The Effect of Dietary Protein Source on Manganese Bioavailability to the Rat (43140)

PHYLLIS E. JOHNSON AND EUGENE D. KORYNTA
United States Department of Agriculture, ARS Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota 58202

Abstract. **Rats were fed diets containing 20% protein from casein, beef, chicken, tuna, or soybean. All diets contained 15% fat and were supplemented with limiting amino acids as necessary to meet National Research Council requirements. In Experiment 1, the manganese content of all diets was the same; manganese content was 5 mg/kg. In Experiment 2, a basal (adequate) level of minerals was provided in each diet but total mineral content varied depending on the contribution of the protein source; manganese was added to achieve a concentration of 5 mg/kg. In both experiments, ^{54}Mn absorption was greatest from tuna (8.54% and 7.71%) and least from beef (4.57% and 4.14%) ($P < 0.0001$). In both experiments, biologic half-life of ^{54}Mn was longest in rats fed beef (18.5 and 26.9 days) and shortest in rats fed soy (14.5 and 16.2 days) ($P < 0.0002$). Except for beef, biologic half-life was similar for dietary groups between the two experiments. In Experiment 1, only kidney manganese concentration was significantly affected by diet and was highest in soy-fed animals. In Experiment 2, plasma, kidney, and liver manganese were all significantly affected by diet and were highest in soy-fed animals and lowest in beef-fed animals.** [P.S.E.B.M. 1990, Vol 195]

The effect of meat or animal foods on manganese bioavailability has been little studied, probably because foods of animal origin are poor sources of manganese. However, there are reports that the addition of meat to vegetarian or meat-free diets improved manganese balance in humans (1, 2). Conflicting results have been reported concerning the effect of feeding soy-based feeds on manganese absorption and retention compared with the effect of feeding casein-based feeds. Manganese absorption was lower from soy-based infant formula than from human milk, cow's milk and cow's milk-based infant formulas in suckling rats (3). We found that manganese was more available to weanling rats from a soy-based diet than from a casein-based diet (4). The difference was not related to the differences in methionine, arginine, or phytate content of the two diets. This study was designed to extend the comparison between soy diets and diets based on animal protein to include meat, poultry, and fish as protein sources.

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Methods

The protein sources in these experiments were chicken, beef, tuna, soy, and casein. Skinless, boneless chicken breasts, extra-lean ground beef, and canned tuna in water were purchased from a local store. High protein casein and soy protein were purchased from Teklad (Madison, WI[1]).

The ground beef was cooked at 350°F for 1 hr, crumbled, and freeze-dried. The tuna was drained and freeze-dried; the chicken was cooked at 350°F for 1 hr, cubed, and freeze-dried. Samples of each protein were powdered and wet digested for mineral analysis (Table I) as described below. In Experiment 1, mineral mixes were formulated for each diet (Table II) so that the final mineral content was similar in all diets. In Experiment 2, mineral levels in the diets were allowed to vary depending on the mineral content of the protein source; one exception was that a basal amount of manganese (5 mg/kg of diet) was provided to equalize the manganese content of all diets.

Diets were formulated to contain 20% protein, 15% fat, 5% fiber, and 53–54% carbohydrate (Table III). Diets were supplemented with amino acids as

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

necessary to meet the requirements of the rat as defined by the National Research Council (NRC) (5). Corn oil was added to some diets to equalize the total fat content, but no attempt was made to equalize the amounts of saturated and unsaturated fats in the various diets. Fat and amino acid composition of the proteins used were obtained from published sources (6–9) or from Teklad, and these data were used in calculating amino acid and fat supplements for each diet.

Male weanling Long-Evans rats (Harlan Sprague-Dawley, Indianapolis, IN) were used in these experiments. In each experiment, there were five groups of rats with eight rats/group. In Experiment 1, the mean weight was 57.4 ± 0.3 g, and in Experiment 2, it was 59.6 ± 1.10 g, at the start of the experiment. Food consumption was monitored to ensure that all rats were eating approximately the same amounts of diet. Rats were housed individually in stainless steel wire cages in a temperature-controlled area with 12-hr light:dark cycles. Demineralized water was available at all times, and diets were fed *ad libitum*. Weight gain was recorded weekly.

After 2 weeks on their respective diets, rats were fed radioactive test meals composed of 4 g of diet with 3 μCi of ^{54}Mn (DuPont, NEN Research Products, Boston, MA) per rat. Test meals were fed after an overnight fast.

