Selective excretion by the kidney is a major mechanism by which the concentrations of many minerals are regulated in mammalian tissues, but significant excretion also occurs via the gastrointestinal tract. The latter route is especially important in the elimination of alkaline earths and metals of the periodic table's first transition group, of which one, the essential element manganese, appears to be almost totally excreted into the gastrointestinal lumen (8).

Mammalian liver and pancreas, both of which originate from the primordial gut (1, 19), have been reported to excrete manganese (6, 13). It is not known however, whether one or both of these organs regulate the concentration of this metal in mammalian tissues, and if so, whether the gut participates in this regulation.

The accompanying papers (5, 15) indicated that the turnover of manganese depends extensively on the dietary supply of this metal, whereas the tissue concentrations tend to remain stable primarily by virtue of excretion of excess metal. The present studies examine the effect of obstruction of the rectum and of the bile flow on the distribution and excretion of this nutrient. The findings suggest the existence of a rapid enterohepatic circulation which regulates the excretion of manganese as one among several gastrointestinal routes.

METHODS

Animals. Male, albino Sprague Dawley rats, weighing 200–250 g, were observed for 1–2 weeks prior to experimentation. They were housed in steel cages, some of which permitted separate collection of urine and feces. Purina laboratory chow (32.5 μg Mn^{55}/g) and tap water were offered ad lib., except that some groups were fed milk containing approximately 36 μg Mn^{55}/liter as determined by neutron activation analysis (10, 12, 18). Manganese sulfate was added as noted. The results on 325 of the animals studied are reported.

Operations. Laparotomies were performed under ether anesthesia. In some groups, the common bile duct was ligated with 5-0 silk at the juncture of the middle to the proximal third; in a few of these, double ligatures were applied also to the lower segment of the rectum. In another series, the liver was prevented from receiving MnE^{4+} by clamping the hepatic artery and the portal vein which followed the intravenous injection of the isotope (2 and Fig. 4). The control animals of this series received the Mn^{4+} after the release of the clamp, at which time the viscera had begun resuming their normal appearance.

All but two animals were decapitated by guillotine. Surgery seemed to affect the distribution of manganese and it was suspected that the agonal stages might do so as well. To vary the agonal stages one animal was killed by electrocution and one by oxygen deprivation.

Isotopes. The Mn^{48}SO_{4}·H_{2}O (J. T. Baker Chemical Co.) was added to the milk and was given by stomach tube or injected intravenously in 0.1 ml 0.9% NaCl solution. In one experiment this salt was first incubated
with rat serum aerobically at 20°C for 1 hr and the serum was injected intravenously.

The Mn\textsuperscript{54}Cl\textsubscript{2} (T\textsubscript{1/2} = 3.14 days) was the carrier-free isotope used in earlier work (5, 15). Standardized doses of 1.0–1.5 μg in 0.1 ml 0.9% NaCl were injected.

The operated animals were given the radioisotope either into the vena cava or into the portal vein; the intact animals were injected into a leg or a tail vein. Animals were excluded if the success of these steps was in doubt.

**Neutron activation analysis.** The methodology has been discussed earlier (10, 12, 16). The precautions in obtaining the samples and the reasons for not computing “specific activities” of Mn\textsuperscript{54} are indicated in the preceding paper (15). Figure 1, curve A, shows that the measurement of Mn\textsuperscript{54} was independent of sample size, the variance of which had been suspected to affect the metal's activation in the nuclear reactor.

**Measurement of radioactivity.** One-half hour or 1 hr after injection of the isotope each rat was placed in a Bollman cage (4) which held the midpoint of the animal's trunk fixed 6 cm above the center of a flat NaI-Tl crystal measuring 11 x 8.8 cm. Figure 1, curve B, shows the diminution in recorded radioactivity when the animal's midpoint was moved from the center of this crystal. The crystal was connected to the circuitry discussed elsewhere (9). The total-body radioactivity and the Mn\textsuperscript{54} of the tissues were counted in the manner stated in the second paper of this series (15). There was some dependence of the counts recorded on the position of a given organ in relation to the center of the crystal (Fig. 1, curve C). Hence this center was utilized as much as possible for the measurement of all radioactivities. Because the various organs approximated a point source better than did intact animals, the total-body radioactivity was again (5) invariably lower by 10–17% than the sum of its fractions recovered with isolated organs.

By comparison to the position of animals or organs over the crystal, the changes in counting geometry which followed the distribution of the injected tracer in a given animal were minor indeed (Fig. 1, curve D).

