Effect of Dietary Calcium and Phosphorus on ^{54}Mn Metabolism Following Single Tracer Intraperitoneal and Oral Doses in Rats[1] (36140)

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Numerous studies have shown a relationship between manganese and other nutrients (1–4), especially calcium and phosphorus (2, 5, 6). Generally it has been assumed, but not proven, that the effects were in the digestive tract and not in the tissues (7, 8). This study was to determine whether dietary calcium and phosphorus level affected ^{54}Mn metabolism at the tissue level as well as in the intestine.

Materials and Methods. In the first experiment, 24 weanling male Cherokee S-D albino rats, initially averaging 78 g, were fed diets containing low (0.1%) or normal (0.6%) calcium and normal (0.4%) or high (0.9%) phosphorus in a two-by-two factorial design. The basal diet is shown in Table I. Calcium was varied in the diets by substituting calcium carbonate for corn and phosphorus by replacing corn with H_3PO_4. By analysis, the diets contained 0.09 or 0.62% calcium, 0.40 or 0.94% phosphorus and 59 ppm manganese. The rats were maintained in stainless steel cages and individually fed 10 g of the diet daily in glass containers plus distilled water *ad lib* in glass containers having stainless steel sipper tubes. After receiving the diets for 46 days, 1 μCi of carrier-free ^{54}Mn as the chloride in acetate buffer, was administered to each rat intraperitoneally and feces and urine quantitatively collected for 4 days. At this time the rats were killed with ether for determination of tissue ^{54}Mn.

In a second experiment, 24 male albino rats of the same stock, initially averaging 127 g in weight were used. They were fed the same diets in the same manner as in the first experiment for 21 days prior to oral administration of 3.90 μCi of ^{54}Mn by gavage. After dosing, feces and urine were quantitatively collected for three days at which time the rats were sacrificed for determination of tissue ^{54}Mn.

TABLE I. Composition of Basal Diet.

Ingredient	Percent
Ground shelled corn	72.63
Soybean meal	23.60
Corn oil	3.00
Salt	0.60
Minerals[a]	+
Vitamin mix[b]	0.17

[a] Furnished in mg/kg of the complete diet: Mn, 47; Zn, 55; Fe, 40; Mg, 301; Cu, 4.4; I, 0.74; Co, 0.33.

[b] Furnished per kg diet: riboflavin, 2.2 mg; calcium pantothenate, 4.4 mg; niacin, 9.9 mg; choline, 99 mg; vitamin A, 2200 iu; vitamin D-3, 880 iu; vitamin B-12, 6.6 μg; Santoquin, 125 mg.

The ^{54}Mn concentration of the samples was determined with an automatic gamma test tube changer system with a NaI (Tl) well crystal[2]. Stable manganese in the liver was determined by atomic absorption spectrophotometry after nitric–perchloric–sulfuric acid wet ashing of samples.

Results. The low dietary calcium level increased fecal and urinary excretion of the administered ^{54}Mn (Table II). Rats that had been given the low calcium diets for 46 days excreted twice as much ^{54}Mn in feces and in urine during the 4 days as did those fed the normal calcium diets. Dietary phosphorus level had no significant ($p \geqq .05$) effect on fecal ^{54}Mn excretion or tissue ^{54}Mn concentrations. However, within each dietary calcium level the urinary ^{54}Mn excretion of

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[2] Manufactured by Baird Atomic, Cambridge, MA.

