Bull. Environ. Contam. Toxicol. (1991) 46:894–900

Environmental Contamination and Toxicology

Sequestration of Copper and Zinc in the Hepatopancreas of *Armadillidium vulgare* Latreille Following Exposure to Lead

M. Tomita,[1] R. Heisey,[1] R. Witkus,[1] and G. M. Vernon[2]

[1]Department of Biological Sciences, Fordham University, Bronx, New York 10458, USA and [2]Division of Science & Mathematics, Fordham University, New York, New York 10023, USA

Heavy metals have been found in tissues of a variety of terrestrial isopods recovered from heavy-metal polluted sites (Martin et al. 1976; Hopkin and Martin 1982; Prosi and Dallinger 1988; Vernon et al. 1989). One of the difficulties encountered in these studies is the interpretation of the influence of multiple non-essential heavy metals on concentrations of essential metals.

The primary soft tissue site of heavy metal storage in the isopod is the hepatopancreas, which stores more heavy metal than any other soft tissue of any other animal (Hopkin and Martin 1982). Within the hepatopancreas essential and non-essential heavy metals are stored in copper granules or cuprosomes of S cells (Coughtrey et al. 1977; Hopkin and Martin 1982; Prosi et al. 1983) and iron granules of the B cells (Hopkin and Martin 1982, 1984). According to Hopkin and Martin (1982) iron granules in B cells and copper granules in S cells accumulate lead and zinc when isopods are exposed to food contaminated with these metals. Ultrastructural and biochemical analyses of granules isolated from isopod hepatopancreas reveal that the metals are localized in both membrane bound granules and smaller non-membrane bound aggregations (Prosi and Dallinger 1988). It has been suggested by Hopkin and Martin (1984) that once deposited in the granules the metals are retained in the isopod until its death. In some cases the hepatopancreas becomes so saturated with metals that the excess is found in the hemolymph (Hopkin and Martin 1982).

In a preliminary study by Tomita et al. (1990) isopods exposed to lead nitrate showed increased copper and zinc concentrations in hepatopancreas tissue. As a result of these findings, this more extensive study was undertaken.

Send reprint requests to R. Witkus at the above address.

MATERIALS AND METHODS

Adult Armadillidium vulgare isopods (N=57) were collected from an uncontaminated site in the Bronx, New York. They were reared in the laboratory in 5-inch glass fingerbowls with No. 2 filter paper, leaf litter from the collection site and 5 ml of spring water per bowl for moisture. A control group and four treatment groups were set up in duplicate. A common pool of leaf litter was subdivided equally among the fingerbowls. Three ml of a solution of lead nitrate in spring water were added to the treatment groups in the following concentrations: 0.5, 1, 10 and 100 mg/l of spring water. The control group received 3 ml of spring water. All isopod groups were maintained for 28 days at 24.5-27.0°C under a 12/12 hour light dark regimen. Only spring water was added to each of the fingerbowls to maintain moisture.

At the end of 28 days, control and treatment group isopods were processed for scanning electron microscopy/energy dispersive x-ray analysis (SEM/EDX) and atomic absorption spectroscopy (AAS). For SEM/EDX analysis 23 isopods were dissected to remove the hepatopancreas. The organs were fixed in 3.5% glutaraldehyde in 0.1M Na cacodylate buffer with 0.5% CaCl2 added (pH 7.4) for one hour at 4°C and processed as previously described (Witkus et al. 1969), omitting postfixation in osmium tetroxide and [en] bloc staining with uranyl acetate. Five micron thick sections were cut from the proximal and mid-glandular regions of the hepatopancreas. Sections were placed on glass slides and stained with 1% methylene blue in 1% sodium borate for light microscopy study. Subsequently, slides were etched in a solution of 1:1 NaOH/absolute ethanol. Areas of slides containing sections were mounted on carbon stubs, sputter-coated with gold palladium and studied with an Hitachi S-510 SEM operating at 25kv with an HNU 5000 EDX spectrophotometer.

Thirty-four control and treated isopods were prepared for AAS. Isopods were dissected in distilled water, and the hepatopancreas from each organism was placed on preweighed, dried filter paper, dried in an oven at 60°C for one week and removed to a desiccator until needed. Dried specimens were weighed, digested in 2 ml of concentrated nitric acid (spectral grade) for 2 hours at 120-125°C and diluted with filtered distilled water for a total volume of 5 ml. Concentrations of metals were determined by the flame method using a Perkin-Elmer atomic absorption spectrophotometer. Data were analyzed with SPSSX (Statistical Package for the Social Sciences) on a mainframe Digital Vax.

RESULTS AND DISCUSSION

Accumulation and distribution of essential metals in isopod hepatopancreas is influenced by lead dose. Multivariate analysis of energy dispersive x-ray analysis (EDX) data (Table 1) and atomic absorption results (Table 2) show that there are significant differences in concentration and distribution of copper, zinc and calcium as lead dose increases.

The hepatopancreas consists of S and B cells (Frenzel 1884; Clifford and Witkus 1971). S cells are the primary site of storage of essential metals such as copper, zinc and calcium as well as potentially toxic metals such as lead (Brown 1978; Hopkin and Martin 1982; Witkus et al. 1987).

