Bull. Environ. Contam. Toxicol. (1989) 42:699–706

Bulletin of Environmental Contamination and Toxicology

Magnesium and Manganese Regulation during Moult-cycle in *Porcellio spinicornis* Say (Porcellionidae, Isopoda)

M. A. Alikhan

Department of Biology, Laurentian University, Sudbury, Ontario P3E 2C6, Canada

One of the most obvious features of the lives of Crustacea is the way in which processes of growth and development are punctuated by the cyclic need to deposit a new cuticle and shed the old one. Much attention has been devoted to the endocrinology of this cyclic phenomenon (Kleinholz and Keller 1979), but rather less to physiological events accompanying actual events of ecdysis, the act of shedding the old cuticle.

In earlier work, Alikhan and Pani (1988) report a significant increase in tissue Mg and Mn concentrations in Porcellio spinicornis reared on diets rich in these two metals, and that oxygen consumption and ammonia excretion are significantly affected by these accumulations. The present study provides information on the regulation of Mg and Mn tissue concentrations in adult P. spinicornis Say (Porcellionidae, Isopoda) during proecdysis, ecdysis, and postecdysis (A and C-H stages, Alikhan 1972).

MATERIALS AND METHODS

Seventh growth-stage intermoult P. spinicornis, used in the study were procured from a stock colony maintained on carrots, at 20°C (± 0.5°C) and 85 - 96% relative humidity. For each experiment, unless otherwise stated, 10 isopods of the same sex and approximately of the same age were weighed to nearest 0.0l mg, and caged individually at 20°C (± 5°C) in 10-cm petri dishes lined with moistened filter papers. Growth-instar of the isopod was approximated by the criterion of Alikhan (1972), while moult-stages were determined by the criterion of Stevenson (1961).

Carrot powder mixed with analytical grade $MgSO_4.7H_2O$ and $MnSO_4.4H_2O$ (BDH Chemicals, Toronto, Ontario) was used to prepare experimental diets, containing 50, 100 and 150 ppm Mg or Mn salts. Fresh carrots were cut into slices, oven - dried at 60°C (± 5°C), powdered and mixed with trace metal salts. The mixture was pressed, in a hydraulic press, into 0.2 g pellets. Each isopod was starved for 96 h to clear its gut, and was presented with a single food-pellet every 24 h.

Pellet-residues were removed, oven-dried at 60°C and weighed to determine daily food consumption. Faecel pellets were removed and filter papers were changed daily.

At the conclusion of each moult-stage, isopods were divided into two groups:one was allowed to consume their exuviae, while from the other exuviae were immediately removed. Both groups were then killed, and isopods along with their exuviae were placed on dry Whatman (No:41 ashless) filter paper of known weight, and oven dried at 60°C for 12 h. Food, faecal, cast skin and whole isopod samples were digested in boiling concentrated aqua regia (3 ml Merck's "suprapur" HNO_3 65% : 1 ml BDH analytical grade concentrated HCl), diluted to 20 ml with 1 M HNO_3, and were assayed for Mg and Mn contents in a Perkin-Elmer atomic absorption spectrophotometer by the flame method .

Statistical analysis of the data was computed with the aid of a DEC-VAX/VMS computer, using $SPSS^X$ software (SPSS, Chicago, Ill., U.S.A.). A three-way ANOVA evaluated effects of diet, sex and exuviae consumption. Within dietary metal levels and tissue concentrations, comparisons were made using one-way ANOVA with Duncan's Multiple Range test. All data were checked for normality (Kolgomorov-Smirnoff test) and homogeneity of variance (Bartlett-Box F test).

RESULTS AND DISCUSSION

Among isopods on Mg-enriched diets, highest food consumption was observed in the control and in individuals on 50 and 100 ppm dietary Mg, and lowest in those on 150 ppm (Table 1). Highest Mg tissue concentrations were observed among isopods on 50 and 100 ppm dietary Mg, and lowest among those in control and on 150 ppm dietary Mg (Table 1).

At 50 ppm dietary Mg, Mg concentrations in the hepatopancreas ranged from 19.6% of the total body accumulation in females to 26.7% in males (compared to 11.5% in males and 15.0% in females in control); exoskeletal Mg concentrations varied from 61.5% in males to 78.4% in females (66.8% in males and 71.0% in females in the control) (Table 3). At 150 and 100 ppm dietary concentrations, they ranged from 34.0% and 40.6%, respectively, in male hepatopancreas to 38.0% and 47.5%, respectively, in females; exoskeletal Mg concentrations varied from 52.3% in males to 45.9 and 50.8%, respectively, in females (Table 3). Differences between the two sexes, however, were not significant at $P > 0.05$.