TABLE II. Effect of Dietary Calcium and Phosphorus on Total Fecal and Urinary Excretion of Intraperitoneally Administered ^{54}Mn During Four Days After Dosing.[a]

		% of ^{54}Mn dose excreted			
		Phosphorus level			
Item	Calcium level	0.4%	0.9%	Mean	SE[b]
Feces[c]	0.1%	55.7	54.9	55.3	
	0.6%	30.4	23.5	27.0	
	Mean	43.0	39.3		
					3.1
Urine[d]	0.1%	0.11	0.03	0.07	
	0.6%	0.05	0.01	0.03	
	Mean	0.08	0.02		
					0.017

[a] Each value represents an average of 6 animals.
[b] Standard error; $N = 6$.
[c] Significant ($p < .01$) difference between calcium levels.
[d] Significant ($p < .01$) difference between phosphorus levels and ($p < .05$) between calcium levels.

rats given high phosphorus diets was only about one-fourth that of rats given normal phosphorus diets. The low dietary calcium level greatly and differentially reduced tissue ^{54}Mn concentration (Table III). Upon sacrifice 4 days after dosing, the livers of rats given the low calcium level had only one-seventh as much ^{54}Mn as livers of rats receiving the normal calcium diet. Spleen, heart, lung and adipose tissues were significantly affected but to a smaller degree.

TABLE III. Effect of Dietary Calcium and Phosphorus on Tissue Concentrations of Intraperitoneally Administered ^{54}Mn Four Days After Dosing.[a]

	% of ^{54}Mn dose/g of dry tissue		
Tissue	Low (0.1%) calcium	Normal (0.6%) calcium	SE[b]
Liver	2.80	20.58[c]	.57
Spleen	4.75	11.62[c]	.92
Heart	0.92	1.36[d]	.11
Kidney	4.38	5.78	.60
Testis	3.94	4.76	1.11
Tibia	0.82	0.36	.16
Lung	0.90	1.39[d]	.15
Adipose	0.33	1.03[c]	.15

[a] Each value represents an average of 12 animals, with 6 on normal P (0.4%) and 6 on high P (0.9%). There were no significant differences due to P levels and no significant interactions between P levels and Ca levels.
[b] Standard error; $N = 12$.
[c] Significant ($p < .01$) difference between calcium levels.
[d] Significant ($p < .05$) difference between calcium levels.

In the second experiment the low dietary calcium level significantly increased fecal and urinary excretion of the orally administered ^{54}Mn (Table IV). As determined by difference from excretion data during the three-day period after dosing, only about half as much ^{54}Mn was retained by rats fed low calcium diets for 3 weeks as by those receiving normal calcium diets. Tissues of rats receiving low calcium contained as little as one-third that of rats receiving normal calcium with the greatest effect in the liver (Table V). Stable manganese in the liver was significantly ($p<.05$) higher in rats given low calcium diets (15 μg/g D.M.) than in those fed normal calcium (12 μg/g).

TABLE IV. Effect of Dietary Calcium and Phosphorus on Total Excretion of Orally Administered ^{54}Mn into Feces and Urine During Three Days After Dosing.[a]

		% of ^{54}Mn dose excreted		
		Phosphorus level		
Item	Calcium level	0.4%	0.9%	Avg
Feces[b]	0.1%	89.0	98.4	93.7[c]
	0.6%	80.4	82.2	81.4
Urine[b]	0.1%	0.107	0.108	0.108[c]
	0.6%	0.040	0.021	0.028

[a] Each value represents an average of 5 animals except feces and urine on normal calcium–high phosphorus was 6 animals and urine on normal calcium–low phosphorus was 4 animals.
[b] Standard error, $N = 5$ was 4.9% for feces and 0.036% for urine.
[c] Significant ($p < .05$) difference between calcium levels.

In rats fed the high phosphorus level, the low calcium diet increased the ^{54}Mn concentrations of the walls of the jejunum, ileum, cecum and colon. High dietary phosphorus significantly increased ^{54}Mn concentrations

TABLE V. Effect of Dietary Calcium and Phosphorus on Tissue Concentrations of Orally Administered ^{54}Mn Three Days After Dosing.[a]

