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Studies on Poisonous Metals. X.[1] Metabolic Fate of Manganese after Oral Administration of Excessive Manganese Chloride in Rats

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The metabolic fate of manganese after the oral administration of excessive manganese chloride in rats was studied. After a single oral administration of manganese chloride (100 mg Mn/kg), the manganese levels in most tissues temporarily increased and then rapidly decreased to the control values within 24—48 h; however, the manganese levels in the pancreas, brain, and bone were considerably higher than the control values even at 48 h after the administration. The tissue levels of manganese in rats given a single oral administration of 200 or 400 mg Mn/kg were markedly higher than those after 100 mg/kg and remained much higher than the control and 100 mg Mn/kg values at 24 h after the administration. The increase in the tissue retention of manganese at such high doses was found to be due to a decrease in the biliary excretion of manganese, which is the major excretion route of the metal. On the other hand, when manganese (100 mg Mn/kg) was given every other day for three months, little tissue accumulation of manganese was observed in rats.

Keywords——manganese; absorption; distribution; excretion through bile and gastrointestinal mucosa; rat

Manganese is an essential metal for animals, but excessive exposure to manganese through inhalation has been shown to have effects on the lungs,[2-6] and, in addition, to cause an irreversible brain disease, somewhat similar to Parkinson's disease.[2,7-9] Exposure to manganese metal and salts primarily occurs through inhalation in industrial plants and mines.[2-4,10] However, Kawamura *et al.*[11] reported that systemic effects, such as lethargy and mental disturbance, were observed in humans following long-term ingestion of water which had been contaminated with maganase dissolved from dry cell batteries buried near the wells. Most of the information on the fate of orally administered manganese has been derived from research with the ingestion of minute doses of manganese salts.[12-14] Therefore, this study was designed to examine the metabolic fate of manganese in rats after single or continuous oral administration of excessive manganese chloride.

Experimental

Materials——Manganese chloride was obtained from Wako Pure Chemical Industries, Ltd. All other chemicals were of reagent grade.

Single Oral Administration Experiment——Male Wistar rats, weighing about 150 g, were fasted for about 20 h with drinking water *ad libitum* prior to use. Manganese chloride (100—400 mg Mn/kg) was given to the rats as an aqueous solution (10—40 mg Mn/ml) by stomach tube. The rats were housed in individual metabolic cages with water *ad libitum* and the urine was collected. The rats were killed with urethane at designated times, and various tissues were collected.

Continuous Oral Administration Experiment——Male Wistar rats, weighing about 150 g, were housed in individual metabolic cages. Manganese chloride (100 mg Mn/kg) was given to the rats as an aqueous solution

(10 mg/ml) by stomach tube every other day for three months. The rats were killed with urethane 3 h or 48 h after the final administration and various tissues were removed.

***In Situ* Rat Biliary Excretion Experiment**——Male Wister rats, weighing about 200 g, were anesthetized with ethyl ether and the bile duct was cannulated with polyethylene tubing (PE 10) as described previously.[15] After the oral or intraperitoneal administration of manganese, each rat was housed in a Bollman cage with water *ad libitum*, and the bile was collected at designated times.

Gastrointestinal Excretion Experiment——Male Wistar rats, weighing about 200 g, were fasted for 20 h prior to use, but drinking water was allowed *ad libitum*. The rats were anesthetized with urethane and the gastrointestinal tract was exposed by a midline abdominal incision. The bile duct was cannulated with polyethylene tubing. The cardia of the stomach was ligated and the pylorus was ligated around a short polyvinyl cannula equipped with a stopcock. The gastric lumen was washed with saline. Then 2 ml of saline was introduced into the stomach and the stopcock was closed. Two L-shaped glass cannulae were inserted at the duodenal and ileal ends and the cannulae were ligated. Saline was passed slowly through the gut and out of the ileal cannula and discarded untill the effluent solution was clear. Then, 10 ml of saline was immediately introduced into the intestine by means of the syringe.

Bile was collected continuously for 2 h after the intraperitoneal administration of manganese. Then, at 2 h after the administration, the stomach and small intestinal contents were sampled separately by withdrawing the luminal solutions into the syringe.

Analytical Procedures——The blood and urine samples were diluted with 1 N HNO_3. The tissues were wet-ashed by the $HClO_4$–HNO_3 method described previously.[16] Then, the content of manganese in the specimens from blood and urine was determined by Zeeman atomic absorption spectrometry using a Hitachi graphite furnace atomizer, model 170-70. Manganese in the specimens from other tissues was chelated with sodium diethyldithiocarbamate, extracted with methyl isobutyl ketone, and determined by the use of a Shimadzu AA-610S flame-type atomic absorption spectrometer.