The presence of relatively high Mg concentrations in the exoskeleton may be due to the physiological functional similarity between Mg and Ca

(Bagatto and Alikhan 1987). Elamin and Wilcox (1986) report that Mg mimics Ca, and as such may be preferentially stored in high concentrations in the cuticle. Among isopods on Mg-enriched diets, highest food consumption was observed in control, as well as in those on 50 and 100 ppm dietary Mn, and lowest in those on 150 ppm Mn (Table 2). Highest Mn tissue concentrations were recorded among males on 100 ppm Mn, and lowest among females on 50 ppm Mn (Table 2).

At 50 and 100 ppm dietary concentrations, hepatopancreatic Mn concentration in males amounted to 44.4% and 64.44%, respectively (against 40% in control), and in females 72.79% and 51.16%, respectively (against 75.92% in control) (Table 4). At 150 ppm dietary concentration, it ranged from 38.31% in males to 47.03% in females (Table 4). Exoskeletal Mn concentration, on the other hand, varied in males from 3.8% of the total tissue accumulation in the control to 3.33% in isopods on 150 ppm dietary Mn; in females, it ranged from 4.02% in control to 4.85% in those on 150 ppm Mn (Table 4). This is not surprising since Mn is especially active in digestive and catabolic enzymes (Vallee

Table 1. Comparison of rates of magnesium uptake, assimilation and accumulation in whole Porcellio spinicornis on different dietary concentrations over a 7-day period. Data on males and females were pooled.

	Total of 7 experimental days			
Treatment (ppm)	body weight concentration (mg)	Food consumption (mg)	Faeces excretion (mg)	Mg tissue ($\mu g g^{-1}$ tissue wt.)
Control (1)	25.9 ±2.9^{a}	33.0 ±1.8^{a} (2.23 ± 0.09)^{*a}	2. 4 ± 0.05^{a} (0.03 ± 0.001)^{*a}	70.35 ±30.1^{a}
50	25.8 ±2.4^{a}	30.8 ±2.1^{a} (15.4 ± 2.70)^{*b}	3.9 ± 0.12^{b}	(0.05 ±0.002)^{*b} 148.03 ± 96.2^{b}
100	26.6 ±2.1^{a}	35.2 ±1.8^{a} (35.2 ± 4.70)^{*c}	3.2 ± 1.2 ^{c}	(0.05 ± 0.003)^{*b} 148.88 ± 52.3^{b}
150	29.4 ±1.9^{b}	9.8 ± 3.2 ^{b}	(14.3 ± 3.80)^{*b} 4.1 ± 0.9^{b}	(0.06 ± 0.010)^{*c} 75.04±17.0^{c}

* n = 10 males and 10 females in each case. Values within brackets represent µg magnesium in food consumed and faeces excreted. Mean Mg concentrations in each column followed by the same letter are not significantly different at 5% level.
(1) Mean Mg concentration in a 0.2 g carrot powder pellet in the control = 13.47 µg (67.39 $\mu g\ g^{-1}$ dry wt).

1970).The presence of Mn in the exoskeleton may be related to its limited ability to compliment or substitute for Mg (Bagatto and Alikhan 1987).

In agreement with the studies on Porcellio scaber, Porcellio muscorum, and Oniscus asellus (Joosse and Van Vliet, 1984), Mn accumulation by various tissues in Porcellio spinicornis , is significantly lower than that

Table 2. Comparison of rates of manganese uptake, assimilation and accumulation in whole Porcellio spinicornis from different dietary concentrations over a 7-day period. Data from males and females were pooled.