**Typical protocol.** A rat was weighed, anesthetized, and operated on according to one of the procedures discussed above. The tracer was then injected intravenously and the animal was placed over the crystal 20 min later. The first count served as the 100% retention for the subsequent ones and the decline of the radioactivity was plotted semilogarithmically as a function of time. After a period of observation, the animal was decapitated and autopsied: the liver, kidneys, thoracic and abdominal viscera, and the remainder of the carcass were dissected and counted separately on the apparatus used for the intact animal. If the bile duct was ruptured, the animal was excluded from the study.

**RESULTS**

**Abolition of the excretion of Mn\textsuperscript{54} by rectal ligation.** Normally, Mn\textsuperscript{54} is excreted into the gastrointestinal tract (8), but this remained to be shown in jaundiced and in manganese-loaded animals. Groups of four rats with rectal obstruction were given Mn\textsuperscript{54} intravenously and manganous sulfate by stomach tube (3.2, 6.4, and 12.0 mg Mn\textsuperscript{54}/animal). The total-body radioactivity remained unchanged during the subsequent 5 days of observation. A similar almost total retention of the isotope was observed in four animals after both the common bile duct and the rectum were ligated. Analyses of the urine of these latter animals and analyses of both urine and feces from three animals with biliary ligation showed that the urine contained almost no measurable radioactivity, whereas the fecal Mn\textsuperscript{54} accounted for the total-body loss.
REGULATION OF MANGANESE EXCRETION BY LIVER

**Diminished excretion of Mn⁴⁺ following biliary ligation.** The previous experiments confirmed the gastrointestinal excretion of manganese. The contribution of the bile was studied thereafter by means of biliary ligation in animals receiving the isotope into either a peripheral or the portal vein. The total-body retention curves of the ensuing four groups of three animals are shown in Fig. 2. Biliary ligation diminished but did not abolish the excretion of this isotope whether or not it had been injected intravenously or intraportally. The two routes of injection reflected themselves in the relative rates by which this isotope became excreted: intraportal injection was followed by more rapid excretion or by more marked retention, depending on whether the bile duct was open or shut. These experiments indicated that, although the liver was only one of the excretory routes, it excreted its manganese uptake and thus induced the transport of Mn⁴⁺ from the tissues to the liver might assume the form of a wave when the tracer’s flow into the bile was blocked. Hence a different method of blocking was tried: A single intravenous injection of Mn⁴⁺ was given to each of 34 rats either before or after the application of a clamp to the hepatic blood vessels for the 10 min during which 90% of the isotope is cleared (2 and Fig. 4). Five minutes later, the livers of the clamped animals contained 2−3.5% of the injected Mn⁴⁺, whereas those of the corresponding controls contained 32%. Eleven days later, the respective values were 18 and 24%. These results are summarized in Fig. 4 as liver-to-carcass ratios (14, 15). The total-body retention of Mn⁴⁺ was measured in the animals sacrificed on the 5th and 11th days (4 groups of 3 animals each). The animals that had received the isotope with the clamp on showed identical total-body retention curves of Mn⁴⁺, as had those with biliary ligation (Fig. 2, curve C). Those that had received the isotope after the release of the clamp behaved identically to the sham-operated animals (Fig. 2, curve D). Evidently, some Mn⁴⁺ was being excreted via extrahepatic routes even when the bile duct was patent; transport from the tissues to the liver accounted only for the rise shown in Fig. 2, but not for the fall.

A second possibility was that cellular damage (3) diminished the manganese uptake and thus induced the decline under investigation. This was tested in 18 bilaterally ligated, 13 sham-operated, and 6 control animals, all of which were given 1 µCi Mn⁴⁺ intravenously 2 hr prior to sacrifice. Sacrifice took place after the following time intervals: 2 hr (6 ligated, 6 sham operated, 6 controls); 1 day (6 ligated, 2 sham operated); 5 days (6 ligated, 5 sham operated). The percent uptake by the liver was computed for each animal. The data were paired on all permutations and subjected to statistical analysis (t test). No significant differences were found between groups: the ligated group as a whole showed liver uptake with a mean and standard deviation of 96.5 ± 8 while the corresponding numbers for the other two groups were 29.1 ± 7 and 21.4 ± 3, respectively.

Now it became probable that manganese absorbed from the diet accumulated in the obstructed liver and...
eventually displaced the radioisotope. This could be shown only by quantifying the stable isotope Mn$^{55}$ present in foods and not by injecting an artificial radioisotope. Figure 5 shows the concentration of manganese in eight livers from intact and laparotomized animals, with and without biliary obstruction. The expected rise of this element’s concentration did occur following biliary obstruction but it is less certain whether this rise was a temporary one. In addition, some of the nonobstructed animals showed a diminution of the Mn$^{55}$ concentration in the liver. These findings agreed with the results obtained with Mn$^{54}$ (Fig. 3) in that biliary ligation and surgery had again affected the metabolism of this metal in opposite directions.