Results and Discussion

Single Oral Administration of Manganese

Figure 1 shows the manganese contents in various tissues of rats given a single oral administration of manganese chloride (100 mg Mn/kg). The blood level of manganese was maximum at 3 h after the administration and then rapidly decreased, indicating rapid distribution of manganese into the various tissues. The maximum levels of manganese in the liver, kidney, lung and heart were observed at 1—3 h after the administration, and the levels then decreased rapidly with time, returning to approximately the control levels at 24—48 h later. The manganese levels in the spleen, pancreas and bone were also maximum at 1—3 h after the administration but decreased only slowly. The manganese levels in the liver, kidney and pancreas were much higher than those in the other tissues. The manganese levels in the testis and brain reached the highest values at 5 and 24 h, respectively, after the administration, although the levels were much lower than in the other tissues. Such decreased accumulation of manganese in these two organs might be due to the blood-brain and blood-testes barriers.[17-19] The urinary excretion of manganese was small (about 1.14 μg in a 48 h period).

Thus, the manganese levels in most tissues of rats given the excessive manganese temporarily increased and then rapidly decreased to control values, but the manganese levels in the pancreas, brain and bone were considerably higher than the control values even at 48 h after the administration, indicating much slower elimination of maganese from these organs, as is the case after the administration of small amounts of manganese.[20,21]

Moreover, we determined the tissue distribution of manganese at 3 h and 24 h after a single oral administration of excessive manganese chloride (200 or 400 mg Mn/kg) (Table I). The levels of manganese in the pancreas, spleen, heart and lung at 3 h after the administration of 200 mg Mn/kg were 5—20 times those after the administration of 100 mg/kg (Fig. 1). The administration of 400 mg Mn/kg resulted in a marked increase in the manganese levels in the tissues, such as heart, bone, spleen and lung. However, the manganese level in the liver did not increase with increase in the dose of manganese, suggesting that only a limited accumulation of manganese in the liver is possible, as reported by Johnson.[22] At 24 h after the

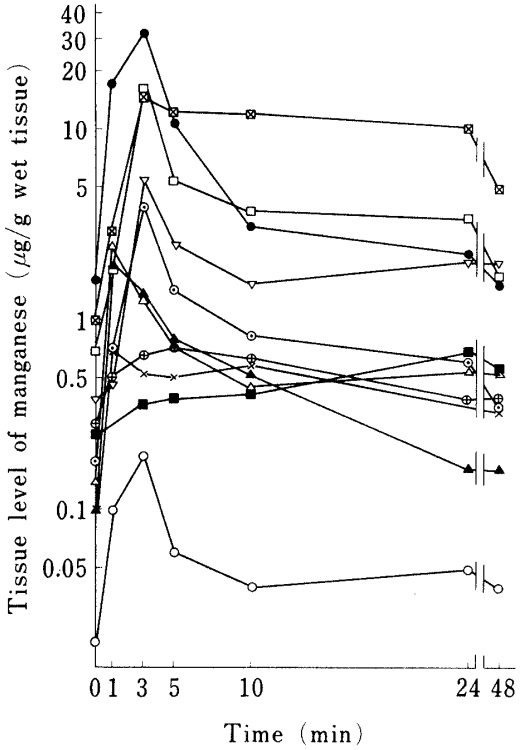

Fig. 1. Tissue Levels of Manganese after a Single Oral Administration of Manganese Chloride (100 mg Mn/kg)

Each value is the mean for 3 to 6 animals.
○, blood; ●, liver; □, kidney; ⊠, pancreas; ▽, bone; ⊙, heart; ▲, lung; ■, brain; ⊕, testis; ×, spleen; △, carcass.

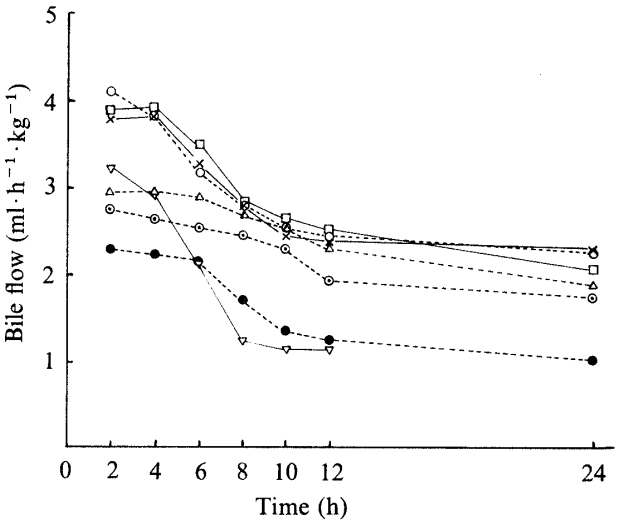

Fig. 2. Bile Flow following Oral or Intraperitoneal Administration of Manganese

Each value is the mean for 3 to 5 animals.
○, control; ×, 50 mg/kg, *p.o.*; □, 100 mg/kg, *p.o.*; ▽, 200 mg/kg, *p.o.*; △, 1 mg/kg, *i.p.*; ⊙, 5 mg/kg, *i.p.*; ●, 10 mg/kg, *i.p.*

TABLE I. Tissue Distribution of Manganese after a Single Oral Administration of Manganese Chloride (200 or 400 mg Mn/kg)

