# Manganese Metabolism in Rats: An Improved Methodology for Assessing Gut Endogenous Losses (43518)

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*Abstract.* **Manganese homeostasis is believed to be maintained by excretion of excess absorbed manganese through the gut, but the extent of endogenous gut losses of manganese has not been quantitated. We developed a model with rats to quantitate endogenous gut losses of manganese in which the parenterally administered isotope was distributed like fed isotope. Intraportally injected $^{54}$Mn complexed to albumin distributed in tissues like the fed isotope, but carrier-free $^{54}$Mn injected intraperitoneally, intravenously, or intraportally, or $^{54}$Mn complexed to transferrin and injected intraportally did not. Thus, manganese appears to be complexed to albumin or an albumin-like protein when it leaves the intestine. A mathematical model of manganese metabolism in rats fed $^{54}$Mn was developed using the SAAM and CONSAM computer programs. It was determined that the liver, not the pancreas, was the major source of endogenous gut losses of manganese. Young, growing rats fed 45 μg of Mn/g diet were calculated to absorb 8.2% of their manganese intake and then to lose 37% of the absorbed manganese through gut endogenous losses.** [P.S.E.B.M. 1993, Vol 202]

Estimation of human requirements for manganese in the past has been based primarily on balance studies. However, balance studies have produced conflicting results as to the amount of dietary manganese that is required to maintain positive balance (1–4). In balance studies, fecal losses include unabsorbed manganese and endogenous manganese that is absorbed and then excreted into the gut. The latter is potentially important because the main excretory route for manganese is not urine. Rather, excess manganese is excreted in bile (5, 6), pancreatic secretions (7), and sloughed intestinal cells (8). Understanding the effect of variations in manganese intake on gut endogenous losses in a rat model will suggest the importance of these losses in humans.

Weigand *et al.* (9–11) have attempted to quantify gut endogenous losses of manganese of rats by modifying radioisotope tracer techniques that have been used to measure zinc endogenous losses (12). They assumed that intramuscularly injected radiomanganese was distributed in endogenous pools the same way as orally administered radiomanganese. However, the route of administration of $^{54}$Mn may affect its excretion. Carter *et al.* (13) noted that calves injected intravenously with $^{54}$Mn rather than fed $^{54}$Mn accumulated 3-fold more $^{54}$Mn in their liver but 13-fold more $^{54}$Mn in their pancreata when fed a control diet of milk. When the milk was supplemented with 15 μg of Mn/g, the pancreata of rats injected intravenously rather than fed $^{54}$Mn accumulated 40-fold more $^{54}$Mn. Thus, the previous application of the radioisotope dilution technique may be invalid.

Inconsistencies among observations concerning manganese transport also need to be resolved. Turnover of $^{54}$Mn from the plasma compartment has been reported to be extremely rapid in humans, with only 1% of an intravenous dose of $^{54}$Mn found in the blood 10 min after injection (14). Gibbons *et al.* (15) found that a manganese-transferrin complex was removed from blood of cows and goats much more slowly than either free manganese or a manganese-$\alpha_2$-macroglobulin com-

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plex. Davidson *et al.* (16) identified transferrin as the major plasma carrier protein for manganese in rats. Perhaps the rapid turnover of plasma manganese can be explained on the basis that a different protein than transferrin transports manganese from the small intestine to the liver.

The purpose of these studies was to develop a model of tracer administration that bypassed the gut but in which the parenterally administered tracer was metabolized and distributed to tissues like orally administered tracer. With the elimination of unabsorbed manganese in the gut, endogenous gut losses of manganese could be quantitated. We also wanted to determine the relative importance of the liver and the pancreas as reservoirs of endogenous manganese.

## Materials and Methods

**Animals and Diets.** Male Sprague-Dawley rats (Harlan Sprague-Dawley, Madison, WI) weighing ≈100 g were housed individually in stainless steel, wire-bottomed cages. An AIN-76A diet (17) and deionized water were offered *ad libitum* for at least 5 days before and throughout the experimental period.

