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# Responses of the Hepatopancreatic 'B' Cells of a Terrestrial Isopod, *Oniscus asellus,* to Metals Accumulated from a Contaminated Habitat: A Morphometric Analysis

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The four-lobed hepatopancreas of terrestrial isopods is a highly specialized enzyme secretory, digestive and absorptive region of the midgut (Hames and Hopkin, 1989). The walls of these blind-ending tubes are composed of two distinctive epithelial cell types: large, lipid-rich 'B'-cells projecting into the lumen, and containing (Fe and P)-rich multivesicular granules; small 'S' - cells containing (Cu+S)-rich (cuprosome) granules (Hopkin and Martin, 1982a; Storch, 1982; Prosi, Storch and Janssen, 1983; Morgan and Winters, 1987).

It has been shown (Hopkin and Martin, 1982, 1984; Morgan and Winters, 1987) that although 'B'- and 'S'-cells differentially accumulate Fe and Cu, respectively, both cells accumulate Pb and Zn within their constituent granules when woodlice are exposed to contaminated food. The functions of the Fe-and Cu-rich granules have not been fully described, although they may represent storage sites for essential metals, and loci for the long-term immobilization of potentially toxic metals. However, it is evident that the two cell types are functionally as well as structurally different. For example, the 'B' -cells respond drastically to the nutritional composition of different diets, whereas the 'S'-cells remain more or less unaffected (Storch, 1982, 1984; Prosi et al., 1983).

This study investigated the possible stressful effects of accumulated Pb and Zn on the metabolically highly responsive, 'B'-cells. Three major objectives were pursued. First, quantitative electron probe X-ray microanalysis (EPXMA) was employed to determine whether the accumulation of Pb and Zn changes the Fe concentration within individual 'Fe-granules'. Morgan and Winters (1987) observed that the accumulation of metal contaminants does reduce the Cu concentration within cuprosomes. Second, simple morphometric techniques were applied to transmission electron micrographs to determine whether competition between essential (Fe) and pollutant (Pb,Zn) metals for binding ligands resulted in an increased granule volume per individual cell.

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Qualitative scanning electron microscopic observations on the factured hepatopancreases of juvenile woodlice indicate that the 'S'-cells of animals mantained on contaminated food contain far more cuprosomes than the S-cells of specimens maintained on uncontaminated food (Hopkin and Martin; 1984). Third, to measure the effect of Pb and Zn accumulation of the amount of stored lipid, in the form of discrete droplets, within the 'B'-cells.

MATERIALS AND METHODS

Female <u>O.asellus</u>, each weighing about 60 mg, were collected in the month of October from a control site (Dinas Powys, O.S. Ref No. = ST 146723) and Pb/Zn polluted disused mine site (Llantrisant = ST 048822) in South Wales, U.K.

Total metal concentrations in 0-10 cm soil samples were determined in ground and homogenised briquettes by X-ray fluorescence analysis (XRF, Philips PW 1400).

The elemental composition of the hepatopancreases was determined by EPXMA in sprayed microdroplets of conc. $HNO_3$ digested tissue samples pooled from 3 individual animals. This technique entailed digestion to dryness in boiling acid, re-solubilisation in 0.5N $HNO_3$ containing cobalt (conc. = 478 µg/g) as an internal calibration element. Microdroplets of these solutions were produced and analysed as previously described (Morgan, 1983).

Samples from the proximal region of the hepatopacreas tubules of 5 individual animals from each site were fixed in 3% glutaraldehyde in 0.1M cacodylate buffer, pH 7.4; post-fixed in osmium tetroxide; embedded in Spurr resin; thin transverse sections were mounted on 100-mesh nickel grids, stained and examined in a JEOL 100S transmission electron microscope for morphometric analysis. Since the 'B' cells are large and structurally polarized, and since the floccular 'Fe - granules' are small, it was necessary to photograph at a magnification of 5,000x with the randomly orientated sampling 'field' placed in the peri-nuclear cytoplasm with a similar proportion of fields photographed in the apical cytoplasm (where the majority of the granules and liquid droplets are distributed) in the cells from control and contaminated animals. <u>Surface densities</u> ($S_v$ = 2 x total number of intersections and divided by total line length, corrected to scale) and <u>volume fractions</u> ($V_v$ = number of points on a feature ÷ total number of points) of 'Fe-granules,' multivesicular bodies with no visible mineral content, and lipid droplets were measured in one or more cell from at least one section from each animal by projecting the negative images (magnification 3.5x) onto a grid drawn on a transparent film (grid dimensions = 20 cm x 14 cm; total line length = 280 cm; number of equally spaced points = 70) placed on a white background. The morphometric methods are fully described by Steer (1981).

