bryan et al 1985), and pulmonate gastropods have been proposed in soil pollution assessment: Cepaea hortensis (Williamson 1980); Helix aspersa (Coughtrey and Martin 1977, Russell et al 1981); Arion ater (Schoettli and Seiler 1970; Ireland 1979; 1981). Popham and D´Auria (1980) have recommended the use of A. ater as an indicator species of metal pollution in soils because this species fulfils most of the characteristics of a bioindicator species. Particullarly, this species is very resistant to Zn pollution (Marigómez et al 1986b), histological damage being only observed after very high Zn exposures (part 2 of this investigation). Thus, it could be found in Zn polluted sites, showing a linear accumulation of this metal (Popham and D´Auria 1980; Ireland 1982).

The knowledge of biological mechanisms of accumulation and elimination of environmental pollutants is essential to get a proper use of bioindicator species. Histological procedures may indicate (a) the events involved in the metabolic regulation of pollutant bioavailability and release (Phillips and Segar 1986), and (b) their specific toxic effects (Simkiss and Mason 1984).

The relevance of the digestive gland as a major site for Zn accumulation has been reported by Schoettli and Seiler (1970) and Ireland (1982). The Zn distribution in this organ might indicate the level of

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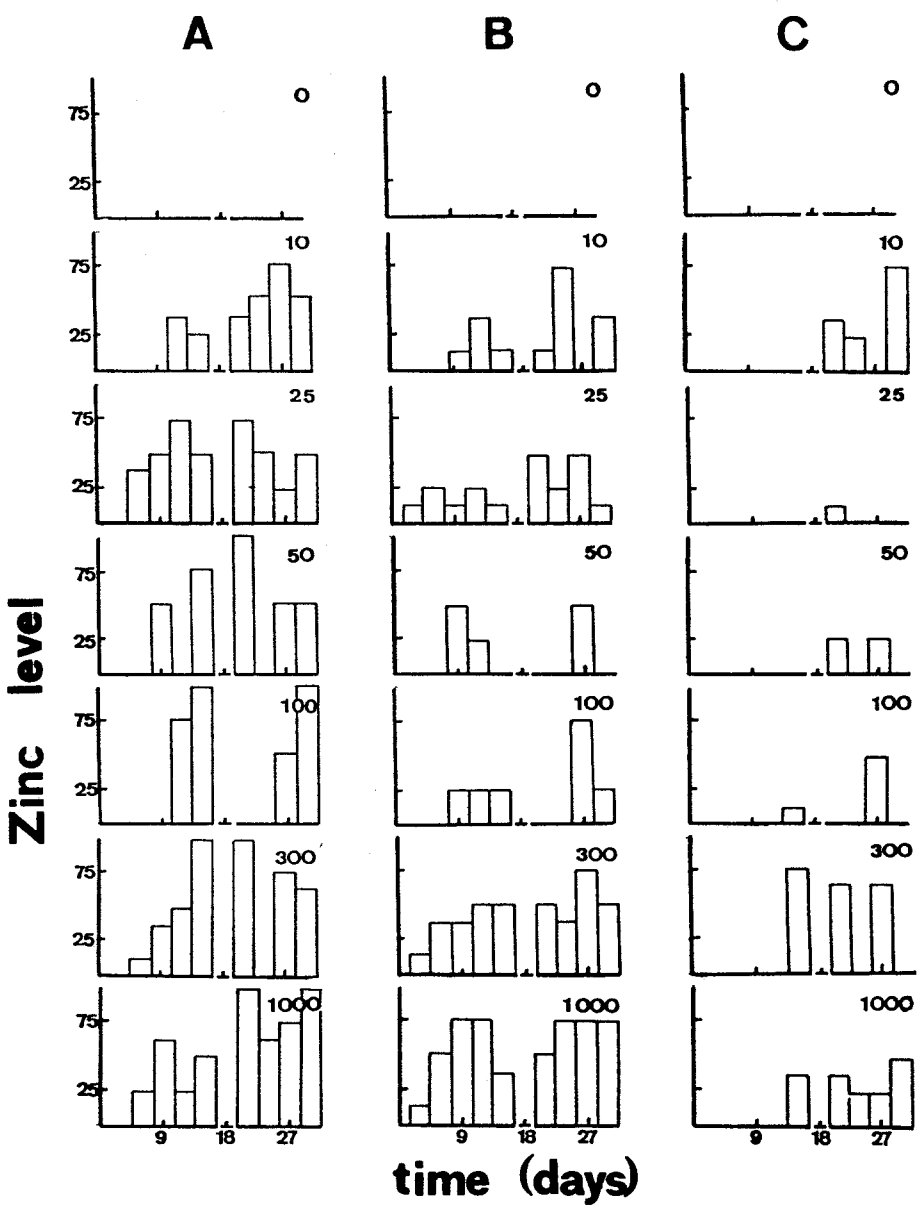

Figure 1. Histochemical levels of zinc (arbitrary units) in the digestive gland vs. bioassay time for each group of studied slugs (from 0 to 1000 mg Zn/kg food). A, lipofuscines of the excretory cells; B, cytoplasm of the calcium cells; C, spherules of the calcium cells.