The concentrations of Mn$^{54}$ in tissues other than the liver are shown in Fig. 6. Effects of biliary obstruction reflected themselves only in muscle, with a significant elevation of Mn$^{54}$. On the other hand, surgery resulted in progressively increasing concentration of Mn$^{55}$ in the kidneys from both operated groups. Two similar groups had shown a rapid initial loss of Mn$^{54}$ from the kidneys by comparison to intact animals.

This increased concentration proximally to the obstruction of one among several excretory routes did not indicate whether the obstructed pathway simply permitted the flow or whether it also regulated the excretion rate of the metal. This was investigated by varying the excretion rate while the bile duct was either open or shut.
obstructed animals showed no discernible increase in the rate of total-body loss of Mn⁴⁺, whereas a marked enhancement of the excretion was exhibited by the controls (Fig. 7).

This striking retention could have been a reflection of diminished dietary (manganese) intake rather than of excretory consequences of biliary ligation. Therefore evidence for isotopic exchange was sought in the distribution of the radiotope among the organs of these rats (Table 1). Again (2, 5), the carcasses of all these animals showed similar retention of this isotope regardless of jaundice or dietary manganese. However, the percent of the Mn⁴⁺ administered was smaller in the liver, after the manganous supplement was given. Biliary obstruction again induced a relative increase of the radioactivity remaining in the liver, regardless of the manganous supplement. The pooled viscera showed the same percent retention of the administered Mn⁴⁺ in all groups, except for the one that had both biliary obstruction and the manganous supplement: here the viscera (including the gastrointestinal tract) contained the balance of the radioactivity lost by the livers.

These findings ruled out diminished food intake as the only reason for the insensitivity of the Mn⁴⁺ turnover to manganese loading. Still, this insensitivity could have been feigned by diminished mobility of the intestines of these jaundiced rats. If this were the case, the ligated animals should remain insensitive to even larger amounts of manganese as well as to parenterally injected carrier. Administration of graded doses of metal, orally and parenterally, were preferable to fractionation of the Mn⁴⁺ between feces and intestinal wall, since washings extracted significant amounts of radioactivity.

The amount of orally administered carrier was varied as follows: Rats maintained on milk for 1 week were divided into sham-operated and ligated groups. These were injected iv with calibrated doses of Mn⁴⁺ and were further divided into subgroups of three. Each was given by stomach tube 1 ml of water or graded amounts of MnSO₄ solution and were sacrificed after recording their total-body radioactivities for 5 days. These radioactivities are shown as percents of the six animals killed at 2 hr after tagging, in Table 2. Biliary ligation resulted in slower excretion of Mn⁴⁺ regardless of how much natural manganese was given.

The above experiments would have proved that the bile flow can be bypassed under conditions of heavy manganese loading, if the following were true: that the isotope lost by these animals had been actually excreted, and that the percent of isotope lost by these animals had been actually excreted. However, the percent of isotope lost by these animals had been actually excreted, and the ligated animals should remain insensitive to even larger amounts of manganese loading. Still, this insensitivity could have been feigned by diminished mobility of the intestines of these jaundiced rats. If this were the case, the ligated animals should remain insensitive to even larger amounts of manganese, regardless of how much natural manganese was given.

### Table 1. Distribution of injected dose of Mn⁴⁺ at end of experiment summarized in Fig. 7, as percent of initial dose given

<table>
<thead>
<tr>
<th>Biliary obstruction</th>
<th>Liver</th>
<th>Viscera</th>
<th>Carcase</th>
<th>No biliary obstruction</th>
<th>Liver</th>
<th>Viscera</th>
<th>Carcase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(milk)</td>
<td>45 (44-48)</td>
<td>21 (20-22)</td>
<td>22 (19-24)</td>
<td>27 (26-27)</td>
<td>42 (41-43)</td>
<td>29 (19-26)</td>
<td></td>
</tr>
<tr>
<td>(milk + MnSO₄)</td>
<td>27 (26-27)</td>
<td>42 (41-43)</td>
<td>29 (19-26)</td>
<td>27 (26-27)</td>
<td>42 (41-43)</td>
<td>29 (19-26)</td>
<td></td>
</tr>
<tr>
<td>No biliary obstruction</td>
<td>9 (6-14)</td>
<td>20 (17-25)</td>
<td>23 (21-24)</td>
<td>9 (6-14)</td>
<td>20 (17-25)</td>
<td>23 (21-24)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means, with ranges in parentheses. Under viscera there is listed the sum of the determinations of the radioactivity in the gastrointestinal tract, kidneys, spleen, pancreas, testes, adrenals, and thoracic organs for each animal.

