Bull. Environ. Contam. Toxicol. (1989) 43:151–158

Bulletin of Environmental Contamination and Toxicology

Effect of Forced Fasting on Magnesium and Manganese Regulation in a Terrestrial Isopod, *Porcellio spinicornis* Say (Porcellionidae, Isopoda, Crustacea)

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The amount of toxic and non-toxic elements assimilated by primary consumers from their environment depends as much on the form, as on concentration of these elements in the food. In superficially contaminated sites, the majority of elements detected in plant material are present as a blanket deposit of fine particles on leaf surfaces (Hopkin et al. 1986), and these are easily removed as the consumed material passes through the alimentary canal. In contrast, trace metals stored in the plant tissue are not readily available as they have been taken up via roots and are firmly bound within the plant tissue (Wieser 1979). Other factors, such as the feeding behaviour of the consumer, the stage of the moult-cycle (Alikhan 1989), interspecific differences in metal uptake, interference between elements and restriction of leaf uptake due to tolerance (Brown and Martin 1981) all influence the concentration of trace elements in the body tissue of primary consumers.

Earlier studies by Alikhan and Pani (1988) have shown that mean concentrations of magnesium (Mg) and manganese (Mn) in whole woodlice are correlated with levels in their diet. Alikhan (in press) reports that both metals are regulated by terrestrial isopods during their intermoult- and moult-cycles. The present study provides information on the regulation of Mg and Mn tissue concentrations during forced fasting in adult, intermoult male and female Porcellio spinicornis Say (Porcellionidae, Isopoda). Mg, the principal cation in the soft tissues is a well known activator of many enzymes of the glycolytic systems (Williams 1970). Mn, on the other hand, plays a special role in digestive and catabolic processes (Williams 1970).

MATERIALS AND METHODS

Intermoult, 7th growth-stage Porcellio spinicornis, used in the study were reared in the laboratory, in a glass tank on pieces of carrots, at 20°C (±0.5°C) and 85-96 per cent relative humidity. In each experiment,unless otherwise stated, ten isopodsof the same sex and approximately of the same age were weighed, and caged individually at 20°C in 10-cm petri dishes lined with moistened filter papers. The

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age and the growth-stage of the isopod were approximated by the criterion of Alikhan (1972).

Apple powder (208.91 ppm Mg and 2.0 ppm Mn) mixed with analytical grade $MgSO_4.7H_2O$ and $MnSO_4.4H_2O$ (BDH Chemicals, Toronto, Ontario) was used as experimental diets. Macintosh apples were cut into thin slices, oven-dried at 60°C (± 5°C), powdered and mixed with trace metal salts to obtain three experimental dietary concentrations (50, 100 and 150 ppm), excluding control. The mixture was pressed, in a hydraulic press, into 0.2 g pellets. Each isopod was starved for 72 h and then presented with a single food-pellet every 24 hours during the 72 h and 144 h feeding periods. The 72 h feeding period was followed by a 144 h fasting period.

Pellet-residues were removed, oven-dried at 60°C (±5°C) and weighed to nearest 0.01 mg to determine daily food consumption for each isopod. To avoid fungal growth, faecel pellets were also removed and the filter paper was changed daily.

At the conclusion of each fasting and feeding period, 10 males and 10 females were dissected alive, and their hepatopancreas and remaining tissues were placed on dry Whatman (No:41 ashless) filter paper, and oven dried at 60°C (± 5°C) for 12 h. After cooling to room temperature, samples were weighed on a microbalance. Food, faecal and isopod tissue 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 magnesium and manganese contents in a Perkin-Elmer atomic absorption spectrophotometer by the flame method .

Statistical analysis of the data was computed with the aid of a Macintosh XL computer, using Bio∑stat software (Pimentel and Smith 1985, Sigma Soft, Placentia, CA). An initial three-way ANOVA evaluated the 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's test).