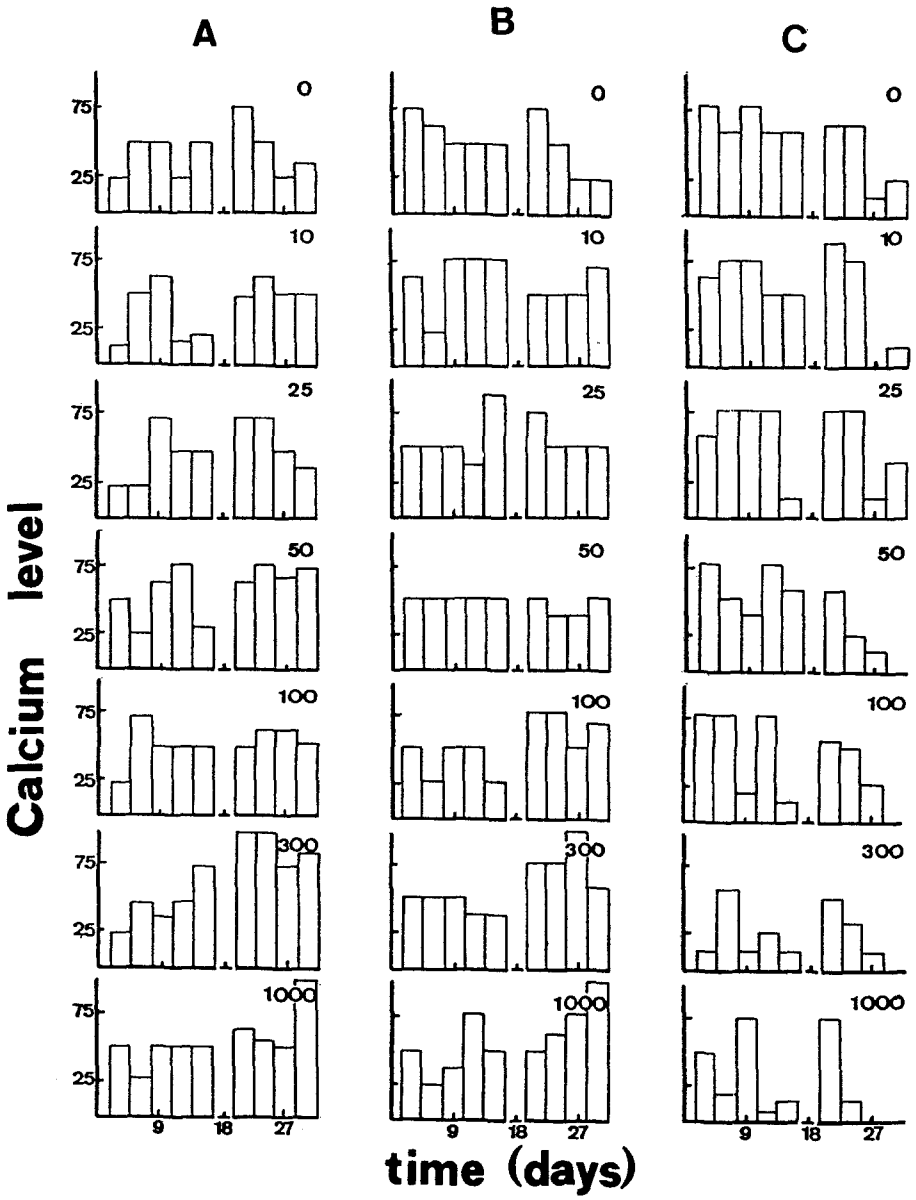

Figure 2. Histochemical levels of calcium (arbitrary units) in the digestive gland vs. bioassay time for each group of studied slugs (from 0 to 1000 mg Zn/kg food). A, lipofuscines of the excretory cells; B, calcium cells; C, yellow granules of the digestive cells.

environmental bioavailable Zn. The present study is a histochemical investigation of the distribution and release of zinc by the different cell types of the digestive gland of A. ater.

MATERIALS AND METHODS

Individuals (198) of A. ater (4-5 cm length, 5-7 g weight) were collected near the university campus. "Natural diet" (equiproportional triturate of lettuce, apple, carrot, and pumpkin with a 1.5% agar aqueous solution) was applied to slugs mixed with zinc chloride in concentrations of 0, 10, 25, 50, 100, 300, and 1000 mg Zn/Kg food for 1 month with a replicate series of experiments. Twelve slugs were kept in 1.5 l. plastic boxes under natural photoperiod (August) at 20°C in a saturated humidity. Before experimentation slugs were starved for 1 week to minimise physiological differences among individuals and to provide acclimatization to laboratory conditions (Akerlund 1969).

Animals were removed every third day at the same hour (17:00-19:00). The posterior lobe of the digestive gland was dissected out and fixed in cold absolute alcohol. Paraffin sections (8 µm) were stained with dithizone to demostrate zinc (Lillie 1965), and with the Stoeltzner's method (plus citrate control) for calcium (Martoja and Martoja-Pierson 1970).