**Experimental Design.** In Study A, rats ($n$ = 3/time point, except as follows: $n$ = 6 for 3, 12, and 24 hr and $n$ = 10 for 48 hr) were fed carrier-free $^{54}$Mn and exsanguinated at various time points between 0.5 hr and 8 days after isotope administration. In Study B, rats ($n$ = 3/treatment/time point, except as follows: $n$ = 6/treatment for rats injected intraperitoneally on Day 2 or fed isotope on Day 4 and $n$ = 2 for rats injected intravenously on Day 2) were fed or injected either intraperitoneally or intravenously in the tail vein with carrier-free $^{54}$Mn and sacrificed 2, 4, 6, and 8 days after isotope administration. In Study C, animals ($n$ = 5/treatment/time point) were either fed carrier-free $^{54}$Mn, injected intraperitoneally with carrier-free $^{54}$Mn, injected intraportally with carrier-free $^{54}$Mn, injected intraportally with $^{54}$Mn complexed to albumin, or injected intraportally with $^{54}$Mn complexed to transferrin and sacrificed 2, 4, 6, and 8 days after isotope administration.

**Isotope Preparation and Administration.** To prepare the manganese-protein complexes, 20 μCi of carrier-free (>40 Ci/g) $^{54}MnCl_2$ in 0.5 *M* HCl (Dupont New England Nuclear Products, Boston, MA) were incubated with either 4 mg/ml of albumin or transferrin in Tris-buffered KCl (pH 7.2) at 37°C. Trisodium citrate (0.05 *M*) and $KMnO_4$ (0.03 *M*) were added to the $^{54}$Mn and transferrin incubation mixture. After 1 hr, the incubation mixture was placed on a Sephadex G-25 column (Pharmacia LKB, Piscataway, NJ) to separate $^{54}$Mn from the $^{54}$Mn complexed to either albumin or transferrin (15).

All animals were fasted 16 hr before isotope administration. Rats injected in the portal vein were anesthetized with a cocktail containing 0.05 ml of xylazine (Rompon; Mobay Co., Shawnee, KS) and 0.1 ml of ketamine (Ketaset; Aveco Co., Fort Dodge, IA) before surgery. Animals were injected with either 2 μCi of $^{54}$Mn complexed to 0.4 mg of albumin or transferrin. Rats fed the isotope were given 5 μCi of carrier-free $^{54}$Mn in 2 g of diet. The animals injected with the isotope were refed *ad libitum* immediately after isotope injection, and the animals fed the isotope were given *ad libitum* access to diet 15 min after finishing their labeled 2-g meals.

A preliminary study was conducted to determine whether the stress of the intraportal injections affected manganese utilization. Five animals were fed the isotope and then anesthetized, and their portal veins were surgically exposed. There were no differences in isotope distribution between these animals and those fed isotope without surgery. Furthermore, there were no differences in weight gain and food intake after isotope administration between animals fed the isotope (no sham operation) and animals injected intraportally. Thus, we concluded that sham operations were unnecessary.

**Sample Collection and Analysis.** Rats were anesthetized with $CO_2$ and blood was collected by cardiac puncture with heparinized syringes. Stomachs, small intestines, ceca, large intestines, feces, livers, pancreata, kidneys, heart, and 1-g muscle samples (Studies B and C) were cleansed of adhering materials and counted in an automatic gamma-well scintillation counter (Gamma Trac 1191; TM Analytic, Elk Grove Village, IL). Radioactivity in the red blood cells, plasma, and urine was too low to quantitate (<100 cpm over background). A standard sample of $^{54}$Mn was counted before each set of samples to correct for decay.

Radioactivity in each organ was expressed as a percentage of the administered dose. Syringes and diet samples were counted before and after isotope administration to determine the exact dose each animal received. To correct for differences in absorption and excretion among the different routes of isotope administration, the distribution of $^{54}$Mn in the different tissues was expressed as a percentage of the soft tissue pool, which was the sum of the radioactivity in the liver, pancreas, kidneys, heart, and muscle. Muscle was considered to be 41% of the rats' body weight (18).

Livers, feces, diet, and reference material were analyzed for manganese content using an atomic absorption spectrophotometer with a graphite furnace atomizer (model 170-70 polarized Zeeman; Hitachi, Tokyo, Japan), as described previously (19). Bovine liver (SRM 1577a) standard obtained from the National Institute of Standard and Technology was determined to contain 103% ($n$ = 6) of the certified value for manganese.

