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BIOREDUCTION OF ZINC AND MANGANESE ALONG A MOLLUSCAN FOOD CHAIN

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Abstract—1. Digestive glands of the gastropod mollusc *Littorina littorea* contain intracellular phosphate granules which bind metals.

2. This tissue was fed to a carnivorous gastropod *Nassarius reticulatus*; the granules passed through the gut and appeared in the faecal pellets.

3. The granules in the faecal pellets retained about 50% of the original zinc and 33% of the original manganese. All the potassium was removed.

INTRODUCTION

Marine invertebrates accumulate and detoxify metals in intracellular phosphate granules in various tissues. These include the digestive gland of gastropods, kidney of bivalves and gut parenchymous tissue of barnacles (reviews—Nott, 1991; Viarengo and Nott, 1992). When these tissues are fed to carnivorous gastropods the granules pass through the gut and appear in the faecal pellets (Nott and Nicolaidou, 1990). Granules in the tissues of origin and in the faecal pellets have been examined in the scanning electron microscope and the elemental composition determined by X-ray microanalysis (Nott and Nicolaidou, 1990). These investigations have shown that in invertebrate tissues the granules consist of magnesium, phosphorus, potassium and calcium together with lesser amounts of sulphur and chlorine. After passage through the gut of a carnivorous gastropod the granules produce peaks for magnesium, calcium and phosphorus but not sulphur, chlorine and potassium. Further to this, granules which contain zinc and manganese in the winkle *Littorina littorea*, clam *Chlamys opercularis* and barnacle *Balanus balanoides* produce peaks for the metals after passing through the gut of either *Nassarius reticulatus* or *Nucella lapillus*. This manganese and zinc in phosphate granules cannot be assimilated into the tissues of the carnivorous molluscs and has, in effect, been rendered biologically unavailable.

However, X-ray microanalysis of spherical granules in a scanning electron microscope provides only qualitative data. In the present work granules are embedded in resin and sectioned for semi-quantitative analysis in the transmission electron microscope. The object of this investigation is to determine the effects of digestive processes in carnivores on the elemental composition of granules and, in particular, to assess the proportion of retained zinc and manganese.

MATERIALS AND METHODS

Adult *Littorina littorea* were divided into three groups of ten in separate aquaria containing aerated seawater at 12°C. The control group were kept in clean seawater and for two treatments the seawater contained either 1 ppm zinc or 1 ppm manganese. After the dosage systems had run for 16 days, samples of digestive gland were taken for microanalysis in the transmission electron microscope (TEM) and the remainder was fed to *Nassarius reticulatus* to produce faecal pellets.

Small pieces of fresh digestive gland and faecal pellets were cryofixed by quench-freezing in liquid ethane to prevent loss of elements from granules and tissues. Full details of the method as applied to molluscan tissues are given in Nott and Langston (1989) and relevant general references on cryopreparation are given in Ryan *et al.* (1990). Frozen specimens were stored at liquid nitrogen temperature and subsequently freeze-dried at $-80°C$. Dry specimens were brought slowly to room temperature and embedded under vacuum in low viscosity epoxy resin.

Sections were cut dry at a thickness of $1.5\ \mu M$ and examined in a Jeol 200CX TEM at 200 kV in the scanning transmission (STEM) mode. Specimen grids were tilted to 30° and analysed with the stationary STEM probe. Analysis times were pre-set to 50 sec.

X-rays which fluoresce from the specimen can be divided into non-specific background radiation, also referred to as Bremsstrahlung or continuum, and peaks occurring at energies which are characteristic for the probed elements.

Total counts were recorded for the peaks and, in each case, the underlying background was subtracted to give a net integral for each element. An integral from an extended, uninterrupted region of background was recorded as a relative measure of atomic mass of the probed material. Ratios of peak integrals to extended background integral (b) gave a measure

Table 1. Comparison of analyses of granules in digestive glands and faecal pellets. No metal treatment. Significance of the difference between mean values for glands and pellets is calculated by Student's *t*-test

	Mass fractions				Elemental ratios		
	Mg/*b*	P/*b*	K/*b*	Ca/*b*	P/Ca	P/Mg	Ca/Mg
Litt. d.g. Clean	1.8 ± 0.5	7.9 ± 1.0	1.5 ± 0.1	0.5 ± 0.1	15.8 ± 3.3	4.6 ± 0.7	0.3 ± 0.1
Nass f.p.	1.1 ± 0.3	5.8 ± 0.8	0.2 ± 0.1	3.1 ± 0.9	2.0 ± 0.6	6.1 ± 1.3	3.6 ± 1.9
t-test	$P = 0.01$	$P = 0.02$	$P = 0.001$	$P = 0.001$	$P = 0.001$	n.s.d.	$P = 0.01$

