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Some Factors Influencing Cadmium–Manganese Interaction in Adult Rats

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Our recent data show that even a low dose of cadmium (20 µg/day/rat) significantly suppresses manganese transduodenal transport when administered during a three-day period (Gruden, 1987). The inhibitory effect of cadmium upon manganese absorption is enhanced by concurrently administered iron-fortified milk diet (Gruden and Munić, 1987). This suggests that the (synergistic) action of cadmium and iron upon manganese and the competition between these (three) ions in the intestine depend on their relative concentrations and affinity for the binding sites within the intestinal mucosa. For this reason we considered it worthwhile examining whether this inhibitory effect of cadmium would be affected by simultaneously administered manganese-fortified milk. Since the absorption of heavy metals and, at the same time, the demand for manganese is higher in the young than in the old animals (Jugo, 1977; Baly et al. 1985; Zidenberg-Cherr et al. 1985) we also studied how this interaction depends upon the animals' age and sex and whether it is the same in the whole small intestine.

MATERIALS AND METHODS

Two types of experiments were performed in which the experimental procedure was slightly different:
Group 1/ Experiments were performed on 6- and 26-week-old albino rats of both sexes. One half of the animals received 0.2 mg cadmium by gastric intubation for three days, while the other half (controls) received distilled water in the same manner. For this period all the animals were fed on cow's milk instead of on a stock diet. All animals were given milk ad libitum.
Group 2/ Six week-old female albino rats of 100 to 130 g body weight were used. The animals were separated

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into groups of ten animals according to the cadmium dose they received by gastric intubation for three days (0.002, 0.02, 0.2 and 2.0 mg Cd/d/rat), and according to the amount of manganese in their milk diet: 0.64, 1.07, 2.14 and 4.28 mg Mn (in the form of chloride)/100 ml cow's milk. The controls were fed plain cow's milk and received no cadmium.

On the 4th day of the experiments all the animals were killed and a segment of the duodenum was processed by the "everted gut sac" method (Wilson and Wiseman,1954). In ten 6-week-old females (Group 1) a segment of jejunum (20 cm distantly from the duodenum) and a segment of ileum (20 cm above the ileocaecal connection) from each rat was also processed. Samples were incubated in a modified Krebs-Ringer solution to which $MnCl_2$ labelled with Mn-54 was added. The same K.R. solution without manganese was inside the tied intestinal segment. The manganese-54 content in the serosal and mucosal solutions and its retention in the intestinal wall were determined after incubation. The results were calculated as S/M (serosal over mucosal) activity ratios for ion transport, and as percentages of the initial mucosal solution activity for its intestinal retention. Student's t-test was used to calculate the statistical significance of the differences between the groups within the same experiments.

To facititate comparison between the control and experimental groups, the results for the latter are presented as percentages of the corresponding control values taken as 100 per cent.

RESULTS AND DISCUSSION

The inhibitory effect of cadmium on manganese transfer was significant throughout the small intestine, but it was highest in its most proximal parts: manganese-54 transfer was 60, 20 and 30 per cent lower in the duodenum, jejunum and ileum, respectively, in the cadmium treated animals than in corresponding controls (Table 1). However, there was no relationship between manganese retention within the intestinal wall and the particular parts of the intestine. As in earlier iron-manganese interaction studies (Gruden and Munić,1987), the inhibitory effect of cadmium upon manganese transfer was more pronounced in the young than in old animals. Manganese transfer was 85 per cent lower in the 6-week-old and 48 and 12 per cent lower in the 26-week-old cadmium treated animals (Table 1). This transport of manganese was the same in male and female 6-week-old animals but the inhibitory effect of cadmium was more pronounced in the older females than in older male rats, although more dependent upon the animals' age than on their sex.

Table 1. The effect of cadmium on manganese-54 transfer and intestinal retention in 6- and 26-week-old rats of both sexes*

Animals' Sex	Age (weeks)	Duodenum	Jejunum	Ileum
TRANSFER OF MANGANESE-54**				
F	6	14.15 ± 1.04^{s}	79.32 ± 6.88^{s}	69.76 ± 7.12^{s}
	26	52.63 ± 5.92^{s}		
M	6	15.00 ± 1.96^{s}		
	26	88.00 ± 9.00		
RETENTION OF MANGANESE-54				
F	6	80.67 ± 4.32^{s}	103.06 ± 5.61	93.25 ± 3.89
	26	84.51 ± 4.14^{s}		
M	6	73.92 ± 3.80^{s}		
	26	91.74 ± 4.72		

*All rats were given 0.2 mg cadmium daily by gastric intubation for three days.

**Data for experimental animals presented as percentages of the corresponding controls - taken as 100 per cent (Mean/$\bar{x}_{10}$/$\pm$ S.E.)