RESULTS AND DISCUSSION

The data on the uptake and accumulation of Mg and Mn in whole isopods exposed to various dietary Mg and Mn concentrations for 72 h and 144 h , aswell as at the end of 144 h of forced fasting are presentedHighest Mg accumulation was observed among isopods on a mixture of 500 ppm Mg and 500 ppm Mn for 144 h , and the lowest in those feeding for 72 h on apple powder only (Figs. 1 and 2; Tables 3 and 4). Differences between the males and females, as well as between isopods feeding on apple-powder alone for 72 and 144 h were not significant at $P > 0.05$. This implies that the presence of Mn in the diet has a stimulatory effect on Mg assimilation and accumulation. Williams (1970) suggests that "selective pumping" of Mg^{2+} (along with other cations, like K^+, Na^+ and Ca^{2+}) into both bacterial and higher cells is brought about by the hydrolysis of adenosine triphosphate (ATP) through enzymes called Na^+/K^+ ATP-ases found in the outer cell membrane.

Some of these enzymes, especially those involved with Mg^{2+} and Ca^{2+} transfer into the cell, have been shown to require activation by Mn^{2+} (William, 1970). This mechanism, therefore, may the main reason for relatively greater deposition of Mg into the isopod tissues from diets containing a mixture of Mn and Mg.

The presence of relatively higher Mg concentrations in the remaining tissue is probably a reflection of Mg stored in the exoskeleton. Bagatto and Alikhan (1987) suggest that Mg, because of its physiological functional similarity with Ca, is preferentially stored in the exoskeleton. Williams (1970) reports that in animals

Table 1. Mg and Mn accumulation by the hepatopancreas and the remaining body tissue of a 7th growth-stage intermoult Porcellio spinicornis after 3 days on Mg-enriched diets.

Dietary Mg conc. (ppm)	Tissue	Mg (ppm)	Mn (ppm)
Apple	1(1)	2458.8 ± 193.9	90.1 ± 14.5
	2(2)	1884.9 ± 92.8	24.8 ± 2.7
500	1	2319.3 ± 143.8	64.6 ± 15.3
	2	1867.5 ± 120.1	28.9 ± 3.5
150 Mg + 500 Mn	1	2949.2 ± 475.2	39.1 ± 5.6
	2	3825.8 ± 221.6	49.9 ± 2.0
500 Mg + 500 Mn	1	2593.4 ± 475.2	56.4 ± 4.9
	2	3741.3 ± 154.2	60.8 ± 6.3

Average of 10 males and 10 females in each case.
(1) Hepatopancreas; (2) Remaining body tissue, including exoskeleton.

Table 2. Mg and Mn accumulation by the hepatopancreas and the remaining body tissue of a 7th growth-stage intermoult Porcellio spinicornis after3 days on Mn-enriched diets.

Dietary Mg conc. (ppm)	Tissue	Mg (ppm)	Mn (ppm)
Apple	1(1)	2458.8 ± 194.49	90.1 ± 14.4
	2(2)	1884.9 ± 92.82	24.8 ± 2.6
500	1	1464.3 ± 161.21	94.9 ± 12.7
	2	3568.8 ± 80.46	31.3 ± 2.8
150 mg + 500 Mn	1	1918.1 ± 203.72	87.4 ± 19.7
	2	3820.3 ± 74.51	34.2 ± 0.9
500 Mg + 500 Mn	1	1610.8 ± 287.76	294.9 ± 44.6
	2	3529.7 ± 261.95	187.8 ± 25.7

Average of 10 males and 10 females in each case.
(1) Hepatopancreas; (2) Remaining body tissue, including exoskeleton.

in Fig. 1-4. The data on the tissue distribution of these two trace metals at the end of the two feeding periods are summarized in Tables 1-4.

Table 3. Mg and Mn accumulation by the hepatopancreas and the remaining body tissue of a 7th growth-stage intermoult Porcellio spinicornis after 6-days on Mg-enriched diets.

Dietary Mg conc. (ppm)	Tissue	Mg (ppm)	Mn (ppm)
Apple	1(1)	2134.5 ± 102.6	112.0 ± 11.9
	2(2)	4255.4 ± 271.2	10.7 ± 0.1
500	1	3253.7 ± 263.8	151.7 ± 12.6
	2	18060.1 ± 2322.5	23.1 ± 1.7
150 Mg + 500 Mn	1	3409.2 ± 235.2	95.6 ± 8.6
	2	6123.8 ± 111.4	29.9 ± 2.0
500 Mg + 500 Mn	1	3213.3 ± 276.6	156.4 ± 24.3
	2	11989.6 ± 1569.7	60.7 ± 6.3

Average of 10 males and 10 females in each case.
(1) Hepatopancreas; (2) Remaining body tissue, including exoskeleton.