By using a custom-built small animal whole-body counter equipped with a ND62 multichannel analyzer (Nuclear Data Instrumentation, Schaumburg, IL), whole body ^{54}Mn of each rat was measured 2 hr after the test meal was administered. Four to five hours after ^{54}Mn administration, rats were fed their experimental diets *ad libitum*. Retention of ^{54}Mn was measured for each rat for 21 days at 2-day intervals. The multichannel analyzer was calibrated with ^{137}Cs. Radioactivity of ^{54}Mn was measured between 694 and 974 keV which includes the gamma peak of ^{54}Mn (834 keV). The measured radioactivity was corrected for background and decay.

Percent apparent absorption of ^{54}Mn was calculated by extrapolating the linear portion of a plot of ln (percentage of retention) versus time from days 11 to 22 after ^{54}Mn administration (10). The excretion rate of ^{54}Mn was expressed as the biologic half-life (BH), which was calculated by using the slope of the linear portion of the same plot, with the following equation:

$$BH = \frac{-\ln 2}{\lambda_b}$$

where λ_b is the slope of the linear portion of a plot of ln versus time. Apparent absorption was not corrected for endogenous excretion by using a similar ^{54}Mn retention curve for injected ^{54}Mn, because the slopes of retention curves for orally administered and injected

Table I. Mineral Content of Protein Sources and Total Diets[a]

Diets	Protein sources					Experiment 1					Experiment 2				
	Manganese	Zinc	Iron	Magnesium	Copper	Calcium	Manganese	Zinc (mg/kg)	Iron	Magnesium	Copper	Calcium	Manganese	Zinc (mg/kg)	Iron	Magnesium	Copper
				(μg/g dry wt)													
Beef	0.71	168	67	555	4.83	4990	4.72	50.2	35.9	443	4.00	6032	4.96	78.8	71.9	722	6.29
	± 0.13	± 5	± 0	± 0	± 0.41	± 458	± 0.37	± 4.2	± 2.7	± 38	± 0.58	± 142	± 0.16	± 9.5	± 6.4	± 31	± 0.22
Chicken	0.45	21	12	923	1.92	5065	5.30	55.0	39.3	444	4.58	6026	5.02	23.9	47.1	746	6.18
	± 0.24	± 1	± 2	± 88	± 1.49	± 46	± 0.16	± 0.9	± 0.8	± 22	± 0.89	± 68	± 0.14	± 3.6	± 1.1	± 9	± 0.30
Tuna	0.45	23	38	834	2.28	4898	4.42	50.1	35.8	392	4.34	5725	4.52	21.6	51.9	686	5.75
	± 0.20	± 1	± 4	± 24	± 0.04	± 283	± 0.88	± 2.9	± 4.0	± 35	± 0.42	± 326	± 0.20	± 2.1	± 3.3	± 46	± 0.21
Soy	9.61	35	150	339	14.7	5549	5.16	58.6	37.9	461	5.04	5953	6.94	21.9	76.0	581	8.87
	± 0.61	± 3	± 15	± 28	± 1.1	± 78	± 0.10	± 0.9	± 1.1	± 8	± 0.11	± 208	± 0.18	± 0.5	± 2.8	± 24	± 0.36
Casein	0.28	41	4	14	2.28	4975	5.52	59.3	40.7	429	4.70	5072	4.43	21.6	38.6	474	5.41
	± 0.12	± 1	± 3	± 0	± 0.98	± 193	± 0.10	± 1.0	± 3.3	± 4	± 1.35	± 175	± 0.30	± 0.6	± 1.4	± 20	± 0.35

[a] Values represent mean ± SD.

Table II. Composition of Mineral Mixes in Experiment 1

	Diet (g/kg)				
	Beef	Chicken	Tuna	Soy	Casein
$CaHPO_4$	500	500	500	500	500
NaCl	74	74	74	74	74
Potassium citrate	220	220	220	220	220
K_2SO_4	52	52	52	52	52
MgO	15.1	14.0	16.0	20.3	23.8
$MnCO_3$	0.33	0.34	0.34	0.20	0.34
Ferric Citrate	2.1	5.5	4.7	0.10	6.0
$ZnCO_3$	0	2.90	2.90	2.70	2.70
$CaSO_4 \cdot 5H_2O$	0.40	0.5	0.5	0.2	0.5
KIO_3	0.01	0.01	0.01	0.01	0.01
$Na_2SeO_3 \cdot 5H_2O$	0.01	0.01	0.01	0.01	0.01
$CrK(SO_4)_2 \cdot 12H_2O$	0.55	0.55	0.55	0.55	0.55
Sucrose	135.50	130.19	128.99	129.93	120.09