X-ray microanalyses of resin embedded sections of phosphate granules in digestive glands of *Littorina littorea* (Litt. d.g.) and faecal pellets from *Nassarius reticulatus* (Nass. f.p.). Ten granules analysed in each gland and pellet and 5–10 samples taken from each treatment. Results are means ± SD. Mass fraction is a ratio of the net peak integral for an element to extended region of background in the X-ray energy spectrum (see Figs 1–6). Elemental ratios are derived from net peak integrals for the elements.

of element mass fractions, Mg/*b*, P/*b*, K/*b*, Ca/*b*, Mn/*b* and Zn/*b*.

All the results refer to the X-ray microanalysis of individual granules in resin-embedded sections of either digestive gland from *Littorina littorea* or faecal pellets from *Nassarius reticulatus*. Ten different granules were analysed in each digestive gland or faecal pellet and the mean value calculated for the sample. Between 5–10 samples were taken for each treatment and the mean value calculated for the treatment. The standard deviation (sn-1) was calculated for each mean.

RESULTS AND DISCUSSION

Results of analyses of the granules in the digestive gland and the faecal pellets are given in Tables 1–3.

In the control situation (Table 1) the mass fractions of magnesium, phosphorus, potassium and calcium in the granules are changed as a result of passage through the gut of *Nassarius reticulatus*. Mass fractions of magnesium, potassium and phosphorus are reduced and this causes an increase in the mass fraction of calcium. It is assumed that magnesium and potassium are more soluble and leached out by the digestive processes of *Nassarius*. Loss of phosphorus indicates that magnesium and possibly potassium are removed as phosphates. However, the massive loss of potassium is out of proportion to the loss of magnesium and phosphorus which suggests that potassium is associated with a ligand other than phosphate. The large increase in the mass fraction of calcium must reflect primarily the loss of potassium.

These numerical results can be assessed qualitatively from spectra derived from granules in the digestive gland and faecal pellets (Figs 1–3). In the digestive gland the peak for phosphorus is dominant with magnesium and potassium also having major peaks (Fig. 1). Calcium has a small K peak at 3.69 keV which is overlapped by a potassium KB peak at 3.59 keV. After the granules have been digested in *Nassarius* the phosphorus peak remains dominant but calcium is now a major peak (Fig. 3). As it can be assumed that calcium is not added to the granules during digestion, the spectrum indicates that the amounts of phosphorus and magnesium are reduced. There is a complete loss of the major peak for potassium. In Fig. 2, the vertical scale of the spectrum in Fig. 1 is expanded to match the height of the calcium peak with the height of the same peak in Fig. 3. If it is assumed that no calcium has been removed from the granules by *Nassarius* then a comparison of Fig. 3 with Fig. 2 (matched calcium peak) gives a graphic indication of the loss in magnitude of the peaks for magnesium, phosphorus and potassium relative to calcium. Indeed, calcium changes from a minor proportion of the mass in Figs 1 and 2 to become a major proportion in Fig. 3.

Table 2. Same as Table 1 but *Littorina* is dosed with zinc

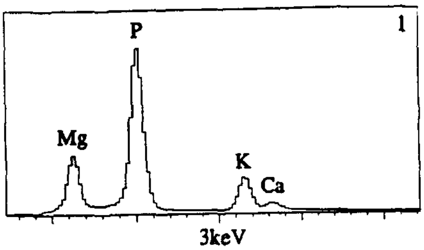
	Mass fractions					Elemental ratios			
	Mg/*b*	P/*b*	K/*b*	Ca/*b*	Zn/*b*	P/Ca	P/Mg	Ca/Mg	Zn/Ca
Litt. d.g. + Zn	1.3 ± 0.4	5.2 ± 1.6	0.8 ± 0.3	0.6 ± 0.2	0.7 ± 0.1	10.6 ± 3.0	6.0 ± 3.8	0.5 ± 0.1	1.5 ± 0.9
Nass. f.p.	1.4 ± 0.5	5.3 ± 1.3	0.2 ± 0.0	1.1 ± 0.3	0.6 ± 0.2	5.5 ± 1.9	5.4 ± 2.5	1.1 ± 0.4	0.7 ± 0.3
t-test	n.s.d.	n.s.d.	$P = 0.001$	$P = 0.01$	n.s.d.	$P = 0.01$	n.s.d.	$P = 0.02$	n.s.d.