In the present EDX study, S cell granules contained copper, zinc, calcium and sulfur (Table 1; Figure 1). Control results compare favorably with those described by Witkus et al. (1987). Lead was not detected in either S or B cells from treatment groups. Judging by the AAS results (Table 2; Figure 2), it is likely that lead levels were below EDX detectable levels. Relatively low lead concentrations may be attributed to a relatively low rate of assimilation by isopods (Beeby 1978). A comparison of concentrations of metals in S cell granules in control and each lead concentration shows that relative weights of copper, zinc and sulfur differ significantly. AAS results of control vs treatment groups did not show significantly different metal concentrations with the exception of the 100 mg/l group (Table 2). In the 100 mg/l group both copper and lead were significantly higher ($p<.02$; $p<.001$), while only lead was significantly different between the 100 mg/l and the 1 or 10 mg/l groups.

Table 1. Summary of EDX Elemental Analysis (N=70 cells).

	(Weight Percent Mean ± SD)			
Groups	Sulfur	Calcium	Copper	Zinc
Control	1.10±1.69[1]	7.58±3.73	1.33±1.73[2]	1.02±1.69[3]
0.5mg Lead	9.76±6.68	8.49±2.46	9.23±8.27	6.50±4.29
1.0mg Lead	11.45±1.57	8.60±0.53	10.66±2.32	6.43±1.64
10mg Lead	7.90±4.38	8.38±1.33	6.68±5.17	5.73±3.97
100mg Lead	17.39±7.09	5.24±2.82	15.56±6.39	11.08±5.37

Median [1] 7.25; [2] 5.62; [3] 4.67

Table 2. Summary of Atomic Absorption Analysis (N=34).

Groups	Calcium	Copper	Zinc	Lead
	(μg/g Mean ± SD)			
Control (N=10)	12,401±6,080	9,156±5,925	6,297±3,231	27±28[1]
1mg Lead (N=6)	8,052±3,000	7,853±3,569	5,756±3,505	24±20
10mg Lead (N=10)	11,369±4,974	13,521±10,082	6,900±6,171	40±23
100mg Lead (N=8)	20,809±17,336	19,550±11,707	14,767±16,495[2]	224±36

Median [1]35; [2] 6,071

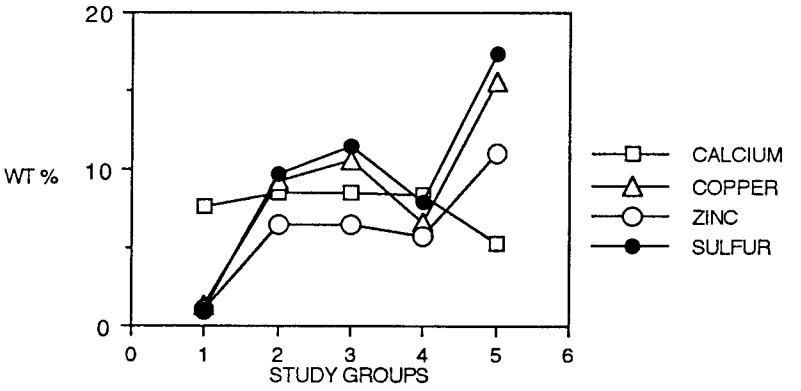

Figure 1. Comparison of weight percent distribution of elements in S cells of control (1), 0.5 mg/l (2), 1 mg/l (3), 10 mg/l (4), 100 mg/l (5).

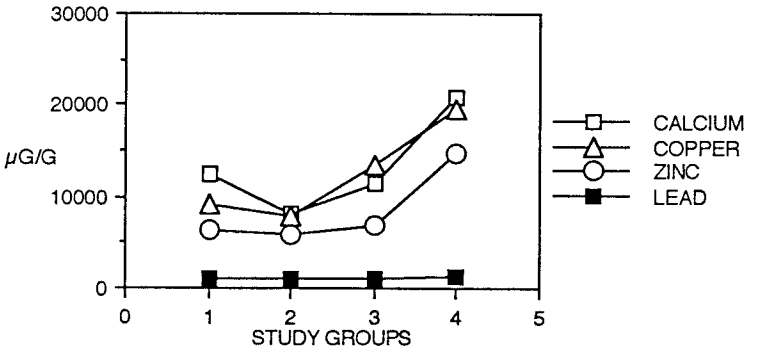

Figure 2. Comparison of metal concentrations in control (1), 1 mg/l (2), 10 mg/l (3), 100 mg/l (4).

Correlational data analysis of AAS results reveals interesting relationships between the concentration of different metals. An analysis, without regard to groups, shows significant positive correlations between calcium and copper, zinc and lead as well as between copper and zinc and copper and lead (Table 3). Analysis of isolated groups reveals that among controls and 100. mg/l group there is also a positive correlation between calcium and copper as well as zinc and between copper and zinc. However, the 1 and 10 mg/l groups have only copper and zinc positively correlated. Regression analyses reveal significances for copper and zinc (Table 3).