		Total of 7 experimental days		
Treatment (ppm)	body weight concentration (mg)	Food consumption (mg)	Faeces excretion (mg)	Mn tissue ($\mu g\ g^{-1}$ tissue wt.)
Control(1)	28.7± 3.2[a]	27.7± 2.1[a]	(1.86 ± 0.09)*[a]	1.78 ± 0.03 [a] (0.006 ± 0.001)*[a]	1.22 ± 0.1[a]
50	28.3± 2.7[a]	27.3 ± 2.7[a]	(13.62 ± 2.70)*[b]	1.95 ± 0.13[b] (0.011±0.002)*[b]	1.76 ± 0.2[b]
100	29.9± 4.2[a]	31.6 ± 3.8[a]	(31.64 ± 3.60)*[c]	1.53 ± 0.12[c] (0.011±0.003)*[b]	3.43 ±0.9[c]
150	26.7± 1.9[a]	10.9 ± 1.7[b]	(14.3 0 ±3.80)*[d]	3.10 ± 0.9 0[d] (0.066±0.011)*[c]	2.67±0.8[d]

* n = 10 males and 10 females in each case. Values within brackets represent µg Mn in the food consumed and faeces excreted. Mean Mn concentrations in each column followed by the same letter are not significantly different at 5% level.
(1) Mean Mn concentration in a 0.2 g carrot powder pellet in the control = 1.54 µg (7.9 $\mu g\ g^{-1}$ dry wt).

recorded for Mg. This may imply that most of the ingested Mn, unlike Mg, does not exist as free ion, but is tightly bound either to a metallothionein or a metallothionein-like protein, and, therefore, cannot be detected by normal atomic absorption spectrophotometric methods. Additionally, Mn assimilation, as suggested by Stauber & Florence (1985), may have been affected either by the presence of Cu (part of haemocyanin) in hepatopancreas or by the free Mg ion, or both. Antagonistic interactions between Cu and Mn, and between Mg and Mn have been reported in algal growth (Sunda et al., 1981), and enzymes involved in the ATP hydrolysis from bacteria to mammals (Williams, 1970). Sunda et al. (1983) propose a competition betweenMn and Cu ions for a critical intracellular site . The recent suggestion by Simkiss (1983) that trace metals like Zn, Cd, Cu, Mg and Mn pass across cell membranes more than a million times faster than would Na, K, and Ca, implies that non-

Table 3. Tissue magnesium concentration in 7th growth-stage intermoult (moult-stages C2 - C4) male and female Porcellio spinicornis as a function of dietary magnesium concentration (ppm).

Sex	Diet	Mg concentration (μg g^{-1} dry wt ± SE*)(1) in Hepatopancreas	Exoskeleton	Remaining Body Tissue
Male	Control(2)	129.59 ± 18.86[a]	749.76 ± 37.48[a]	243.5 ± 22.18[a]
	50	888.38 ± 117.14[b]	2,050.37 ± 92.27[b]	392.92 ± 50.08[a]
	100	1,692.97 ± 1,042.12[c]	2,182.29 ± 91.66[b]	299.78 ± 14.99[a]
	150	925.90 ± 31.28[b]	1,421.70 ± 76.73[c]	372.31 ± 20.85[a]
Female	Control(2)	332.75 ± 99.45[d]	1,580.77 ± 79.03[c]	312.55 ± 81.37[b]
	50	652.49 ± 62.08[d]	2,608.47 ±130.42[b]	65.69 ± 37.96[b]
	100	4,477.98 ± 178.89[e]	4,782.10 ± 204.19[d]	171.14 ± 25.69[b]
	150	986.85 ± 96.03[d]	1,192.96 ± 96.47[e]	419.76 ± 82.95[b]

(1) n = 12 isopods in each case. Means within each column followed by the same letter are not significantly different at 5 per cent level.
(2) Mean magnesium concentration in a 0.2 g carrot powder pellet in the control = 13.47 μg (or 67.39 μg g^{-1} dry wt).

Table 4. Tissue manganese concentration in 7th growth-stage intermoult (moult-stages C2 - C4) male and female Porcellio spinicornis as a function of dietary manganese concentration (ppm).

Sex	Diet	Mn concentration (μg g^{-1} dry wt ± SE*)(1) in Hepatopancreas	Exoskeleton	Remaining Body Tissue
Male	Control(2)	16.74 ± 4.09[a]	1.27 ± 0.09[a]	23.22 ± 3.67[a]
	50	33.85 ± 14.94[a]	2.51 ± 0.17[b]	39.87 ± 4.31[b]
	100	91.41 ± 29.87[b]	4.39 ± 0.91[c]	46.06 ± 1.53[c]
	150	37.05 ± 5.94[a]	3.23 ± 1.01[c]	56.69 ± 5.99[d]
Female	Control(2)	36.05 ± 13.12[e]	1.91 ± 0.12[d]	9.52 ± 3.56[e]
	50	38.61 ± 28.93[e]	2.28 ± 0.27[b]	12.15 ± 0.67[f]
	100	48.85 ± 7.27[e]	3.89 ± 0.87[c]	42.73 ± 6.22[c]
	150	52.31 ± 9.31[e]	5.40 ± 1.02[d]	53.52 ± 6.06[d]