Apparent absorption of manganese was calculated as manganese intake minus fecal losses of manganese. The true absorption of dietary manganese was calculated as manganese intake minus total fecal losses of

manganese plus endogenous gut losses of manganese ($m$), where $m = F^* S_f/S_m$ (12). $S_f$ represents the specific activity of manganese ($^{54}$Mn/total manganese) in the feces; $S_m$ represents the specific activity of manganese of endogenous origin in the feces. The specific activity of the liver was used as $S_m$ when animals were injected intraportally with $^{54}$Mn bound to albumin.

**Statistical Analysis.** Data were analyzed by a SAS general linear model program (20). Tests for least significant differences ($P < 0.05$) were used to differentiate among means for variables that had been significantly affected by the treatments.

**Kinetic Analysis.** The time course of $^{54}$Mn distribution in the sampled tissues and excreta of animals fed the isotope was analyzed by compartmental analysis using the SAAM 30 and CONSAM modeling programs run on an IBM PS/2 model 386 computer (21, 22). Best fit was judged by minimizing the residual sum of squares for error between observed data and model predictions. The fractional exchange rate constants in the model are referred to as the L(I,J) and defined as the fraction of manganese in Compartment J transferred to Compartment I per unit time.

## Results

The movement of $^{54}$Mn through the gastrointestinal tract in animals fed the isotope and sacrificed at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, or 48 hr after isotope administration is shown in Figure 1. Overall, 91 ± 1% (range, 87–95%) of the isotope could be accounted for in the gastrointestinal tract and the feces of animals fed the isotope at each time point. At 0.5 hr, 72% of the fed isotope was found in the stomach. Approximately one third remained in the stomach until 3 hr. Significant quantities (21–59%) of the isotope were found in the small intestine from 0.5 to 6 hr. Large amounts of $^{54}$Mn did not appear in the cecum until 4 hr (32%) and significant (17%) amounts of $^{54}$Mn remained in the cecum at 24 hr. Isotope began appearing in the feces at 12 hr. By 48 hr, rats had excreted 71% of the fed isotope in their feces; 22% more of the isotope could be accounted for in the gastrointestinal tract. Little (<10%) of the fed dose of isotope remained in the gastrointestinal tract 3 days after the oral dose.

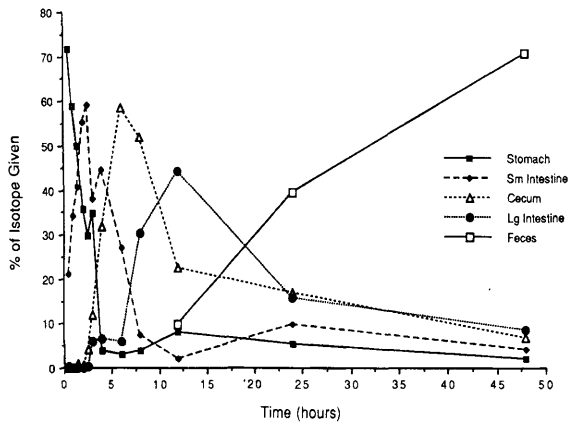


**Figure 1.** Movement of $^{54}$Mn through gastrointestinal tract of rats fed $^{54}$Mn in Study A. Values are means ($n$ = 3/time points), except as follows: $n$ = 6 for 3, 12, and 24 hr and $n$ = 10 for 48 hr.

We then compared the fecal excretion and tissue distribution of fed $^{54}$Mn to that of injected isotope after 2, 4, 6, and 8 days in Study B (Table I). We chose these times because significant amounts of the isotope remained in the gastrointestinal tract for 2 days when rats were fed the isotope in Study A. Animals fed the isotope excreted significantly ($P < 0.05$) more $^{54}$Mn in their feces than animals injected intraperitoneally or intravenously with $^{54}$Mn at 2 days (66 ± 5% vs 37 ± 6% and 46 ± 3%, respectively) and at 8 days (94 ± 1% vs 83 ± 3% and 78 ± 2%, respectively). Fecal losses of $^{54}$Mn by rats injected with the isotope represented gut endogenous losses rather than nonabsorbed manganese.