Table 4. Mg and Mn accumulation by the hepatopancreas and the remaining body tissue of a 7th growth-stage intermoult Porcellio spinicornis after 6 days on Mn-enriched diets.

Dietary Mg conc. (ppm)	Tissue	Mg (ppm)	Mn (ppm)
Apple	1(1)	3214.6 ± 189.06	192.2 ± 16.74
	2(2)	1136.3 ± 98.43	35.8 ± 3.26
500	1	3282.0 ± 112.33	118.8 ± 18.53
	2	7719.3 ± 515.21	53.1 ± 4.79
150 mg + 500 Mn	1	4235.2 ± 212.34	19.1 ± 0.09
	2	1500.7 ± 92.09	2.1 ± 0.02
500 Mg + 500 Mn	1	3211.1 ± 52.34	42.5 ± 1.23
	2	10056.3 ± 324.25	16.0 ± 2.34

Average of 10 males and 10 females in each case.
(1) Hepatopancreas; (2) Remaining body tissue, including exoskeleton.

living in terrestrial and freshwater habitats, K^+ and Mg^{2+} are accumulated preferentially as compared with Na^+ and Ca^{2+}, while in animals living in the sea Na^+ and Ca^{2+} are strongly rejected, Mg^{2+} is somewhat rejected or held closely in balance, while K^+ is accumulated. Elamin and Wilcox (1986) maintain that both Mg and Ca have special affinities to organic sulphonic acid residue ($Ca^{2+} > Mg^{2+}$, through a radius ratio effect) , and as such both are deposited as carbonates and phosphates in high concentrations in the hard external cuticle.

Highest Mn tissue concentrations in isopods on manganese-enriched diets were

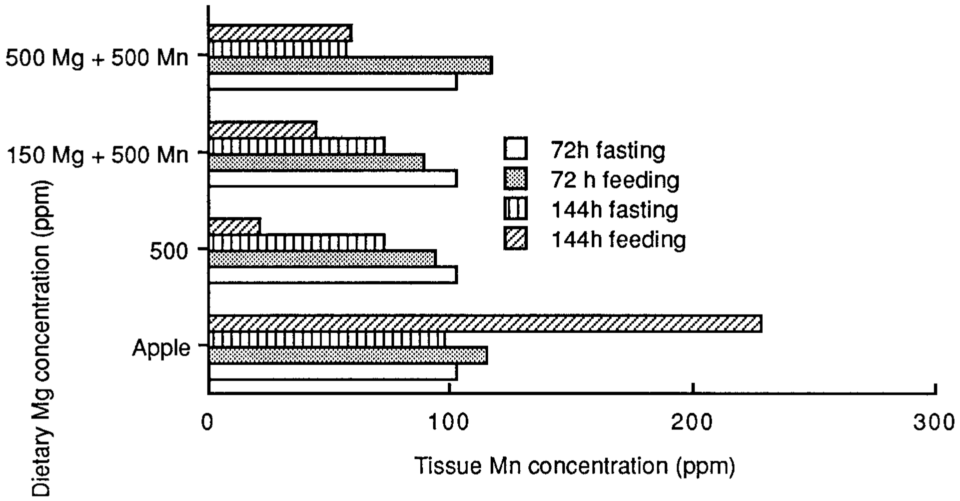

Figure 3.-Effect of various dietary magnesium concentrations on the tissue manganese levels in a 7th growth-stage intermoult Porcellio spinicornis. Each bar in the figure represents an average of 20 (10 males + 10 females) isopods. Apple = 217.6 μg Mg g^{-1} dry wt.