(1) Average of 12 isopods in each case. Means within each column followed by the same letter are not significantly different at $P > 0.05$.
(2) Mean Mn concentration in a 0.2 g carrot powder pellet in the control = 1.54 μg (or 7.9 μg g^{-1} dry wt).

essential elements, as well as essential heavy metals which are surplus to requirements, must be rapidly excreted, or stored in an insoluble form to prevent them from diffusing throughout the body to interfer with biochemical reactions within the tissues. To adopt the first solution to this problem, according to Hopkin and Martin (1984), would involve

Table 5. Tissue magnesium concentration in moulting 7th growth-stage male and female Porcellio spinicornis as a function of dietary magnesium concentration (ppm).

		Mg concentration ($\mu g\ g^{-1}$ dry wt ± SE*)(1) in		
Sex	Diet	Group one(2)	Group two(3)	Exuviae
Male	Control(4)	2,261.55 ± 115.17[a]	1,583.11 ± 114.12[a]	982. 72 ± 47.23[a]
	50	4,283.05 ± 230.34[a]	1,400.05 ± 63.54[b]	2,527.32 ± 117.91[b]
	100	6,389.99 ±1,052.18[b]	4,390.76 ± 98.05[c]	2,567.21 ± 876.67[c]
	150	3,091.51 ± 353.27[a]	2,119.85 ± 237.39[c]	1,128.89 ± 76.29[d]
Female	Control(4)	1,720.21 ± 81.98[e]	1,583.51 ± 74.9[d]	679.67 ± 52.34[e]
	50	2,417.18 ± 118.48[e]	1,675.07 ± 89.76[b]	1,969.87 ± 98.74[f]
	100	3,771.29 ± 231.45[e]	1,182.25 ±112.23[c]	1,999.27 ± 182.89[c]
	150	1,906.95 ± 155.71[e]	1,883.58 ± 320.52[d]	2,011.93 ± 132.98[d]

(1) Average of 12 isopods in each case. Means within each column followed by the same letter are not significantly different at $P > 0.05$.
(2) Isopods were allowed to feed on their exuviae.
(3) Isopods were not allowed to feed on their exuviae.
(4) Mean Mn concentration in a 0.2 g carrot powder pellet in the control = 1.54 µg (or 7.9 $\mu g\ g^{-1}$ dry wt).

expenditure of energy to maintain concentration gradients between cellular portion of the alimentary canal, including hepatopancreas, and the digestive fluids (see Simkiss 1977, for a more detailed discussion of this concept). The presence of both Mg and Mn within exoskeleton (Table 5) and hepatopancreatic S cells (Table 6) suggests that isopods have adopted the second solution and strictly regulate their concentrations in the haemolymph by controlling the amount that precipitates in the two tissues. Thus, the primary role of Mg and Mn within the exoskeleton and the hepatopancreas is probably the "detoxification" of these metals and not the storage of essential elements as suggested by Hopkin and Martin (1982). Small reserves of free Mg and Mn ions would be maintained in the haemolymph to supply the various cellular biochemical processes, while major acquisitions from food are diverted to either the hepatopancreas or the exoskeleton for

"detoxification", or for excretion during the moult-cycle. Hopkin and Martin (1984) maintain that once heavy metals, whether essential or non-essential, are deposited within exoskeleton and the hepatopancreas,

Table 6. Tissue magnesium concentration in moulting 7th growth-stage male and female Porcellio spinicornis as a function of dietary magnesium concentration (ppm).