The distribution of $^{54}$Mn in different tissues was expressed as a percentage of the soft tissue pool, which was the sum of the radioactivity in the liver, pancreas, muscle, kidneys, and heart. The route of isotope administration significantly affected the tissue distribution of $^{54}$Mn in Study B (Table I). Animals fed $^{54}$Mn had a significantly ($P < 0.05$) greater proportion of $^{54}$Mn in their livers than rats injected intravenously at all time points and a significantly larger proportion than rats injected intraperitoneally at 2 and at 6 days. In contrast, animals fed $^{54}$Mn had a significantly smaller proportion of $^{54}$Mn in their pancreata than animals injected intraperitoneally at 4, 6, and 8 days or those injected intravenously at 2 and 6 days.

There are two possible explanations for the differences in the distribution of $^{54}$Mn when routes of administration differed (i.e., fed versus intraperitoneally and intravenously). First, orally administered $^{54}$Mn was delivered via the portal vein to the liver before circulating to other tissues, as occurs with intravenously (tail

**Table I.** Tissue Distribution of $^{54}$Mn in Rats Either Fed Isotope or Injected Intraperitoneally or Intravenously (Tail Vein) with Isotope in Study B[a]

| Organ | Time after $^{54}$Mn dose (days) | Route of isotope administration (% tissue pool) | | |
|---|---|---|---|---|
| | | Oral | ip | iv |
| Liver | 2 | 78.8 ± 3.4* | 42.7 ± 4.8† | 34.6 ± 3.9† |
| | 4 | 63.0 ± 5.2* | 47.0 ± 4.3*† | 33.9 ± 0.5† |
| | 6 | 58.8 ± 4.1* | 31.2 ± 7.1† | 23.4 ± 1.6† |
| | 8 | 51.1 ± 7.2* | 54.8 ± 2.2* | 18.6 ± 1.7† |
| Pancreas | 2 | 3.2 ± 0.2† | 8.5 ± 1.8*† | 13.5 ± 0.2* |
| | 4 | 6.3 ± 1.3† | 17.6 ± 1.6* | 10.5 ± 0.7† |
| | 6 | 7.8 ± 0.7† | 14.7 ± 4.9* | 12.8 ± 1.0* |
| | 8 | 9.4 ± 1.9† | 22.5 ± 1.3* | 11.9 ± 1.4† |

[a] Values are mean ± SE ($n$ = 3, except for intraperitoneal Day 2 and oral Day 4 where $n$ = 6, and intravenous Day 2 where $n$ = 2). The means in each horizontal row showing the same symbol (*, †) are not significantly different ($P < 0.05$).

vein) and intraperitoneally injected $^{54}$Mn. Second, $^{54}$Mn absorbed from the intestine was probably bound to protein(s). These possibilities were investigated in Study C.

There were no differences in the tissue distribution of $^{54}$Mn in the animals receiving oral isotope and the animals injected intraportally with $^{54}$Mn complexed to albumin at all time points (Fig. 2). Animals injected intraperitoneally with free $^{54}$Mn, intraportally with free $^{54}$Mn, or intraportally with $^{54}$Mn complexed to transferrin had a significantly smaller proportion of $^{54}$Mn in their livers and tended to have a larger proportion of $^{54}$Mn in their pancreata than animals fed the isotope at any time point. Animals injected with $^{54}$Mn complexed to transferrin had a significantly larger proportion of $^{54}$Mn in their muscle at 2 and at 4 days and tended to have more $^{54}$Mn in their muscle at 6 and at 8 days than animals given the isotope by any of the other routes.

A mathematical model of manganese metabolism in rats fed $^{54}$Mn in Study A was developed (Fig. 3). The fractional rate constants, L(I,J), which represent the fraction of Compartment J transferred to Compartment I per hour, are shown in Table II. The fit of the experimentally obtained data to the final mathematical model is shown for liver in Figure 4.

