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Cadmium-induced lipofuscins and effect of zinc on hepatopancreas cells in *Idotea baltica*

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Abstract. Histological investigation revealed cadmium-induced lipofuscin formation in hepatopancreatic cells of the crustacean isopod, *Idotea baltica*. A comparison of lipofuscin induction by cadmium and by copper showed that cadmium has a weaker peroxidative action than copper. The effect of cadmium was reduced by the simultaneous presence of zinc, which on its own was ineffective in lipofuscin induction. A tentative explanation of the interactive effects of these materials is suggested.
Key words. Lipofuscin; cadmium; zinc; isopod.

Xenobiotic substances and environmental stress have been demonstrated to contribute to the formation of free radicals that can peroxidize the lipoproteins contained in cell membranes. The products derived from the interaction of oxidized lipids and cellular proteins are accumulated in the lysosomal vacuolar systems as insoluble granules containing polymeric fluorescent lipopigments, classified as lipofuscin[1]. Lipofuscin has an important role in the compartmentalization of heavy metals. These processes have been observed mainly in mammals[2], whereas data on marine organisms (fish, invertebrates and fungi) are scarce[3–6].

We have previously reported that, like other invertebrates, *Idotea baltica*, a widely distributed crustacean isopod inhabiting coastal waters, and an important link in food webs, accumulates cadmium and zinc preferentially in the hepatopancreatic gland[7–8]. The cells of this target organ appear to be considerably modified by cadmium, which causes alterations in the endoplasmic reticulum, the mitochondria and the lysosomes, as well as an increase in residual bodies rich in electron-dense material. Zinc, on the other hand, produces only a considerable accumulation of inert granules, but it also decreases cadmium cytotoxicity in animals exposed to both metals.

In order to assess whether cadmium-induced cytological damage and the interactive effects of cadmium and zinc are related to the formation of free radicals, we investigated the presence of lipofuscin in the cells of *I. baltica* following exposure to cadmium and/or zinc. The results are presented and discussed.

Materials and methods

Experimental conditions. We used adult male specimens of *I. baltica*, 16–18 mm in length, from a population living in the western area of the Gulf of Naples (Porto di Miseno). They were kept under standard laboratory conditions in 4 Plexiglass experimental vessels (22 × 16 × 8), each containing a liter of filtered sea-water. The animals were separated from one another by Plexiglass partitions to avoid cannibalism.

Groups of animals were exposed to the metals either at a level of 1 mg/l for 5 or 10 days, or 0.5 mg/l for 10 days. One group was exposed to 1 mg/l of cadmium + 1 mg/l of zinc. Another group was exposed to copper at 1 mg/l for 5 or 10 days or 0.5 mg/l for 10 days, to provide a positive control group for the Schmorl reaction, as the ability of copper to induce lipofuscin has been well demonstrated in vertebrates and invertebrates[6,9]. All the solutions were renewed every other day. The animals were fed on *Gracilaria* sp., and the food was also changed every other day. Dead animals were removed daily.

Tissues sampling and histological procedure. After treatment, the animals were starved for 24 h and then anesthetized. Pancreatic glands were removed and fixed in Bouin's fluid for 4 h under vacuum or in formol calcium for 18–20 h. They were then immersed in solutions of saccharose at increasing concentrations in phosphate buffer 0.1 M, at 4 °C, embedded in tissue teck (OCT) and sectioned using a cryostat.

For general histological observations, toluidine blue was employed. Lipofuscin pigments were revealed by Schmorl's reaction[10].

Results

Schmorl's reaction in the digestive cells of control animals revealed the presence of a few lipofuscin granules (fig.), probably a product of aging, since all the animals used had reached maturity.

In animals exposed to 1 mg/l of cadmium for 10 days, an intense reaction for lipofuscin was observed, mostly