[s]Values bearing the superscript letter are significantly different from the corresponding control values ($P<0.05$).

Comparing the inhibitory effect of cadmium with that of iron in our studies (Gruden and Munić, 1987) the inhibitory effect of the former was much greater than that of iron upon manganese transfer. This distinction between iron and cadmium is also evident in that the inhibitory effect of iron upon manganese absorption is significant only in the proximal parts of the small intestine (Gruden, 1987). The data require consideration when dealing with cadmium, especially at a young age.

As in our previous experiments (Gruden, 1987; Gruden and Munić, 1987) the dose of 0.2 mg Cd/d/r was the critical one. This dose of cadmium always significantly decreased manganese-54 transfer (by up to 75 per cent) irrespective of the manganese concentration in the milk diet, as did a higher dose of 2.0 mg Cd/d/r (Table 2).

Table 2. The effect of cadmium and manganese doses upon manganese-54*:

Mn** ↓ / Cd →	0.002	0.02	0.2	2.0	Mn alone°°
duodenal transfer of manganese-54					
0.64	98.1±10.0	128.1±20.1	22.2±2.3^{s}	34.6±3.4^{s}	83.2±9.1
1.07	170.3±30.7^{s}	153.7±18.2	32.2±4.9^{s}	29.2±2.2^{s}	69.3±7.1^{s}
2.14	143.3±17.2^{s}	120.4±11.1	33.4±4.1^{s}	36.1±3.7^{s}	88.0±9.5
4.28	89.1±13.2	121.2±13.2	27.6±3.0^{s}	34.8±2.8^{s}	70.5±14.1
intestinal retention of manganese-54					
0.64	95.4±2.5^{s}	99.1±2.7	79.2±2.5^{s}	60.2±3.7^{s}	91.1±1.9^{s}
1.07	97.5±2.0	101.3±3.1	83.7±2.1^{s}	60.2±3.9^{s}	86.7±1.1^{s}
2.14	97.2±1.8	98.2±3.8	88.8±2.7^{s}	68.1±2.2^{s}	91.2±1.9^{s}
4.28	90.2±2.1^{s}	97.5±3.0	80.6±4.0^{s}	60.5±3.1^{s}	86.2±2.6^{s}

*Data for experimental animals presented as percentages of the corresponding controls - taken as 100 per cent (Mean/$\overline{x}_{10}$/±S.E.)

**Cd=mg/d/rat;

**Mn=mg/100 ml milk

^{s}Values bearing the superscript letter are significantly different from the corresponding control values (P<0.05).

°°Yet unpublished results.

Both cadmium and manganese, administered separately, inhibit the transport and intestinal retention of manganese-54 (Gruden, 1987; and Table 2. the last column). However, in combination, these two ions affect manganese-54 less than cadmium given alone, i.e. their action upon manganese metabolism is not a synergistic one, as distinct from that of the cadmium-iron combination which inhibited manganese absorption synergistically (Gruden and Munić, 1987). A "saturation" effect of cadmium is observed at all levels of manganese when the cadmium dose was kept constant at 0.2 or 2.0 mg/d/r so that the variation of manganese concentration in the milk diet from 0.64 to 4.28 mg/100 ml did not influence significantly either manganese transfer or its intestinal retention at these two higher cadmium doses. Lower cadmium doses (0.02 and 0.002 mg Cd/d/r) generally increased manganese-54 transfer (by up to 70 per cent) leaving the intestinal retention unchanged. No critical manganese concentration was observed for such an effect. This, stimulatory action of low cadmium doses on manganese was observed in earlier studies (Gruden, 1987), and on iron transfer (Gruden and Munić, 1987), and even with larger doses of cadmium, on calcium transfer (Gruden, 1977). It appears that low doses of cadmium (0.002 and 0.02 mg/d/r) do not affect the action of the other ions on manganese metabolism. Iron, either provokes or increases the inhibitory effect of cadmium (Gruden and Munić, 1987), while manganese either decreases the inhibitory action at the high doses of cadmium, or even stimulates manganese-54 transfer at low doses of cadmium (Gruden, 1987 vs. Table 2). Recently reported results suggest that a non toxic dose of manganese exhibits some protective action against cadmium toxicity by decreasing its gastrointestinal absorption (Fischer, 1983; Sarhan et al. 1986). It might be that, as Goering and Klaassen (1985) have assumed, this protective effect of manganese results from an increased synthesis of metallothionein provoked by manganese. Nevertheless, it does not explain why manganese alone, irrespective of its concentration, decreased manganese-54 transfer by up to 30 per cent, while in combination with 0.002 or 0.02 mg of cadmium it even stimulates radiomanganese transfer (Table 2).

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