Movement of the tracer from the stomach to the small intestine, L(3,2), was inhibited when the animals were refed after 6 hr. This was accounted for by decreasing the fractional rate L(3,2) (which was 0.464 $h^{-1}$) to 0.0464 $h^{-1}$ at 6 hr and then increasing it back up to the original conditions by 144 hr. A second peak of $^{54}$Mn appeared in the stomach at 12 hr, which coincided with the appearance of the isotope in the feces. We assumed that the rats were practicing coprophagia because that was the only way more isotope could enter the stomach. P(4) represented the turnover of the cecum and large intestine (Fraction 4) and P(5) represented the fraction of Compartment 4 that was eaten. Using estimates of P(4) in the model, we estimated that the rats were eating 15% of their feces before the feces were collected and counted, even though the rats were in wire-bottomed cages.

We were unable to quantitate $^{54}$Mn transport from the pancreas to the small intestine using this experimental design because the amount of the isotope in the pancreas kept increasing until 48 hr and then remained constant until 6 days. Therefore, the pancreas was probably a reservoir for the excess manganese and not a major source of gut endogenous manganese losses.

In contrast, manganese turnover from the liver was

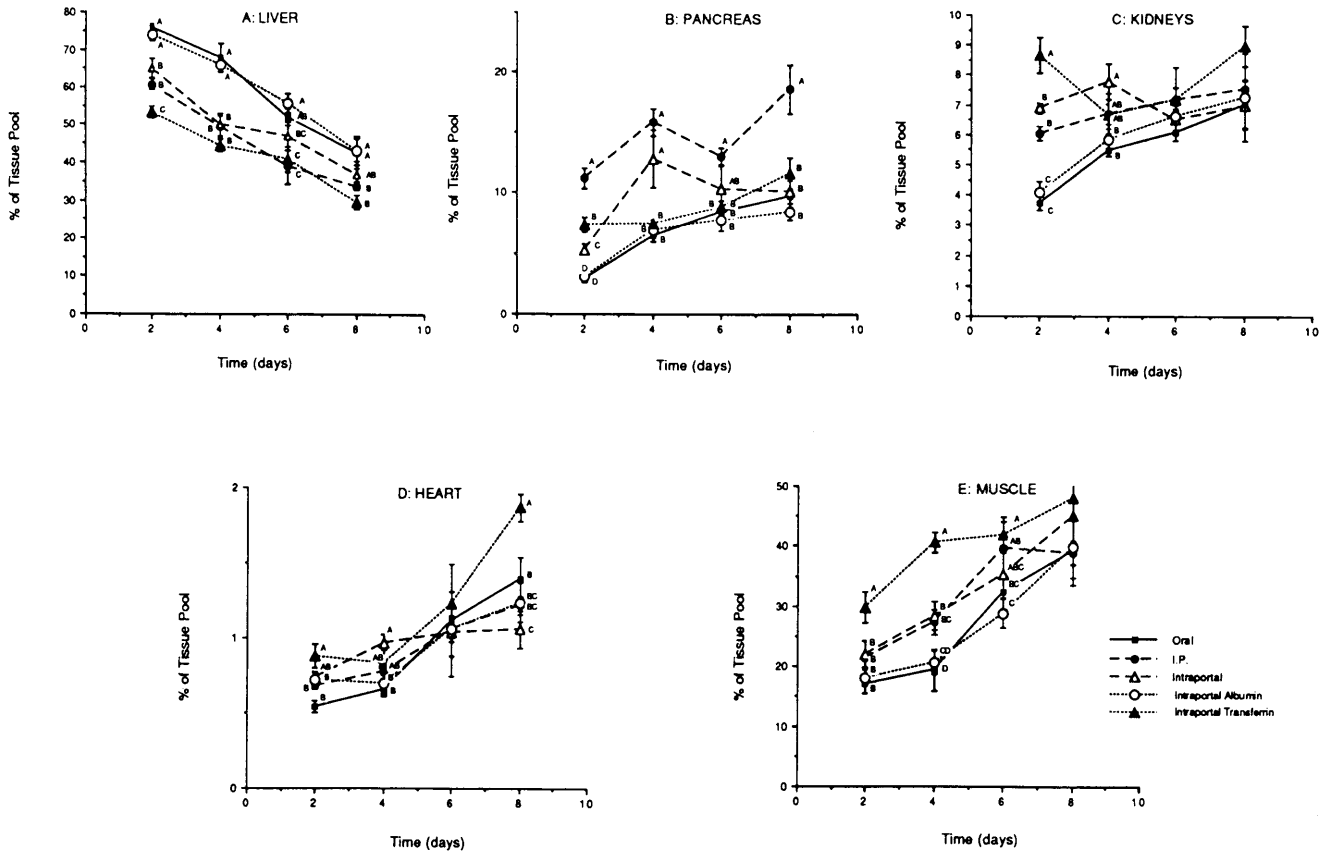


**Figure 2.** Distribution of $^{54}$Mn administered in different forms and by different routes in Study C. Percentage of $^{54}$Mn in the total (A) liver, (B) pancreas, (C) kidneys, (D) heart, and (E) muscle of rats either fed, injected intraperitoneally, or injected intraportally with carrier-free $^{54}$Mn, injected intraportally with $^{54}$Mn complexed to albumin, or injected intraportally with $^{54}$Mn complexed to transferrin. Values are means ± SE ($n$ = 5). Means at each time point without a common superscript are significantly ($P < 0.05$) different.

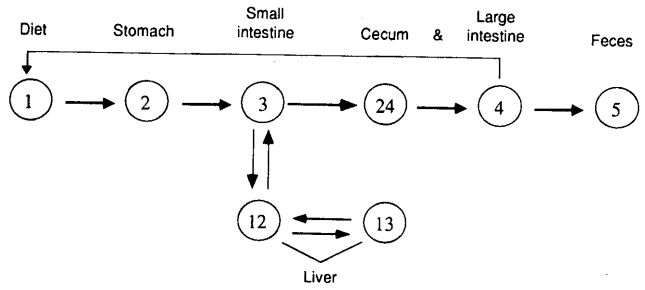