Table III. Composition of Diets

	Composition (g/kg)				
	Beef	Chicken	Tuna	Soy	Casein
Beef	338.4	—	—	—	—
Chicken	—	226.0	—	—	—
Soy[a]	—	—	—	230.4	—
Tuna	—	—	200.0	—	—
Casein[a]	—	—	—	—	200.0
Corn Oil[b]	18.0	127.0	146.5	150.0	150.0
Cornstarch[c]	150.0	150.0	150.0	150.0	150.0
Sucrose[d]	385.6	388.5	395.5	368.1	402.0
Cellulose[a]	50.0	50.0	50.0	50.0	50.0
Glutamic Acid[e]	8.5	10.0	10.0	—	—
D-methionine[a]	2.5	1.5	1.0	4.5	1.0
Choline chloride[f]	2.0	2.0	2.0	2.0	2.0
AIN-76 Vitamin Mix[a]	10.0	10.0	10.0	10.0	10.0
Mineral Mix[g]	35.0	35.0	35.0	35.0	35.0

[a] Teklad, Madison, WI.
[b] Corn oil contained 0.02% ethoxyquin (Pfaltz & Bauer, Inc, Waterburg, CT).
[c] Best Foods, Englewood Cliffs, NJ.
[d] American Crystal Sugar Co., Moorhead, MN.
[e] Ajinomoto Co., Inc., Tokyo, Japan.
[f] Life Technologies, Inc., Chagrin Falls, OH.
[g] Experiment 1, see Table II; Experiment 2, AIN-76A Mineral Mix®; Teklad, Madison, WI.

^{54}Mn are not the same (11). Regression equations were calculated for data from individual rats, and statistical analyses of data were done on the absorption and BH values calculated from these equations. Correlation coefficients (r^2) for the linear regressions on the retention data ranged from 0.91 to 0.99 in Experiment 1 and 0.95 to 0.99 in Experiment 2; the P value was $p < 0.0001$ for all regressions.

With sufficient data, it was possible to resolve the ^{54}Mn retention curve into two linear phases after excretion of unabsorbed dietary ^{54}Mn. In Experiment 1, two BH values were calculated: BH-1 for Days 5 to 8 after dose and BH-2 for Days 11 to 22 after dose. Absorption values shown for both experiments were calculated from data for Days 11 to 22 after dose.

To study manganese balance in Experiment 1, metabolic cages (Nalgene Co., Rochester, NY) were used for a 72-hr collection of urine and feces. The dietary groups were subdivided into equal groups of four rats each. Half of the rats from each diet group were placed in metabolic cages during the first collection, beginning on Day 25 of the experiment, and half from each group were placed in metabolic cages during the second period, beginning on Day 32. Rats were placed in the metabolic cages approximately 72 hr before the collection period began to accustom them to the cages. Whole-body counting continued before and after the collection periods. Feces and urine were stored at −20°C for later measurement of manganese content. Because manganese in urine proved to be undetectable, manganese balance was calculated as intake-fecal excretion of manganese. Balance was not measured in Experiment 2.

After 5 weeks of the experiment, rats were anesthetized with sodium pentobarbital and blood was collected by heart puncture with EDTA as the anticoagulant. Blood was centrifuged at 1000g for 15 min, and the plasma was removed and frozen for manganese analysis. Tissues taken for manganese analysis included those from kidney, femur, and liver. Hearts were used for manganese superoxide dismutase (SOD) determination.

Freeze-dried tissues and diets were ashed with a nitric acid/hydrogen peroxide digestion (12). Manganese was determined by atomic absorption spectrophotometry with bovine liver as the control (National Bureau of Standards). The certified value for manganese in bovine liver is 9.9 ± 0.8 μg/g; we found 10.7 ± 0.2 μg/g.

Plasma was digested by allowing a mixture of 0.5

ml of plasma and 0.5 ml of double-distilled concentrated nitric acid (GFS Chemicals, Columbus, OH) to stand in acid-washed Teflon screw-top vials overnight. The acid-digested samples were analyzed by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer HGA 500-306). Manganese in bovine serum (National Bureau of Standards) was also analyzed. The certified value is 2.6 ± 0.5 ng/ml; we found 2.49 ± 0.11 ng/ml.

