Role of liver in regulating distribution and excretion of manganese\textsuperscript{1,2}

P. S. PAPAVASILIOU, S. T. MILLER, AND G. C. COTZIAS
Medical Research Center, Brookhaven National Laboratory, Upton, New York

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\textsuperscript{55}/g) and tap water were offered ad lib., except that some groups were fed milk containing approximately 36 μg Mn\textsuperscript{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 Mn\textsuperscript{55} by clamping the hepatic artery and the portal circulation which regulates the excretion of manganese. The customary acceleration of excretion following moderate loads with manganese was abolished by blocking the bile duct. Acceleration could be induced in bile-ligated animals provided they were overloaded with metal. The sum of the data indicated that bile formation constitutes the main regulatory route under ordinary conditions, but, under conditions of overloading, auxiliary gastrointestinal routes participate.

\textsuperscript{1}This work was supported by the U.S. Atomic Energy Commission and, in part, by National Institutes of Health Grant OH 00159-03.

\textsuperscript{2}A preliminary report has been published (11).

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.

Received for publication 8 October 1965.
with rat serum aerobically at 20°C for 1 hr and the serum was injected intravenously.

The Mn⁵⁴Cl₂ (T₁₂₂0=31.4 days) was the carrier-free isotope used in earlier work (5, 15). Standardized doses of 1.0-1.5 μCi 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, 18). The precautions in obtaining the samples and the reasons for not computing "specific activities" of Mn⁵⁴ are indicated in the preceding paper (15). Figure 1, curve A, shows that the measurement of Mn⁵⁴ 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 2 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 Mn⁵⁴ 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 Mn⁵⁴ by rectal ligation. Normally, Mn⁵⁴ is excreted into the gastrointestinal tract (8), but this remained to be shown in jaundiced and in manganasc-loaded animals. Groups of four rats with rectal obstruction were given Mn⁵⁴ intravenously and manganasc sulfate by stomach tube (3.2, 6.4, and 12.0 mg Mn⁺⁺/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 Mn⁵⁴ 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 isotope very rapidly, possibly because it received significant amounts of metal from the gut.

The diminished excretion of Mn⁴⁺ following biliary ligation was expected to produce a rise of the isotope’s concentration in the liver. The unexpected findings and their explanation are dealt with below.

Fifteen animals with biliary obstruction were dissected at various intervals in parallel with 2 control groups of 15 laparotomized and 15 intact animals. Figure 3 shows that the radioactivity rose sharply and then declined in the livers of obstructed animals. This differentiated the obstructed group from its two control groups. Surprisingly, the livers of all operated animals differed from the nonoperated ones. The effects of surgery were not restricted to the liver, since the kidneys of these animals showed a significantly higher rate of loss than did the nonoperated controls. The other tissues tested did not show striking differences among these groups.

Several possible mechanisms could account for the striking rise and subsequent fall of the radioactivity in these livers. The first to be investigated was that 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 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 16 bile-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 uptakes 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 non-obstructed 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.

---

**FIG. 5.** Changes in concentration of Mn$^{54}$ in livers of rats. Data which precede zero time represent intact animals, the remainder laparotomized ones. Among the horizontal lines, dashed ones indicate the total envelope, solid ones the mean and 1 SD each, on the data obtained on the nonoperated animals. Asterisk represents one rat in which no evidence of obstruction was elicited in spite of ligation of the bile duct.

**FIG. 6.** Changes in the concentration of Mn$^{54}$ in selected tissues of laparotomized animals.

**FIG. 7.** Total-body retention curves of injected Mn$^{54+}$ in laparotomized rats with (A,B) and without (C,D) biliary ligation. At the arrow groups B and D received the indicated amounts of Mn$^{54+}$ in the milk.