After careful observation of the histochemical results a semiquantitative valuation of zinc and calcium levels in the digestive gland was considered. These subjective histochemical levels were transformed into percentage values after considering the widest range of variation for each metal (minimum value=0, maximum value=100%). Mean values of these percentages (arbitrary units in Figures 1 and 2) were calculated from the replicate series of experimental treatment.

RESULTS AND DISCUSSION

Zinc was mainly located in the lipofuscin material of the excretory cells (Fig. 1). It was also observed in the perinuclear cytoplasm and the spherules of the calcium cells. Ocassionally, although scarcely conspicuous, zinc was detected in the cytoplasm and brush-burder of digestive cells (fig. 3). This does not agree with the results of Schoettli and Seiler (1970) who reported finding zinc only within the calcium spherules of Arion rufus (this could be syn. A. ater, Martin comm. pers.). The location of zinc

only in calcium spherules in the present study was limited to low zinc concentrations and short time exposure times in all concentrations (Fig. 2).

It can therefore be suposed that the difference in zinc distribution is the result of differences in the level of exposure. In fact, the location of metals in molluscs is very variable from one species to another and for different metals and times of exposure. Viarengo et al (1981) found copper in the digestive gland lysosomes of the mussel (Mytilus edulis) while Marigómez et al (1986a) located copper only within calcium spherules of the digestive gland of A. ater. The present results could indicate a difference in the distribution of zinc in the digestive gland cell types as a function of the zinc concentration and duration of exposure (Fig. 1). Initially, zinc would be taken up by the digestive cells and then mobilised either for excretion from the excretory cells or accumulation, regulation, and to a lesser extent excretion in the calcium cells.

Metal binding has been frequently associated with calcium salts, mainly carbonates, in molluscs (Simkiss 1981). This relationship between metallic pollutants and calcium metabolism has been reported by other authors (Ireland 1982; Dallinger and Weiser 1984; Marigómez et al 1986a). Although Marigómez et al (1986a) found a close parallel between calcium and copper levels in copper-exposed A. ater, the present results (Fig. 2) do not indicate that zinc is working in the same way. On the contrary, in several cases we have found an inverse relationship between extreme values for both metals. This event supports the hypothesis that zinc elimination is mainly via excretory cells, and to a lesser extent by competition with calcium inside calcium cells, which results in a returning to normal after zinc extrusion.

As a result, the histochemical detection of zinc in the digestive gland of A. ater is a realistic quick and cheap indication of the presence of high levels of zinc in the environment. The used methodology is an initial approach which does not offer a quantifiable correlation between the environmental zinc levels and the intensity of histochemical detection. However, the distinct cellular distribution of metals might indicate different degrees of exposure, and the quantification of the histological response (part 2 of this invetigation) might indicate the environmental risk of the contamination.

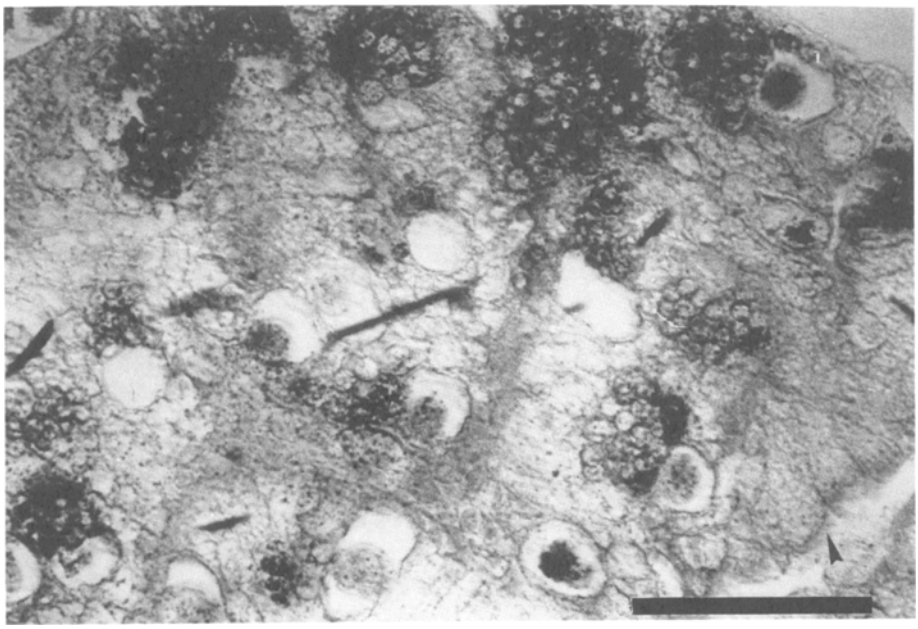

Figure 3. Digestive epithelium after 27 days of treatment with 300 mg Zn/kg food stained with the dithizone method for the demonstration of zinc. A positive reaction is observed in the apex of digestive cells (arrow), in the lipofuscines of excretory cell vacuoles, and in the calcium cells. Scale bar: 100 µm.

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