### Table 2. Percents of total-body Mn⁴⁺ remaining 5 days after injection as a function of single oral doses of manganese carrier

<table>
<thead>
<tr>
<th>Mn⁴⁺ Given, µg</th>
<th>No. of Animals</th>
<th>Mean</th>
<th>Range</th>
<th>No. of Animals</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>79.5</td>
<td>70.0-80.5</td>
<td>9</td>
<td>75.5</td>
<td>70.0-76.0</td>
</tr>
<tr>
<td>5 x 10⁵</td>
<td>3</td>
<td>78.0</td>
<td>75.2-80.5</td>
<td>3</td>
<td>75.0</td>
<td>71.2-83.2</td>
</tr>
<tr>
<td>1 x 10⁵</td>
<td>2</td>
<td>86.5</td>
<td>81.5-83.5</td>
<td>3</td>
<td>76.0</td>
<td>49.5-63.2</td>
</tr>
<tr>
<td>2 x 10⁵</td>
<td>3</td>
<td>76.0</td>
<td>66.4-80.0</td>
<td>3</td>
<td>52.7</td>
<td>47.2-58.6</td>
</tr>
<tr>
<td>4 x 10⁵</td>
<td>3</td>
<td>76.0</td>
<td>78.6-82.0</td>
<td>3</td>
<td>59.0</td>
<td>50.0-67.5</td>
</tr>
<tr>
<td>8 x 10⁵</td>
<td>3</td>
<td>66.0</td>
<td>62.0-72.0</td>
<td>3</td>
<td>65.0</td>
<td>56.0-66.0</td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>6</td>
<td>54.2</td>
<td>46.0-63.0</td>
<td>6</td>
<td>20.8</td>
<td>10.5-22.1</td>
</tr>
<tr>
<td>5 x 10⁶</td>
<td>1</td>
<td>46.0</td>
<td>46.0-63.0</td>
<td>1</td>
<td>31.5</td>
<td>17.5-25.5</td>
</tr>
<tr>
<td>2.5 x 10⁶</td>
<td>6</td>
<td>46.5</td>
<td>30.5-55.0</td>
<td>7</td>
<td>21.8</td>
<td>11.5-32.5</td>
</tr>
<tr>
<td>1.28 x 10⁶</td>
<td>5</td>
<td>52.5</td>
<td>28.2-37.0</td>
<td>6</td>
<td>16.5</td>
<td>9.2-24.0</td>
</tr>
</tbody>
</table>

The above experiments would have proved that the bile flow can be bypassed under conditions of heavy manganese loading, if the following were true: that the isotope lost by these animals had been actually excreted, rather than exchanged across their intestinal mucosas. Hence, in the experiments shown in Fig. 8, the manganous salt was injected intravenously to eliminate isotopic exchange across the gut.

The rate of loss of Mn⁴⁺ by the animals with biliary ligation was similar to the corresponding ones on the milk diet (Fig. 7), whereas the sham-operated controls showed a faster rate of loss. The obstructed ones showed accelerated excretion of Mn⁴⁺ only with doses higher than 50 µg Mn⁴⁺/animal, whereas the controls responded to all doses given. When 200 µg Mn⁴⁺ were given intravenously with rat serum or saline as the vehicle, the responses obtained were less pronounced than those found in the corresponding animals receiving 400 µg Mn⁴⁺ (Fig. 8) but the vehicle induced no differences.
DISCUSSION

The first demonstration to emerge of this work was that the intestine and some of its tributaries constitute a system of multiple excretory routes for manganese. This system contrasted to the kidney which excreted negligible amounts of metal even during jaundice and after loading with manganese. The routes which did excrete this metal had as their common origin the primordial gut. This embryological consideration evoked interest in the relative effectiveness of these routes, as discussed in a companion paper (a).

Additional findings pertained to several effects of surgery. These were generally opposite in direction to those induced by biliary obstruction. Hence, in the present experiments, the normal contribution of the liver to the excretion of manganese had been underrated because of the surgery employed. While attempting to explain these manifestations of stress, one might note the effects of administering cortisol or ACTH (15), since all were in the same direction.

The biphasic change of the concentration of Mn4+ in obstructed livers became important after it led us to show the central role of the liver in the distribution of this metal: the initial rise indicated movement of manganese from the intestine, whereas biliary obstruction impaired primarily the sensitivity with which excretion was normally regulated. Hence, in the presence of administering cortisol or ACTH (15), since all were in the same direction.

REFERENCES