*Regulation of the excretion rate of manganese.* Sixteen rats eating milk for 1 week were laparotomized, injected with tracer, and the bile duct was ligated in half of them. The ligated group showed again a distinctly slower rate of loss of Mn$^{54}$ from their bodies (Fig. 7). On the 3rd day of observation 2.2 µg stable Mn$^{55+}$ were added per milliliter of milk in four of the obstructed animals, whereas four of the sham-operated rats were offered a diet to which only 1.1 µg/ml had been added. The regimen of the remaining animals was not changed. Despite a larger dietary MnSO$_4$ supplement, the bile duct-
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 radioactivity in the gastrointestinal tract, kidneys, spleen, pancreas, testes, adrenals, and thoracic organs for each animal.

<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></td>
<td>Ligated</td>
<td></td>
<td></td>
<td>Non-ligated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5×10²</td>
<td>3</td>
<td>78.0</td>
<td>75.2-80.5</td>
<td>3</td>
<td>57.0</td>
<td>52.2-63.2</td>
</tr>
<tr>
<td>1×10³</td>
<td>2</td>
<td>82.5</td>
<td>81.5-83.5</td>
<td>3</td>
<td>56.0</td>
<td>49.5-63.2</td>
</tr>
<tr>
<td>2×10³</td>
<td>3</td>
<td>76.0</td>
<td>66.4-83.0</td>
<td>3</td>
<td>52.7</td>
<td>47.2-58.6</td>
</tr>
<tr>
<td>4×10³</td>
<td>3</td>
<td>86.0</td>
<td>78.6-93.5</td>
<td>3</td>
<td>59.8</td>
<td>52.0-67.5</td>
</tr>
<tr>
<td>8×10³</td>
<td>3</td>
<td>66.8</td>
<td>62.0-72.0</td>
<td>3</td>
<td>49.5</td>
<td>26.2-56.0</td>
</tr>
<tr>
<td>1×10⁴</td>
<td>6</td>
<td>54.2</td>
<td>45.5-69.0</td>
<td>6</td>
<td>20.8</td>
<td>19.5-22.1</td>
</tr>
<tr>
<td>2×10⁴</td>
<td>3</td>
<td>46.0</td>
<td>42.6-60.5</td>
<td>3</td>
<td>92.5</td>
<td>77.5-75.5</td>
</tr>
<tr>
<td>3×10⁴</td>
<td>6</td>
<td>46.5</td>
<td>30.5-52.5</td>
<td>7</td>
<td>21.8</td>
<td>11.5-32.5</td>
</tr>
<tr>
<td>6×10⁴</td>
<td>6</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>
<tr>
<td>1.28×10⁵</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></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

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 9 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, rather than exchanged across their intestinal mucosas. Hence, in the experiments shown in Fig. 8, the manganese 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 in 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 have 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 tissues to the liver, which was confirmed by excluding the liver from receiving initially the injected radioisotope. Transport in the opposite direction was shown by the evidence summarized in Table 1. In addition, some of the absorbed metal must be normally transported promptly into the bile, in view of the decline of radioactivity and increased manganese concentration which followed biliary obstruction (Figs. 3 and 5).

Several intriguing observations pertained to the regulation of excretion: rectal obstruction abolished the excretion, whereas biliary obstruction impaired primarily the sensitivity with which excretion was normally regulated. Furthermore, preventing the liver from receiving the initial bulk of tracer did not prevent the animals from excreting it, although their livers were accumulating instead of losing radioactivity. Consequently, although these results proved the existence of more than one excretory route (6, 13), they raised valid questions regarding the identity of the tissues drained by each.

The sum of these and some earlier findings (7) indicates that the absorbed manganese reaches the liver, becomes localized in its mitochondria (14, 16, 17, 20), and, although some metal becomes distributed to the tissues, a significant fraction is discharged into the bile. It remains to be seen to what extent this circulation is completed by reabsorption, whether its pump is located in the mitochondria and, if so, of which tissues. The latter is worth ascertaining since the liver was shown by these investigations to be instrumental in the homeostasis of this metal.

The authors thank Mr. Alexander R. Nicholson for securing data on distribution and turnover and for their statistical analysis, and Miss Judith Edwards for analysis of some of the tissues.

REFERENCES