**Figure 3.** Proposed model of manganese metabolism in rats fed $^{54}$Mn. Circles represent theoretical compartments; numbers within circles indicate compartment numbers. Arrows represent transfer pathways. Table II provides data on fractional exchange rates between compartments.

**Table II.** Rate Constants for $^{54}$Mn Movement in Rats

| Model parameter[a] | Model-calculated values | |
|---|---|---|
| | Fractional exchange rate ($h^{-1}$) | Coefficient of variation of the adjustable parameters[b] |
| L(2,1) | 0.213 | 0.008 |
| L(24,3) | 0.213 | 0.011 |
| L(1,4) + L(5,4) | 0.125 | 0.019 |
| L(3,12) | 500.0 | 80.7 |
| L(12,3) | 40.85 | 8.00 |
| L(12,13) | 0.026 | 0.005 |
| L(13,12) | 0.046 | 0.013 |

[a] L(I,J) is defined as the fraction of manganese transferred from Compartment J to Compartment I per hour. As indicated in Figure 3, the theoretical Compartments 1, 2, 3, 24 and 4, and 5 correspond to the manganese pools in the diet, stomach, small intestine, cecum and large intestine, and feces, respectively. The model has two theoretical compartments (12 and 13) in the liver. This model calculated a coprophagy fraction (P[5]) to be 15% of the cecum and large intestine pools.
[b] Measure of variation between observed and model-predicted values.

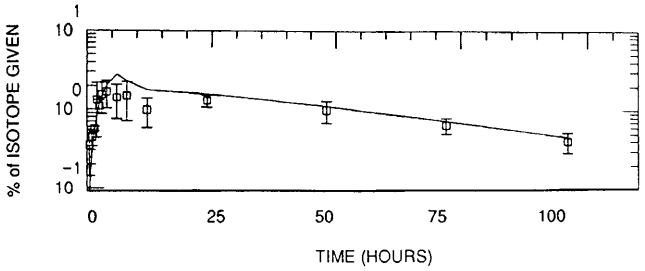


**Figure 4.** Comparison of observed (□) with model-calculated values (—) for $^{54}$Mn activity in the liver. Model-calculated $^{54}$Mn in the liver includes the sum of the activities of Compartments 12 and 13 shown in Figure 3.

extremely rapid. $^{54}$Mn turnover could best be explained mathematically by two theoretical compartments (Compartments 12 and 13). Compartment 12 exchanged rapidly with the small intestine, and Compartment 13 exchanged with Compartment 12 more slowly. We used the specific activity of manganese ($^{54}$Mn/total manganese) in the liver when $^{54}$Mn was complexed to albumin and injected intraportally to estimate the specific activity of the endogenous pool of manganese.