Table 3. Atomic Absorption Correlations/Regressions.

Correlations Variable Pair	r/R^2	Significance
Calcium vs Copper	.5782	.000
Calcium vs Zinc	.6505	.000
Calcium vs Lead	.4071	.008
Copper vs Zinc	.8277	.000
Copper vs Lead	.4717	.000
Regressions Variable		
Copper	.2286	.004
Zinc	.1277	.040

Hopkin and Martin (1982) found that Oniscus asellus collected from a contaminated site accumulated lead. Their report did not specifically address the impact of the concentration of lead on other metals. Their data show that the copper and zinc concentrations were similar in leaf litter from both contaminated and uncontaminated sites. However, isopods from a lead contaminated site concentrated more copper and zinc than isopods from an uncontaminated site.

A comparison of the SEM/EDX data with AAS data reveals inconsistent changes over the range of lead concentrations. Atomic absorption results show increases in mean concentrations of calcium, and calcium is positively correlated with lead. These results are consistent with those obtained by Beeby (1978) in Porcellio scaber. The decrease in relative amounts of calcium as detected by SEM/EDX at the 100 mg/l lead concentration in the S cell granules could be due to a change in the distribu-

tion of calcium between the granules and cytoplasmic matrix. Beeby and Richmond (1987), in their study of the relationship between calcium and lead in the snail Helix aspersa, demonstrated a positive correlation between calcium and lead. In a later study, Beeby and Richmond (1988) found that tissue calcium levels were also higher in snails from a lead contaminated site. Contaminated snails concentrated calcium at a faster rate on a high lead diet than similarly treated uncontaminated snails.

The physiological/biochemical bases for the effect of lead on distribution of copper, zinc and calcium are not yet understood. As a consequence of lead exposure, it is possible that the other metals are redistributed within the hepatopancreas and perhaps within the isopod as well. The mechanisms by which these metals are mobilized, transported and sequestered by the hepatopancreas are not fully understood, and further study is necessary to elucidate the role of the hepatopancreas in heavy metal detoxification.

Acknowledgments. This study was supported by a Research Corporation Cottrell College Science Grant.

REFERENCES

Beeby A (1978) Interaction of lead and calcium uptake by the woodlouse, Porcellio scaber (Isopoda, Porcellionidae) Oecologia (Berlin) 32:143-148

Beeby A, Richmond L (1987) Adaptation by an urban population of the snail Helix aspersa to a diet contaminated with lead. Environ Pollut 46:73-82

Beeby A, Richmond L (1988) Calcium metabolism in two populations of the snail Helix aspersa on a high lead diet. Arch Environ Contam Toxicol 17:507-511

Brown W (1978) Lead detoxification by a copper tolerant isopod. Nature 276:388-390

Clifford B, Witkus ER (1971) The fine structure of the hepatopancreas of the woodlouse, Oniscus asellus. J. Morph 135:335-350

Coughtrey PJ, Martin MH, Young EW (1977) The woodlouse, Oniscus asellus, as a monitor of environmental cadmium levels. Chemosphere 6:827-832

Frenzel J (1884) Uber hie Middeldarmdruse der Crustaceen. Mitt. Zool. Sta. Neapel 5:50-101

Hopkin SP, Martin MH (1982) The distribution of zinc, cadmium, lead and copper within the woodlouse Oniscus asellus (Crustacea, Isopoda). Oecologia 54:227-232

Hopkin SP, Martin MH (1984) Heavy metals in woodlice. In: Holdich DA, Sutton SL (ed) The Biology of Terrestrial Isopods, Sym. Zool. Soc. London, No. 53. Academic Press, London and New York, pp 143-166

Martin MH, Coughtrey PJ, Young EW (1976) Observations on the availability of lead, cadmium and copper in woodland litter and the uptake of lead, zinc and cadmium by the woodlouse Oniscus asellus. Chemosphere 5:313-318

Prosi F, Dallinger R (1988) Heavy metals in the terrestrial isopod Porcellio scaber Latreille. I. Histochemical and ultrastructural characterization of metal containing lysosomes. Cell Biol and Toxicol 4:81-96

Prosi F, Storch V, Janssen HH (1983) Small cells in the midgut glands of terrestrial isopoda: Sites of heavy metal accumulation. Zoomorphology 102:53-64

Tomita M, Vernon G, Heisey R, Witkus R (1990) The effect of lead on other elements in an isopod. AAAS Annual Meeting (abstract)

Vernon G, Greco R, Witkus R (1989) Localization of heavy metals in the hepatopancreas of the terrestrial isopod, Oniscus asellus. Proc. 47th Annual Meeting EMSA 1092-1093

Witkus ER, Grillo RS, Smith WJ (1969) Microtubule bundles in the hindgut epithelium of the woodlouse, Oniscus asellus. J Ult Res 29:182-190

Witkus R, Horgan MJ, Klein M, Faso L (1987) Comparative elemental analysis of S and B cells of the hepatopancreas of A. vulgare, a terrestrial isopod. Comp Biochem Physiol 87C: 149-151

Received October 29, 1990; accepted December 19, 1990.