Representative pieces of proximal hepatopancreas were fixed in glutaraldehyde only, and sectioned on dry glass knives for quantitative electron probe X-ray microanalysis (Winters and

Morgan, 1988) of individual 'Fe-granules'

Statistical comparisons were made by either the two-tailed Student's t-test, or the non-parametric Mann-Whitney 'U'-test.

RESULTS AND DISCUSSION

Table 1. Elemental Composition (mM/kg dry weight) of O. asellus hepatopancreas determined by EPXMA of sprayed microdroplets

| Site | P | S | Ca | Fe | Cu | Zn | Pb |
|---|---|---|---|---|---|---|---|
| Dinas Powys (Control) | 259 ± 26 | 558 ± 59 | 98 ± 34 | 13 ± 4 | 33 ± 12 | 6 ± 3 | 2 ± 6 |
| Llantrisant (Polluted) | 235 ± 49 N.S | 688! ± 125 | 103 ± 24 N.S | 15 ± 4 N.S | 20 ± 6 ** | 19 ± 5 *** | 44 ± 22 *** |

number of observations = 8 in each case; ! = this value is uncertain because of the overlap between S(K) and Pb(M) lines, and the consequent deconvolution difficulties; ** = $P < 0.01$; *** = $P < 0.001$, N.S = non significant (Student's t-test)

Table 2. Elemental composition (mM/kg dry weight) of individual Fe-granules in glutaraldehyde-fixed, resin-embedded sections measured by EPXMA.

| Site | Fe | P | Ca | Zn | Pb |
|---|---|---|---|---|---|
| Dinas Powys (Control) | 156 ±50 | 314 ±50 | 27 ±12 | N.D | N.D |
| Llantrisant (Polluted) | 62 ±27 ** | 213 ±19 ** | 36 N.S. ±10 N.S | 10 ±9 *** | 281 ±78 *** |

number of observations = 5 in each case; N.D. = non-detectable; ** = $P < 0.01$; *** = $P < 0.001$; N.S. = non significant (Mann-Whitney 'U' test).

XRF analyses showed that the salient 'total' soil metal compositions were: Dinas Powys (control) = Pb, 240 ug/g; Zn, 480 ug/g; Cu, 220 ug/g; Fe, 37800 ug/gl Llantrisant (polluted) = Pb, 9820 ug/g; Zn, 3280 ug/g; Cu, 170 ug/g; Fe 51900 ug/g. Thus, Llantrisant soil is contaminated by Pb and Zn.

Whole hepatopancreas elemental compositions are presented in Table 1. Note that the Llantrisant tissue samples contained significantly higher Pb and Zn concentrations (and contents, since the dry weights of the hepatopancreases were very similar) than controls, but the Fe concentrations were not significantly different.

EPXMA of individual 'Fe-granules' (Table 2) indicated that Pb and Zn concentrations were significantly higher than control values in the Llantrisant samples, whilst the Fe (and P) concentrations were signifcantly lower in Llantrisant granules. Since the Fe concentrations in the whole hepatopancreases of Dinas Powys and Llantrisant animals were very similar (13 and 15 mM/kg dry weight, respectively), and since the Fe concentrations in the granules differ by a factor of about x 2.5, it can be surmised from analytical data alone (assuming that the major proportion of hepatopancreatic Fe is located within the granules, and ignoring the 'diluting' effect of the 'S'-cells) that the 'B'-cells of animals from the polluted site (Llantrisant) contain about 3x more granules than the controls.

Morphometric observations (Table 3) indicated that the $V_v$ and $S_v$ values for 'Fe-granules' and multivesicular bodies were significantly higher in the 'B'-cells of woodlice from the Pb/Zn polluted site; in contrast, both morphometric parameters for lipid droplets were significantly lower in the cells of Llantrisant animals. If the contribution of lipid and multivesicular bodies to the measured cytoplasmic volumes (in Dinas Powys 'B'-cells =28.7% of the volume; Llantrisant = 9.4%) is taken into account, we can estimate that 'Fe-granules' occupy about 3.8% of the peri-nuclear cytoplasm volume in Dinas Powys 'B'-cells, and about 20.2% in Llantrisant 'B'-cells i.e a volume density difference of about x5.