Heart tissue for assay of manganese SOD activity was prepared at 4°C or with the tissue on ice. The heart was minced and homogenized in a Potter-Elvehjem homogenizer in 18 ml of Hepes buffer (pH 7.4). The homogenate was transferred to a 50-ml plastic tube and centrifuged at 700*g* for 12 min. The supernatant was decanted to polypropylene tubes and centrifuged at 10,000*g* for 12 min. The mitochondrial pellet obtained was then suspended in 4 ml of cold Tris buffer (pH 8.2) and sonicated to release manganese SOD into solution. One unit of manganese SOD activity was defined as the amount needed to inhibit the oxidation of pyrogallol by 50% in the presence of 1 m*M* cyanide-Tris-HCl buffer (13).

Data were analyzed using analysis of variance. Where significant treatment effects were found, Tukey's studentized range test was used to characterize the differences between groups.

Results

Experiment 1. A typical ^{54}Mn retention curve is shown in Figure 1. Manganese absorption was affected only slightly, but significantly, by the source of dietary protein (Table IV). Absorption was highest from tuna (8.42%) and lowest from beef (4.57%). In contrast, the biologic half-life of ^{54}Mn (Days 11–22) was longest with beef (18.5 days) and shortest with soy (14.5 days) and tuna (15.0 days). These complementary changes in manganese absorption and excretion resulted in little difference in manganese balance among the five dietary groups (Table IV), although balance was significantly greater in soy-fed animals than in tuna-fed animals. There was no apparent relationship between the BH for Days 5 to 8 after dose (BH-1) and the BH for Days 11 to 22 after dose (BH-2) or between BH-1 and ^{54}Mn absorption.

The relative lack of effect of dietary protein on biochemical indices of manganese status (Table V) was consistent with the small differences in absorption, turnover, and balance. Only kidney manganese concentration was significantly affected by diet, with the value being highest for soy-fed animals and lowest for chicken-fed animals. Although the differences were not significant, manganese SOD activity and liver manganese concentration were also greatest in animals fed soy protein. There were no significant differences among dietary groups in the final weights of the rats (data not shown).

Experiment 2. As in the first experiment, percentage of manganese absorption was greatest from the tuna diet (7.71%) and least from the beef diet (4.14%) (Table IV). Again, biologic half-life of ^{54}Mn was longest in rats fed beef (26.9 days) and shortest in rats fed soy (16.2

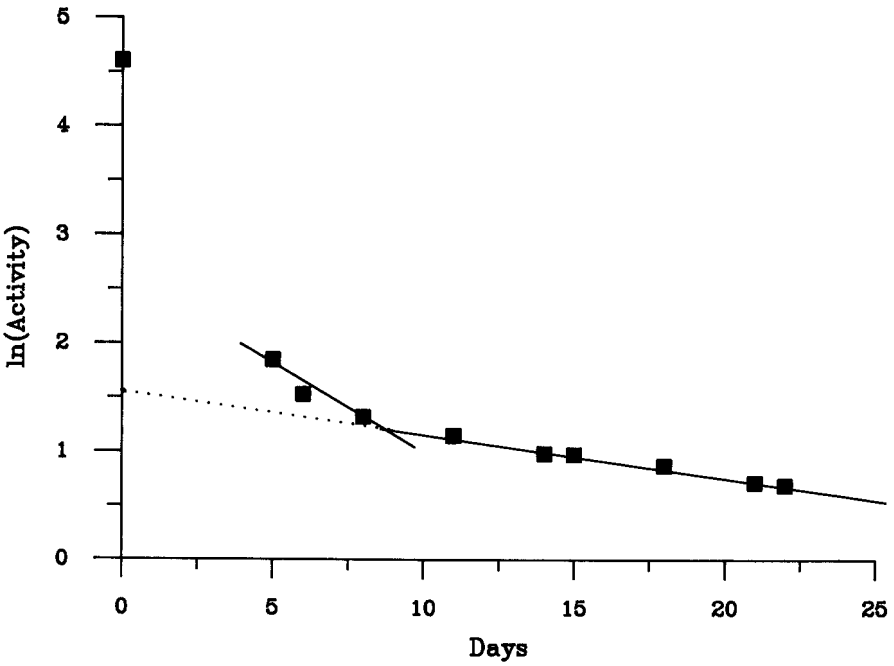

Figure 1. Typical ^{54}Mn retention curve from Experiment 1. Lines indicate linear regressions used to determine BH and percent absorption of ^{54}Mn. The steeper slope of the line from Days 5 to 8 indicates BH-1 is shorter than BH-2 (Days 11 to 22). Percentage of absorption was determined from the intercept of regression from Days 11–22.