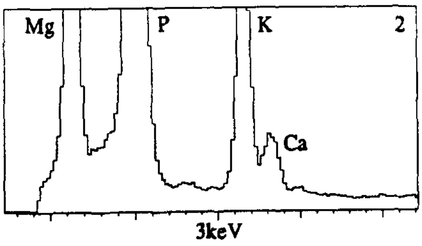
See Table 1. footnote for details.

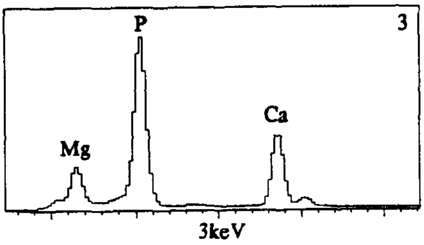
Table 3. Same as Table 1 but *Littorina* is dosed with manganese

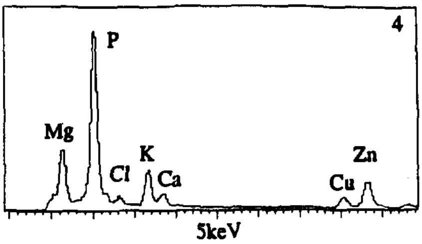
	Mass fractions					Elemental ratios			
	Mg/*b*	P/*b*	K/*b*	Ca/*b*	Mn/*b*	P/Ca	P/Mg	Ca/Mg	Mn/Ca
Litt. d.g. + Mn	4.4 ± 2.0	16.6 ± 4.0	4.5 ± 1.6	0.9 ± 0.3	0.3 ± 0.1	18.4 ± 6.8	4.4 ± 1.6	0.3 ± 0.2	0.3 ± 0.0
Nass. f.p.	4.4 ± 2.0	18.6 ± 4.0	0.4 ± 0.2	6.0 ± 3.2	0.4 ± 0.1	5.0 ± 3.3	5.1 ± 1.5	1.4 ± 0.8	0.1 ± 0.0
t-test	n.s.d.	n.s.d.	$P = 0.001$	$P = 0.02$	$P = 0.10$	$P = 0.01$	n.s.d.	$P = 0.05$	$P = 0.001$

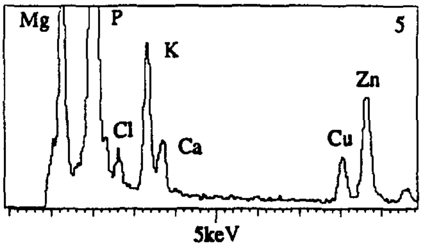
See Table 1. footnote for details.











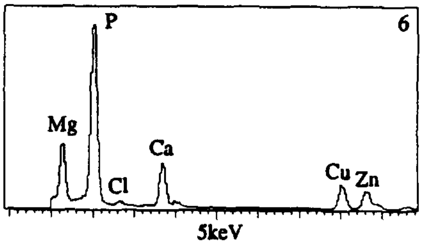


Fig. 1. Granule in digestive gland of *Littorina littorea*. Phosphorus produces a major peak and calcium a minor peak. (v.s. = 16 K). X-ray spectra derived from sections of single resin-embedded phosphate granules. Horizontal scale = X-ray energy; vertical scale (v.s.) = X-ray counts.

Fig. 2. Same spectrum as Fig. 1 but expanded vertically. Size of calcium peak similar to that in Fig. 3. (v.s. = 2 K).

Fig. 3. Spectrum derived from *Littorina* phosphate granule in *Nassarius* faecal pellet. Granule produced in *Littorina* digestive gland and then eaten by *Nassarius*. Potassium is lost. In Figs 2 and 3 calcium peaks are similar height but comparatively, magnesium and phosphorus peaks are reduced in Fig. 3. (v.s. = 16 K).

Fig. 4. Spectrum derived from phosphate granule in digestive gland of *Littorina* dosed with zinc. Copper is derived from the microscope not the specimen. (v.s. = 4 K).

Fig. 5. Same spectrum as Fig. 4 but expanded vertically. Size of the calcium peak is similar to that in Fig. 6. (v.s. = 1 K).