Table 3 Morphometric analysis of 'B' cells: surface densities ($S_v$) and volume fractions ($V_v$).

| Site | Fe-granules | Multivesicular bodies | Lipid droplets |
|---|---|---|---|
| Dinas Powys $S_v$ (23) | 0.095 (a) ± 0.030 | 0.022 (a) ± 0.010 | 0.353 (a) ± 0.060 |
| $V_v$ (23) | 0.027 (b) ± 0.009 | 0.009 (b) ± 0.005 | 0.278 (b) ± 0.040 |
| Llantrisant $S_v$ (29) | 0.484**(a) ± 0.030 | 0.134**(a) ± 0.008 | 0.084**(a) ± 0.020 |
| $V_v$ (29) | 0.183 ± 0.030 | 0.034**(b) ± 0.008 | 0.060**(b) ± 0.020 |

numbers in parentheses = total number of 'fields' measured including 12 in the apical cytoplasm of Dinas Powys cells and 16 in the apical cytoplasm of Llantrisant cells. ** = $P<0.01$ (Mann-Whitney 'U'-test); values designated by the same letter ('a' or 'b') in each vertical column were compared statistically.

Hopkin and Martin (1984) showed that the number of cuprosomes in the 'S'-cells of juvenile O.asellus, reared on litter contaminated with heavy metals, was far greater than in woodlice from the same brood reared on uncontaminated litter. These authors were unable to comment on the responses within the 'B'-cell because 'Fe-granules' begin to accumulate at a later stage in juvenile development. The present paper indicates that environmental exposure to Pb and Zn resulted in an accumulation of both metals within the 'Fe-granules' of adult woodlice, a decrease in the Fe concentration within individual granules, and an expansion of the metal sequestering granule compartment within individual 'B'-cells. These observations suggest that the woodlouse is unable to regulate the net uptake of iron and contaminant metals into the hepatopancreas, and responds to the presence of elevated intracellular burdens by the formation of more membrane-limited, relatively insoluble, granules.

Lipid stores in the 'B'-cells form a major portion of the energy reserves of isopods (Steeves, 1969; Hopkin and Martin, 1984); much of this lipid may be converted to glycogen, and then to glucose, for respiratory energy release (Passano, 1960). The much reduced number of lipid droplets in the 'B'-cells of woodlice from the metal contaminated site suggests that the sequestration of Pb and Zn exerts a significant energy demand upon the 'B'-cells. An analogous reduction has been recorded (Ireland and Richards, 1977; Richards and Ireland, 1987) in the glycogen reserves within earthworm chloragocyte cells in response to Pb accumulation. The observed changes in the ultrastructure of the 'B'-cells of woodlice sampled from the contaminated habitat were unlikely to be the direct result of Pb or Zn inhibition of lipid metabolism. However the 'energy-demand' hypothesis must be tempered by the possibility that the quality and/or quantity of the food available at the two sites differ considerably, although a cursory examination of the calcareous, mixed-deciduous, sites suggests that they are rather similar, and that food was not limited. This is an important consideration, because it has been shown (Storch, 1982, 1984) that the morphology of the 'B'-cells of woodlice fed on diets containing different proportions of protein, fat and carbohydrate can vary enormously.

We conclude that the reduction of lipid reserves, in the 'B'-cell of woodlice living in a metal-polluted habitat, represents an indirect, and probably non contaminant-specific, sub-lethal response to the energy demands associated with the 'housekeeping' of elevated cellular metal burdens. It would, be interesting to determine whether O.asellus from Llantrisant possess (a) a reduced 'scope-for-growth' i.e. the index successfully used for estimating the physiological fitness of animals exposed to pollutant stress (Widdows, 1985), and (b) reduced reproductive performance compared with controls. Woodlice are efficient accumulators of heavy metals (Hopkin and Martin, 1982b; Morgan et al., 1986). The morphometric evaluation of the structural responses within the metabolically active 'B'-cells could potentially have an important role in terrestrial pollution monitoring.

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