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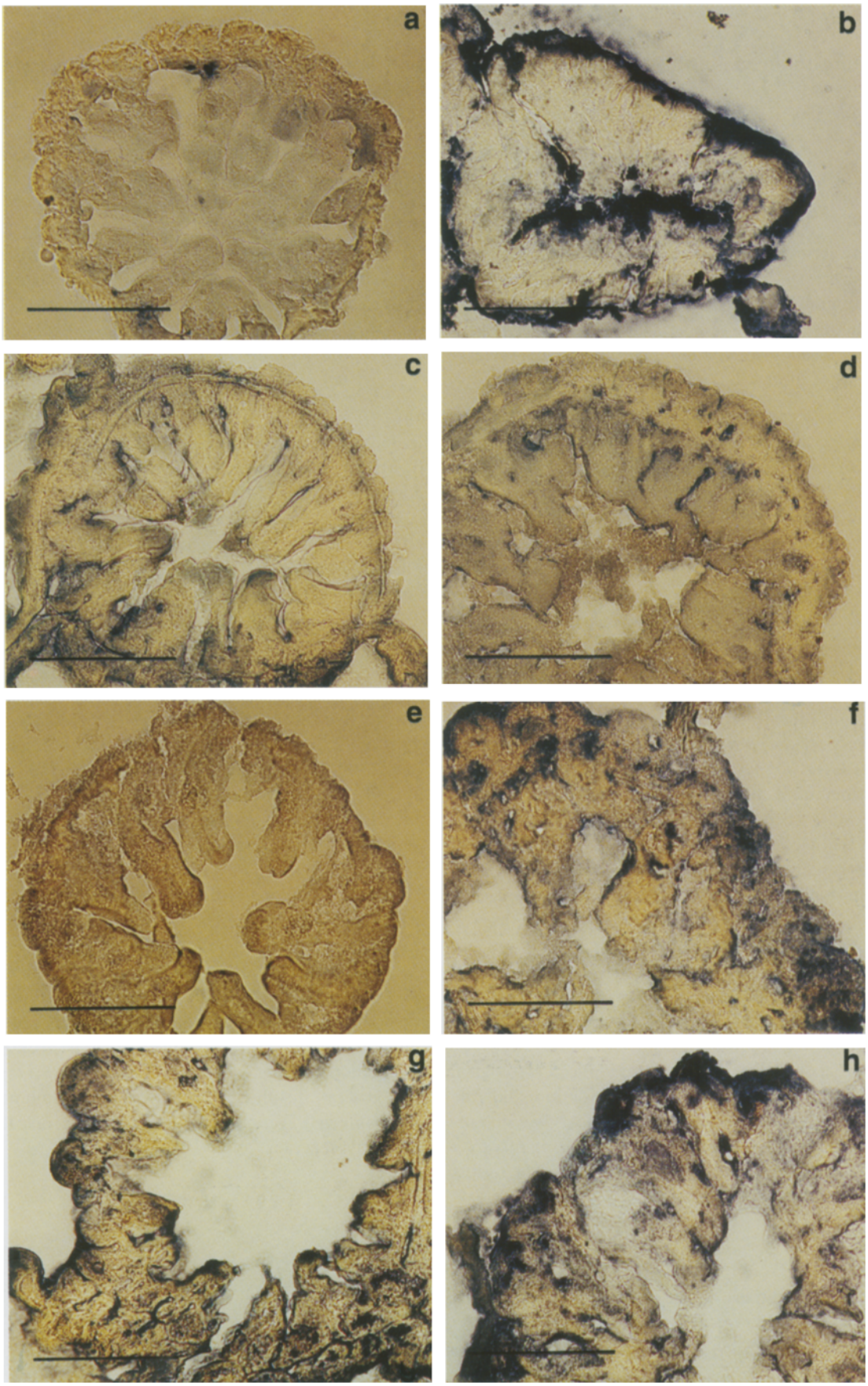
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localized in the basal and apical portions of the cell. When either the exposure period (5 days) or metal concentration (0.5 mg/l for 10 days) was decreased, no difference was observed between treated and control animals.

In animals exposed to the same zinc concentration for the same periods, no difference was observed with respect to the controls.

In animals exposed to zinc and cadmium simultaneously (1 mg/l Cd and 1 mg/l Zn for 10 days), we observed an increase in lipofuscin compared to control animals; this increase, however, was lower than in animals exposed only to cadmium at the concentration of 1 mg/l of cadmium. Lipofuscin was always localized essentially in the basal and apical portions of the cells.

In animals exposed to copper, lipofuscin accumulation was markedly greater than in the controls (fig.), and no variation was observed with varying metal concentrations (1 and 0.5 mg/l) and exposure periods (5 and 10 days).

Discussion

Our previous investigations have demonstrated that in *Idotea baltica* the toxic effects of cadmium and zinc are always accompanied by alterations in the ultrastructure of the cells of the hepatopancreas, the target organ of heavy metals[7,8].

In the present paper we demonstrated that, in these cells, cadmium induces lipofuscin formation; zinc, instead, does not contribute to lipofuscin production, and its presence reduces cadmium effects.

We compared the effects of cadmium to those of copper, which, as has been demonstrated by many literature data, induces a significant accumulation of lipofuscin even at low metal concentrations and following short exposure periods. These effects are produced by cadmium only at high concentrations and following rather long exposure periods. Therefore, it can be inferred that cadmium has a weaker peroxidative action than copper.

Of remarkable interest is our finding that zinc fails to induce lipofuscin formation. This is in agreement with the report of Chvapil[11], who suggested that this metal might not take part in redox cycling. Moreover, we demonstrated that zinc reduces the induction of lipofuscin by cadmium. The protective role of zinc ions against cadmium ions is probably due to stabilization of lysosomal membranes, as has been suggested for other metal ions by Wilson[12] and Gutteridge[13]. In our case, this effect might also be due to the reduced cadmium availabiltiy in cellular liquids in the presence of zinc, since cadmium is sequestered in inert and insoluble granules in the digestive cells, as we have already reported[14]. Interactive effects of these two metals have already been described by us as being due to enzymatic polymorphism[15], strategies of metal accumulation[14], and ultrastructural alterations of the hepatopancreas cells[7,8].

In conclusion, our findings provide the first evidence in the literature of cadmium-induced lipofuscin formation in crustaceans.

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Figure. Cryofixed cross-section of *I. baltica* hepatopancreas stained by Schmorl's reaction: *a* control animals, *b* animals exposed to 1 mg/l cadmium for 10 days, *c* animals exposed to 0.5 mg/l for 10 days, *d* animals exposed to 1 mg/l for 5 days, *e* animals exposed to 1 mg/l zinc for 10 days, *f* animals exposed simultaneously to 1 mg/l of both metals, *g* animals exposed to 0.5 mg/l copper for 10 days, *h* animals exposed to 1 mg/l copper for 10 days.

Note the large lipofuscin accumulation in the basal and apical zones in *b*, *g* and *h*, and the slight accumulation in *f*. No differences with respect to *a* can be observed in the other groups. Scale bar: 100 µm.