Table IV. Effect of Dietary Protein on ^{54}Mn Absorption, BH, and Balance

Diet	Experiment 1					Experiment 2	
	^{54}Mn (% absorption)	BH-1[a]	BH-2[b] (days)	Manganese intake (μg/day)	Manganese balance (μg/day)	^{54}Mn (% absorption)	BH-2[b] (days)
Beef	4.57a[c]	5.5b	18.5c	80.9	3.2a, b	4.14c	26.9a
Chicken	6.57b, c	9.1a	17.4b, c	92.7	3.5a, b	6.39a, b	17.4b
Tuna	8.42c	7.1a, b	15.0a, b	88.4	−5.2b	7.71a	16.7b
Soy	5.46a, b	6.8b	14.5a	83.7	9.8a	4.62b, c	16.2b
Casein	5.30a, b	6.2b	15.4a, b	86.2	2.8a, b	5.88a–c	16.3b
Root MSE	1.33	1.5	1.8	13.4	9.3	1.34	1.7
Analysis of variance (*P*)	<0.0001	<0.0004	<0.0002	NS	<0.05	<0.0001	<0.0001

[a] From Days 5–8 after dose.
[b] From Days 11–22 after dose.
[c] Values in the same column not sharing a common letter are significantly different by Tukey's studentized range test.

Table V. Biochemical Indices of Manganese Nutrition in Rats Fed Different Proteins

Group	Experiment 1					Experiment 2			
	Manganese SOD (units/g)	Plasma manganese (ng/ml)	Femur manganese (μg/g)	Kidney manganese (μg/g)	Liver manganese (μg/g)	Manganese SOD (units/g)	Plasma manganese (ng/ml)	Kidney manganese (μg/g)	Liver manganese (μg/g)
Beef	0.98[a]	4.13	3.44	3.79	6.33	1.65[a]	4.10[b]	3.39b	4.36b
Chicken	1.20	4.25	3.64	3.64b	6.84	1.72	5.21c	3.81	6.15a
Tuna	1.20	4.05	3.48	4.01	7.05	1.91	4.27a, c	3.69	6.62a
Soy	1.28	4.12	3.38	4.16a	7.24	2.10	6.40b	4.00a	6.92a
Casein	1.03	4.44	3.49	4.04	6.60	1.74	4.28a, c	3.82	6.64a
Root MSE	0.47	1.05	0.28	0.34	0.87	0.32	0.66	0.32	0.60
Analysis of variance (*P* value)	NS	NS	NS	0.03	NS	NS	0.0001	0.01	0.0001

[a] One unit of SOD activity reduces the auto-oxidation of pyrogallol (0.2 m*M*) by 50%.
[b] Values within the same column with different letters are significantly different by Tukey's studentized range test ($P < 0.05$).

days) and casein (16.3 days). The BH of ^{54}Mn in rats fed beef was markedly longer in Experiment 2 than in Experiment 1, while the BH in other dietary groups was similar between the two experiments.

Effects on biochemical indices of manganese nutrition were more pronounced in Experiment 2 than in Experiment 1. Concentrations of manganese in plasma, liver, and kidney were all significantly affected by the dietary treatments. Plasma, kidney, and liver manganese were highest in rats fed the soy diet and lowest in rats fed the beef diet. Although differences were not significant, manganese SOD activity was also greatest in rats fed soy protein and lowest in rats fed beef. There were no significant differences among dietary groups in the final weights of the rats. Weights were similar to those in Experiment 1.

Discussion

We observed rapid excretion of ^{54}Mn during the first 7–10 days after each test meal. Much of this rapid decrease can be attributed to excretion of unabsorbed ^{54}Mn in the feces. Our data are somewhat limited, because rats were usually counted only every other day. However, the presence of a "fast" turnover component could be seen from about Days 5 to 9 after the meal. The BH-1 values in Experiment 1 were calculated from this data. This fast component has also been observed in humans (14–17). If the fast component represents very rapid turnover of absorbed ^{54}Mn, then true manganese absorption is much greater than we report here. This problem has been discussed by Davidsson *et al.* (15) and Mena *et al.* (14). However, our use of data from Days 11 to 22 postdose to determine absorption is consistent with the method used by Davidsson *et al.* (15) and our previous work (17, 18) to estimate manganese absorption. For practical purposes, in terms of evaluating manganese absorption or bioavailability from various foods or diets, use of the "slow" component data beginning at 11 days postdose may be the most meaningful approach. However, our understanding of manganese metabolism and its regulation will remain incomplete until we understand what is transpiring to cause the fast turnover.