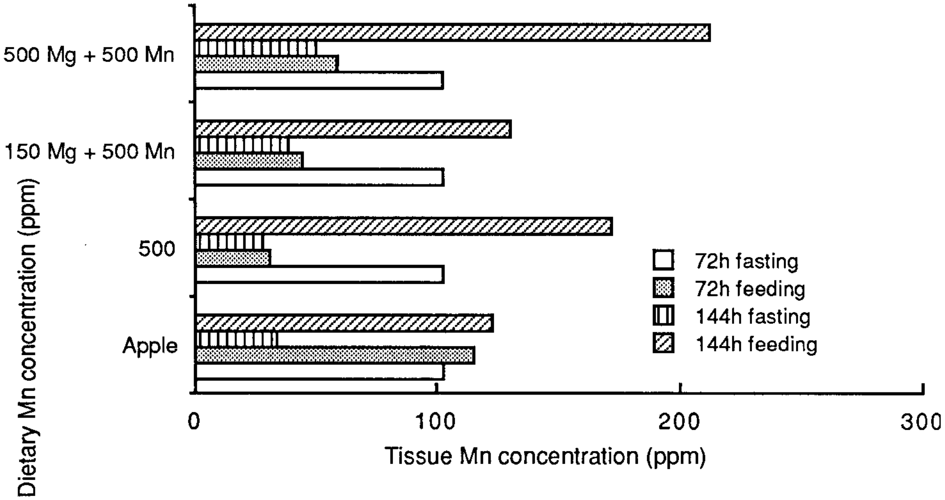

Figure 4.-Effect of various dietary manganese concentrations on the tissue manganese levels in a 7th growth-stage intermoult Porcellio spinicornis. Each bar in the figure represents an average of 20 (10 males + 10 females) isopods. Apple = 2.0 μg Mg g^{-1} dry wt.

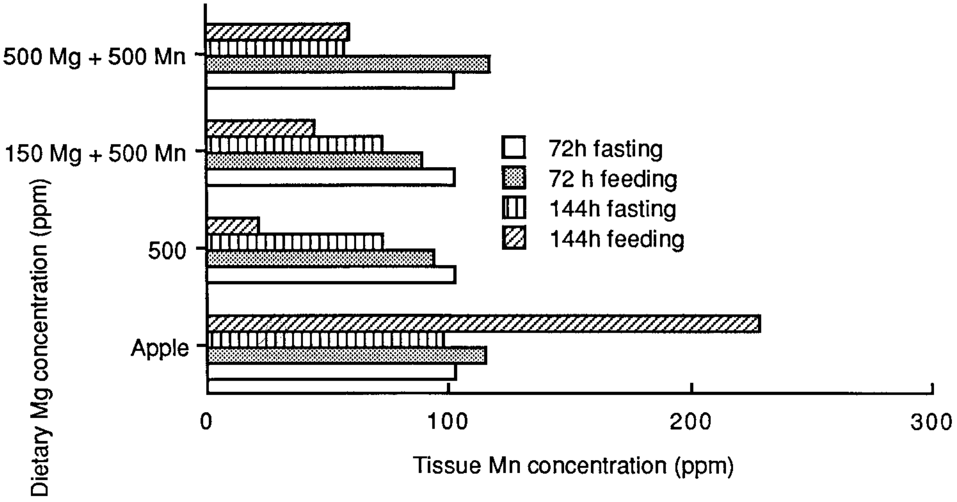

Figure 3.- Effect of various dietary magnesium concentrations on the tissue manganese levels in a 7th growth-stage intermoult Porcellio spinicornis. Each bar in the figure represents an average of 20 (10 males + 10 females) isopods. Apple = 208.9 µg Mg g^{-1} dry wt.