		Mn concentration ($\mu g\ g^{-1}$ dry wt ± SE*)(1) in		
Sex	Diet	Group one(2)	Group two(3)	Exuviae
Male	Control(4)	104.78 ± 5.71[a]	79.18 ± 5.21[a]	3.22 ± 0.67[a]
	50	221.91 ± 6.71[a]	99.11 ± 4.71[b]	9.37 ± 0.31[b]
	100	162.70 ± 2.66[b]	147.54 ± 7.31[c]	6.06 ± 1.53[c]
	150	141.28 ± 2.94[a]	75.20 ± 3.64[c]	1.69 ± 0.09[d]
Female	Control(4)	149.46 ± 7.89[e]	90.96 ± 4.5[d]	1.16 ± 0.06[e]
	50	122.50 ±18.02[e]	126.26 ± 7.2 [b]	2.15 ± 0.61[f]
	100	48.85 ± 7.27[e]	3.89 ± 0.87[c]	42.73 ± 6.22[c]
	150	52.31 ± 9.31[e]	5.40 ± 1.02[d]	53.52 ± 6.06[d]

(1) Average of 12 isopods in each case. Means within each column followed by the same letter are not significantly different at $P > 0.05$.
(2) Mean Mn concentration in a 0.2 g carrot powder pellet in the control = 1.54 µg (or 7.9 $\mu g\ g^{-1}$ dry wt).

they are retained by the animal until death. However, data presented in Tables 5 and 6 suggest that exoskeleton and hepatopancreas are used as sinks to get rid of excessive quantities of both essential and non-essential metals during ecdysis. Similar suggestions concerning Cu excretion during the moult are made by Alikhan (1972) in P. laevis, and for Ca^{45} by Radu et al. (1971) in Trachconiscus balticus . The necessity to eat exuviae in terrestrial isopods in order to help inflate newly formed soft cuticle provides the additional benefit of reacquiring the essential elements stored in the old cuticle and lost during the ecdysis.
Acknowledgments. The study was supported by a grant from the Natural Science and Engineering Council of Canada.

REFERENCES

Alikhan MA (1972) Changes in the hepatopancreas metabolic reserves of Porcellio laevis Latreille during starvation and the moult-cycle. Am Midl Nat 87:503-514

Alikhan MA, Pani AK (1988) The effect of magnesium and manganese uptake and accumulation on oxygen consumption and ammonia excretion in Porcellio spinicornis Say (Porcellionidae, Isopoda). Arch int Physiol Bioch 96 (in press)
Bagatto G, Alikhan MA (1987) Zinc, iron, manganese and magnesium accumulation in crayfish populations near copper-nickel smelters at Sudbury, Ontario, Canada. Bull Environ Contam Toxicol 38:1087-1081
Elamin OM, Wilcox GE (1986) Effect of magnesium and manganese nutrition on muskmelon growth and manganese toxicity. J Amer Soc Hort. 111: 582-587
Hopkin SP, Martin MH (1982) The distribution of zinc, cadmium, lead and copper within the hepatopancreas of woodlouse. Tiss Cell 14:703-715
Hopkin SP, Martin MH (1984) Heavy metal in woodlice. Symp Zool Soc Lond 53:143 - 166
Joosse ENG, van Vliet LHH (1984) Iron, manganese, and zinc input in soil and litter near a blast - furnace plant and the effects on respiration of woodlice. Pedobiologia 26: 249 - 255
Kleinholz LH, Keller R (1979) Endocrine regulation in Crustacea. In: Barrington AG ed. Hormones and evolution. 1:159-198. Academic Press New York
Radu V G, Tomescu N, Racovità N, Imreh S (1971) Radioisotope researches concerning the feeding and the assimilation of Ca^{45} in terrestrial isopods. Pedobiologia 11: 296 - 303
Simkiss K (1977) Biomineralization and detoxification. Calcif Tiss Res 24:199 - 200
Simkiss K (1983) Lipid solubility of heavy metals in saline solutions. J mar biol Assn UK 63: 1 - 7
Stauber JL , Florence TM (1985) Interactions of copper and manganese: a mechanism by which manganese alleviates copper toxicity to the marine diatom, Nitzschea closterium (Ehrenberg) W. Smith. Aquat Toxicol 7:241- 254
Stevenson JR (1961) Polyphenol oxidase in tegumental glands in relation to the molting cycle of the isopod crustacean Armadillidium vulgare. Biol Bull 121:554-560
Sunda W G, Barber RT, Huntsman SA (1981) Phytoplankton growth in nutrient rich seawater: importance of copper - manganese cellular interaction. J Mar Res 39: 567 - 586
Vallee BL (1970) mettallothionein proteins. In: Neurath H, ed. The proteins. vol. 5: 1-192. Academic Press New York
Williams RJP (1970) The biochemistry of sodium, potassium, magnesium and calcium. Quart Rev Chem Soc 24: 331-365
Received June 8, 1988; Accepted August 26, 1988.