Fig. 6. Spectrum derived from *Littorina* granule in *Nassarius* faecal pellet. Granule produced in digestive gland of *Littorina* dosed with zinc and then eaten by *Nassarius*. Potassium is lost. In Figs 5 and 6 calcium peaks are similar height but comparatively, magnesium, phosphorus and zinc are reduced in Fig. 6. (v.s. = 4 K).

These changes in the spectra produce some significant changes in the elemental ratios of the granules in the digestive gland when they reappear in the faecal pellets (Table 1). Calcium is increased relative to phosphorus and magnesium. The ratio of magnesium to phosphorus does not change significantly.

When *Littorina littorea* are treated with zinc (Table 2) the mass fractions of magnesium, phosphorus and zinc in the granules show no significant difference after passage through the gut of *Nassarius reticulatus*. However, there is again a significant reduction in the mass fraction of potassium and a gain in the mass fraction of calcium.

Loss of potassium should automatically increase the mass fraction of the remaining elements; this is obviously the case for calcium. However, the fact that mass fractions of magnesium, phosphorus and zinc do not increase in faecal pellet granules suggests that some fractions of these are lost during the process of digestion. Once again, it is assumed that during digestion, granules do not accumulate any elements.

X-ray spectra show an accumulation of zinc in the granules of *Littorina* after dosing with the metal (Fig. 4). When the analytical electron microscope is operated at 200 kV it is marginally less efficient for detecting calcium X-ray quanta at 3.69 keV than it is for zinc at 8.63 keV. The spectra indicate, therefore, that granules produced in the digestive gland of *Littorina* contain more zinc than calcium. Granules appearing subsequently in the faecal pellets of

Nassarius (Fig. 6) produce spectra that are without potassium and the calcium peak has increased relative to the zinc peak. When the scale in Fig. 4 is expanded (Fig. 5) to match the calcium peak with that in Fig. 6, it is apparent that some magnesium and phosphorus and possibly some zinc, have been lost from the granules during digestion by *Nassarius*. However, there is considerable variation in the relative heights of the calcium and zinc peaks between different spectra and this produces greater variability in the Zn/Ca ratios than in the mass fractions of calcium (Ca/b) and zinc (Zn/b). This suggests that the concentrations of calcium and zinc are not positively correlated.

Elemental ratios derived from spectra show that in faecal pellet granules there is a significant decrease in both phosphorus and magnesium relative to calcium. There is a possible 50% decrease in zinc relative to calcium but the difference is not statistically significant ($P > 0.10$).

When *Littorina* are treated with manganese (Table 3) the mass fractions of magnesium, phosphorus and manganese in the granules show no significant difference after passage through the gut of *Nassarius*. However, there is a significant loss in the mass fraction of potassium and a compensatory gain in the mass fraction of calcium. Lack of any gain in magnesium, phosphorus and manganese suggests that a proportion of these elements has been lost from the granules in the gut of *Nassarius*.

Spectra for manganese repeat the pattern of results for zinc; peaks for magnesium, phosphorus and manganese are reduced relative to calcium in the faecal pellet granules. These results are reflected in the elemental ratios (Table 3). If it is assumed that calcium is not removed from the granules, it can be deduced from the Mn/Ca ratios, namely 0.27 and 0.09, that approximately two thirds (66%) of the manganese is removed from the granules when they pass through the gut of *Nassarius*.

In conclusion it appears that processes of digestion in the carnivore *Nassarius* do affect the elemental composition of the phosphate granules produced in the digestive gland of *Littorina*. Most elements apart from calcium suffer some loss. It appears, also, that more manganese may be removed than zinc. Metal removed from the granules is probably absorbed by the digestive epithelium of the carnivore whereas metal not removed, remains unavailable to the carnivore. Granules in the faecal pellets will become part of the sediment but the bioavailability and likely fate of the constituent metal phosphates is open to speculation. They may represent a significant metal pathway in the environment; it has been proposed that similar phosphate granules which are produced in and excreted from kidneys of marine bivalves (Doyle *et al.*, 1978) are an original, biogenic source of marine phosphorite deposits. In this sense it is possible that metals which are mineralized and thus detoxified within the intracellular environment remain biologically unavailable when returned to the external environment. Metal phosphates are probably insoluble in seawater and if ingested by an animal feeding on particulates, will remain insoluble in the gut. In conclusion it can be suggested that molluscan metal detoxification is effective on an environmental scale.

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