The effect of dietary protein source on the bioavailability of manganese in this study was small. Although

changes in the dietary protein source affected both manganese absorption and turnover, these effects tended to be complementary, so that few differences in manganese balance or indices of manganese status were observed. All of the foods studied, with the exception of the soy, contained rather low concentrations of native manganese. Thus, any effect of these foods on manganese bioavailability would have to be as a result of binding to or interacting with manganese from the rest of the diet. In contrast, about 40% of the manganese in the soy-based diet originated from the soy protein itself. Possibly, the native manganese in the soy protein was bound or complexed in such a way that it was more available to the animal than was the extrinsic ^{54}Mn label and the inorganic manganese provided by the mineral mixes.

The manganese balance values found in Experiment 1 are not correlated with the manganese absorption or BH values measured with ^{54}Mn. As noted above, the changes in absorption and BH tended to be complementary and it is really the net result of both absorption and excretion effects that must be compared with balance values. Thus, the lack of any apparent relationship between the balance data and the ^{54}Mn data is not too surprising. Furthermore, errors are easily introduced into balance data by even small amounts of contamination of feces by rat hair or spilled food or by incomplete collection of feces or measurement of food intake. Therefore, the ^{54}Mn data probably give a better indication of differences in manganese bioavailability among diets than do the balance data.

These results conflict with the report that, in humans, substitution of a combination of ground beef and tuna for soy protein improved manganese balance significantly (1). However, in that study, manganese intake was slightly higher from the meat-containing diet than from the meat-free diet (4.94 vs 4.53 mg/day, respectively) and the difference in manganese intakes was almost equal to the difference in manganese balance between the two dietary treatments (+0.28 vs −0.18 mg/day, respectively). In a study of Indian diets, Rao and Rao (2) found that substitution of mutton or fish for pulses, in a milk-containing diet, improved manganese balance in men consuming about 8 mg of manganese/day.

In contrast to the studies cited above, in which meat or fish were substituted for legumes in practical diets, Experiment 1 used diets in which the concentrations of minerals other than manganese were held constant. We also added amino acid supplements to the diets in order to meet the NRC requirements for rats without feeding them unusually high levels of dietary protein. In mixed diets, such as those used in studies with humans, amino acids are also provided by foods other than the primary protein source and supplementation to meet requirements is unnecessary. It is possible that effects of meat and fish in the human studies were partially caused by interactions of other minerals in the diet with manganese or by differing amino acid compositions of the diets and that these effects were damped by the equalization of mineral and amino acid composition of the diets used in Experiment 1. However, in a previous study with rats (4), supplementation of soy or casein diets with methionine or arginine did not markedly affect manganese absorption or retention. Likewise, Price and Bunce (19) found no effect on manganese balance in young girls when threonine, lysine, or methionine were added to the diet.

Changes in the mineral content of practical diets supplemented with meat or other animal proteins seem a likely explanation for the changes in manganese metabolism reported when such diets were fed. In Experiment 2, when mineral concentrations in the diets were allowed to vary depending on the contribution of the protein source, concentrations of manganese in plasma, kidney, and liver were significantly affected by the dietary treatment. The BH of ^{54}Mn was markedly greater for rats fed beef in Experiment 2 than it was in Experiment 1, while BH for rats in other dietary groups was similar between the two experiments. The beef diet in Experiment 2 contained about 79 mg of zinc/kg, in contrast to 22–24 mg of zinc/kg for other diets. The beef diet contained about 72 mg of iron/kg; the soy diet contained about 76 mg of iron/kg; and other diets contained about 39–52 mg of iron/kg. High amounts of dietary iron are known to inhibit manganese absorption and increase manganese turnover (19–22). The increased BH of ^{54}Mn in rats fed beef and the lack of change in the BH in soy-fed rats between the two experiments argue against the effects of beef being the results of differences in dietary iron. Zinc is not known to affect manganese metabolism. It is possible that a complex interaction of several minerals with manganese is involved in the differences observed among these diets.

Halpin and Baker (23, 24) found that, in chicks, addition of fish meal to a casein/dextrose-based diet resulted in a reduction in bone manganese. This effect was later reported to be caused by the ash component of the fish meal (25). In contrast, the tissue manganese-lowering effect of adding corn-soybean meal to chick diets was found to be a result of the neutral detergent fiber content of the corn-soybean meal (25).

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