Apparent and true absorption of manganese were calculated to be 5.3% and 8.2%, respectively, of total manganese (Table III). Absorption of $^{54}$Mn from one labeled meal was calculated to be 6.4%, which approximated apparent absorption of total manganese. Using our modifications of Weigand and Kirchgessner's (12) formula, we calculated gut endogenous losses of manganese to be 2.9% of manganese intake or 37 ± 4% of the absorbed manganese.

## Discussion

**Manganese Transport.** Intraportally injected $^{54}$Mn complexed to albumin (but not intraportally injected $^{54}$Mn complexed to transferrin) distributed in tissues like fed $^{54}$Mn. Thus, even though transferrin has been identified as the major manganese-binding protein in the plasma (16), albumin, or a protein that behaves like albumin, may be the major manganese transport protein between the small intestine and the liver.

Other nutrients are transported from the gut to the liver by different proteins than those that transport them from the liver. For example, Gordon *et al.* (23) demonstrated that albumin is the plasma carrier of zinc and copper from the intestine to the liver. However, ceruloplasmin is the major transport protein of copper when it leaves the liver (24). Similarly, absorbed retinol is esterified with palmitic acid and transported by chylomicra to the liver (25). In the liver, the retinal ester is hydrolyzed and retinol is complexed with retinol-binding protein and transthyretin for transport to extrahepatic tissues.

**Endogenous Losses of Manganese.** We took an inadequately tested model and modified it so that endogenous gut losses of manganese could be quantitated. The specific activity of the liver when animals were injected intraportally with $^{54}$Mn complexed to albumin was used to calculate the specific activity of the endogenous pool of manganese.

We found that the data were best described by a model with two theoretical compartments in the liver: a rapidly exchanging (Compartment 12) and a slowly

**Table III.** Calculation of Endogenous Loss and True Absorption in Rats Injected Intraportally with $^{54}$Mn Complexed to Albumin and Sacrificed after 6 Days

| | μg/day | Percentage of intake |
|---|---|---|
| Manganese intake | 761 ± 15[a] | — |
| Apparent absorption | 40 ± 16[b] | 5.3 ± 1.3 |
| Endogenous fecal losses | 22 ± 6[c] | 2.8 ± 0.8 |
| True absorption | 62 ± 16[d] | 8.2 ± 1.9 |

[a] Values are the mean ± SE ($n$ = 5).
[b] Apparent absorption: intake − fecal losses.
[c] Endogenous fecal losses: fecal losses × (specific activity of manganese in feces/specific activity of manganese of endogenous origin in feces).
[d] True absorption: intake − (fecal losses − endogenous fecal losses).

exchanging (Compartment 13) compartment. We hypothesized that Compartment 12 represented the manganese that was being excreted in bile. This suggests that the measurement of the specific activity of $^{54}Mn$ in the bile, in addition to whole liver, would improve calculations of endogenous gut losses of manganese.

In future studies, animals may have to be kept in restraining cages rather than wire-bottomed cages to avoid the confounding problem of coprophagy. This is not a perfect solution because the use of restraining cages tends to stress animals and results in lowered food intakes and growth.

Estimates of endogenous losses of other minerals need to be re-evaluated because other investigators have not measured the tissue distributions of orally administered versus injected radioisotope. Our work demonstrated that the mode of isotope administration affects isotope distribution.

We found that rats fed a diet containing approximately 45 $\mu$g of Mn/g diet absorbed 8.2% of their manganese intake and subsequently re-excreted 37% of the absorbed manganese in their feces. If the efficiency of absorption and the excretion of endogenous losses are proportional in rats and humans, then humans consuming 3 mg of manganese/day would absorb 0.24 mg and excrete 0.09 mg through gut endogenous losses. Subjects in balance studies are often in slightly (i.e., 0.08–0.16 mg/day) negative balance when fed 2.9–3.3 mg of manganese daily (4, 26, 27). The negative balances observed in human balance studies are equivalent to potential gut endogenous losses of manganese. It appears that although small, gut endogenous losses of manganese, probably through biliary secretions, are important in the maintenance of manganese homeostasis.

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