	% of ^{54}Mn dose/g of dry tissue				
	Low (0.1%) calcium		Normal (0.6%) calcium		
Tissue	Normal P (0.4%)	High P (0.9%)	Normal P (0.4%)	High P (0.9%)	SE[b]
Liver[cf]	0.066	0.178	0.363	0.487	0.058
Spleen[cd]	0.028	0.052	0.079	0.187	0.008
Heart[cf]	0.020	0.030	0.057	0.067	0.006
Kidney[cd]	0.085	0.163	0.235	0.277	0.023
Lung[c]	0.060	0.021	0.037	0.074	0.015
Tibia[c]	0.008	0.009	0.015	0.016	0.002
Testis[ef]	0.042	0.078	0.082	0.101	0.014
Adipose	0.003	0.004	0.003	0.002	0.001
Stomach[cd]	0.074	0.144	0.159	0.210	0.017
Duodenum[ef]	0.066	0.184	0.208	0.222	0.029
Jejunum[f]	0.051	0.242	0.110	0.124	0.036
Ileum	0.097	0.422	0.194	0.153	0.097
Cecum[ef]	0.161	0.448	0.089	0.099	0.055
Colon	0.180	0.675	0.208	0.200	0.131

[a] Each value represents an average of 5 animals, except all tissues other than spleen on normal Ca–high P were 6 animals and lung tissue on normal Ca–normal P was 3 animals.

[b] Standard error; $N = 5$.

[c] Significant ($p < .01$) difference between calcium levels. [d] Significant ($p < .01$) difference between phosphorus levels. [e] Significant ($p < .05$) difference between calcium levels. [f] Significant ($p < .05$) difference between phosphorus levels.

in liver, spleen, heart, kidney, testes, stomach, duodenum, jejunum and cecum (Table V).

Discussion. With ip as well as orally administered ^{54}Mn, low calcium diets greatly affected ^{54}Mn metabolism, thus showing that manganese metabolism after absorption is influenced by the calcium content of the diet. The effects on tissue ^{54}Mn concentrations were in the same direction with both ip and oral dosing, thus, the general assumption that manganese metabolism is affected only in the gut is not valid (2, 5, 6). No doubt this assumption has been a factor in the interpretation of results which did not differentiate between effects on absorption and those on subsequent Mn metabolism. Research to define where the effect lies has been needed. Apparently, the paper by Cotzias and Greenough (9) has had a profound influence on the thinking of people studying manganese metabolism. However, in their study elements other than Mn were injected and not a part of the diet for weeks prior to ^{54}Mn injection. Recent studies show that dietary minerals are metabolized very differently from injected ones (10, 11).

The mechanism whereby differences in dietary calcium cause the major effect on manganese metabolism at the tissue level was not established. Thus, whether the effects are directly due to dietary calcium *per se* or primarily to changes in tissue levels of stable manganese or other constituents as influenced over a period of time by dietary calcium or its relationship to secondary hyperparathyroidism is unknown. The much lower ^{54}Mn content in liver and other vital organs in rats fed the low calcium diet may have been due to fewer manganese binding sites being available. The higher stable manganese in the liver of low calcium fed rats is consistent with this concept. Normally, manganese content of tissues is not very variable (12). However, since manganese metabolism is so greatly affected by many other nutrients,

even small variations in tissue Mn may materially affect Mn metabolism. The ^{54}Mn concentration was increased in the lower gut walls by the low calcium diet in orally dosed rats, especially in those fed the high phosphorus level. This effect, which is opposite to that in the vital organs such as liver, may have been due either to a higher ^{54}Mn uptake, or to a slower release rate. Miller *et al.* have shown that after uptake the release rate from the intestinal wall is extremely rapid (Miller, *et al.*, unpublished).

Summary. The effects of dietary calcium [0.1% (low) or 0.6% (normal)] and phosphorus [0.4% (normal) or 0.9% (high)] on ^{54}Mn metabolism were determined following single ip or oral dosing in weanling rats. Rats receiving the low calcium diet excreted much more ^{54}Mn from both ip and oral doses via feces and urine, and their tissues retained much less. Within each dietary calcium level, urinary excretion of ip administered ^{54}Mn was far less on the high phosphorus diet. With orally administered ^{54}Mn, high dietary phosphorus increased ^{54}Mn tissue concentrations. These results show that low dietary calcium has a major effect on manganese metabolism at the tissue level as well as within the gut.

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