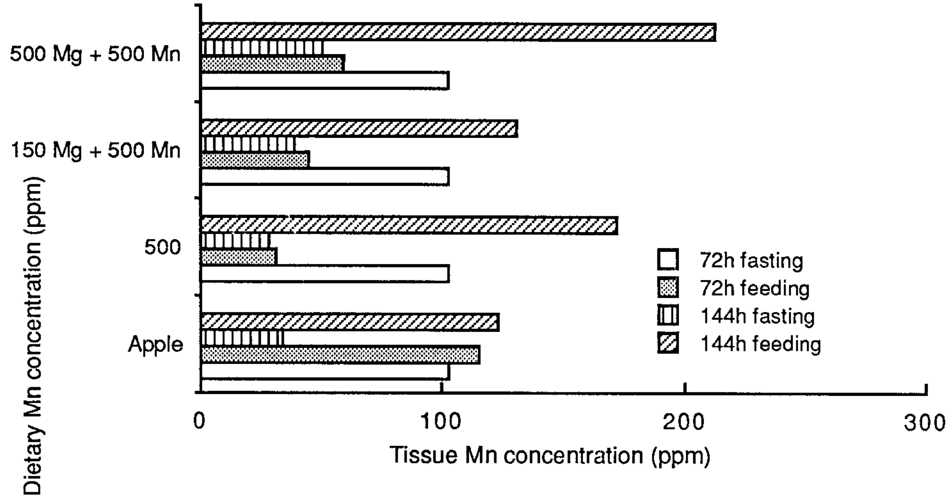

Figure 4- Effect of various dietary manganese concentrations on the tissue manganese levels in aaverage of 20 (10 males + 10 females) isopods. Apple = 2.0 µg Mn g^{-1} dry wt.

recorded for those in the control (apple powder alone) (Fig. 3), and lowest in those feeding for 144 h on the 500 ppm Mg diet (Fig. 3). Highest concentrations of Mn were found in the hepatopancreas, and the least in the remaining tissues including exoskeleton (Table 4). This is not surprising since Mn is known to be active in a number of digestive and catabolic enzymes (Bagatto and Alikhan 1987). The presence of Mn in the remaining tissue may be related to its ability to compliment or substitute for Mg (Bagatto and Alikhan 1987). This replacement is probably successful because the chemistries of Mg and Mn, as opposed to most other bivalent cations of the first transition series, are rather similar (for complete Discussion, see Williams 1970).

Forced fasting of 144 h depleted both Mg and Mn stores, and the decrease was relatively higher in Mn stores. Lowest Mg concentration among 144 h starved isopods were recorded in those on 1:1 mixture of Mg and Mn (Fig. 2), and highest in the control (Figs. 1 and 2). Similarly, highest Mn lose was recorded in isopods in the control (Fig. 1) and those on 1:1 Mg-Mn mixture (Fig. 3), and lowest in those feeding on 3:10 Mg-Mn mixture (Fig. 3 and 4). Under conditions of food deprivation in Armadillidium vulgare, according to Storch (1984), the hepatopancreatic cells, with the exception of those in the regenerative zone (terminology of Alikhan 1969) show the presence of autolysosomes, and a general deterioration and disintegration of the cytoplasm. Other cells, according to Papathanassiou and King (1984) undergo changes associated with a lack of amino acid incorporation and protein synthesis. Under these circumstances, some of the Mg and Mn stored in the hepatopancreatic tissue may be released and excreted out. However, with the advent of re-feeding, the hepatopancreatic cells show enlargement of mitochondria with light cristae (Storch 1984) and concentric whorls in the ergastoplasm (Papathanassiou and King 1984), both associated with hyperactivity of the cell. At the conclusion of the 144 h re-feeding period, highest Mg concentration was recorded in isopods on 500 ppm Mg diet (Table 4) and lowest in the control (Table 3 and 4). Highest Mn concentration, on the other hand, was observed in isopods feeding on 1:1 Mg-Mn mixture (Table 3), and lowest in those on 3:8 Mg-Mn mixture (Table 4). This implies that isopods, inspite of cellular loses during starvation, are capable of re-building their Mg and Mn stores during re-feeding. Mg and Mn not required for normal metabolic purposes and stored in the carapace are easily lost through exuviae during the normal moult-cycle (Alikhan 1989).

Mn, according to Williams (1970), is not a very good match for Mg as judged by its radius, but nickel would be a much closer fit. Nickel and Mg have very similar geometric demands for they both have a strong tendency to octahedral geometry. Nickel absorption into the crustacean carapace, according to Mierle (1981), is not a true intracellular uptake, but probably represents amounts bound at the cell surface. It is probable that most of the cuticular Mg is also an extracellular accumulation. However, more analytical work will be required to confirm this contention.

Acknowledgment. The study was supported by a grant from the Natural Sciences and Engineering Council of Canada. The visit of the senior author (K.B.) to the Laurentian University was partially funded by the Weiller Foundation of Israel.

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Received September 21, 1988; Accepted January 10, 1989.