Tissue	Manganese (μg/g tissue)[a]			
	Control	3 h		24 h
		Dose (mg Mn/kg)		Dose (mg Mn/kg)
		200	400	200
Blood	0.02±0.01	1.52± 0.40	4.85± 0.25	0.56±0.16
Liver	1.57±0.21	42.36± 2.80	77.66±16.66	4.90±0.45
Kidney	0.69±0.12	32.87± 2.69	83.15± 9.86	7.43±0.96
Pancreas	1.00±0.11	62.77± 4.64	130.08±73.77	17.16±4.32
Bone	0.37±0.02	62.46±14.63	160.95±43.21	53.09±9.34
Heart	0.18±0.03	45.03±12.24	255.67±44.60	2.77±1.39
Lung	0.10±0.02	38.28±17.14	331.92±76.74	0.95±0.09
Brain	0.25±0.04	0.88± 0.17	2.54± 0.54	1.44±0.16
Testis	0.28±0.01	1.81± 0.06	4.08± 2.10	0.89±0.12
Spleen	0.10±0.06	1.00± 0.29	26.72±14.87	0.82±0.07
Carcass[b]	0.14±0.05	15.48± 1.46	50.31±10.87	6.39±0.97

a) Each value is the mean±standard deviation for 3 to 6 animals.
b) Residue after removal of the tissues described above and the gastrointestinal tract (including contents) from the whole body.

administration, on the other hand, the manganese level in the bone was much higher after the administration of 200 mg Mn/kg than after 100 mg Mn/kg, indicating relatively slow elimination of manganese. In addition, the tissues levels of manganese at 24 h after the administration of 200 mg Mn/kg were markedly higher than the control value, in contrast with the case of the administration of 100 mg Mn/kg.

To clarify the reason for the higher tissue levels of manganese after the administration of excessive manganese, we examined the biliary excretion of manganese, since this is the main excretion route of manganese,[23,24] in rats given a single oral administration of 50, 100, or 200 mg Mn/kg. As shown in Table II, the biliary excretion (% of the dose) of manganese during 24 h after the administration of 100 mg Mn/kg was nearly identical with that after 50 mg Mn/kg. The biliary excretion of manganese after the administration of 200 mg Mn/kg, in contrast, greatly decreased. In addition, Table II shows the biliary excretion and body retention of manganese during 24 h after a single intraperitoneal administration of 1, 5, or 10 mg Mn/kg. The biliary excretion (% of the dose) of manganese after the administration of 10 mg Mn/kg was greatly decreased compared with that after the administration of 1 mg Mn/kg. However, the body retention of manganese tended to increase with increasing dose of manganese. Witzleben *et al.*[25] have reported that an acute manganese overload (100—200 mg $MnSO_4$/kg, *i.v.*) in rats causes a rapid marked decrease in the ability of the liver to clear bilirubin into the bile and the rapid development of ultrastructural changes characteristically found in many instances of cholestasis. As shown in Fig. 2, a decrease in the bile flow rate was observed in the rats following the oral administration of 200 mg Mn/kg or the intraperitoneal administration of 1—10 mg Mn/kg. These results show that acute excessive doses of manganese decrease the biliary excretion of manganese, as a result of a decrease in the bile flow rate, resulting in an increase in the tissue retention of manganese.

It has been reported that upon the administration of excessive manganese, gastrointestinal routes other than the biliary route may participate in the excretion on manganese.[26] We examined the excretion of manganese through the gastrointestinal mucosa after the intraperitoneal administration of manganese chloride (1—10 mg Mn/kg). As shown in Table III, the small-intestinal excretion of manganese was substantial, although the gastric excretion was very small. An increase in the dose of manganese tended to decrease the biliary excretion of manganese and, in contreast, to incrase the small intestinal excretion of the metal. These results show that upon the administration of excessive manganese, the gastrointestinal mucosa is an important route for the excretion of manganese, as well as the bile.

TABLE II. Biliary Excretion and Body Retention of Manganese 24 h after a Single Oral or Intraperitoneal Administration of Manganese Chloride in Rats

	Dose (mg Mn/kg)	Manganese (% of dose)[a]	
		Bile	Body[b]
Oral	50	5.33±0.37	—
	100	5.47±0.97	0.29±0.01
	200	1.58±0.13[c]	3.90±1.72[c]
Intraperitoneal	1	68.70±4.16	10.07±5.23
	5	51.35±5.43[d]	17.39±7.05
	10	26.67±3.17[d]	25.32±7.81

a) Each value is the mean ± standard deviation for 3 or 4 animals.
b) Residue after removal of the gastrointestinal tract (including contents) from the whole body.
c) Significantly different from 100 mg Mn/kg, $p<0.05$.
d) Significantly different from 1 mg Mn/kg, $p<0.05$.

Table III. Excretion of Manganese through Bile and Gastrointestinal Mucosa after a Single Intraperitoneal Administration of Manganese Chloride in Rats

Dose (mg Mn/kg)	Manganese (μg) excreted[a] (% of dose)[b]		
	Bile	Small intestine	Stomach
Control	0.38 (0.15—0.61)	3.02 (2.20—3.85)	0.05 (0.04—0.05)
1	51.00 (44.00—58.00) (26.15)	3.41 (2.75—4.06) (1.75)	0.08 (0.07—0.09) (0.04)
5	86.49 (73.54—94.90) (8.65)	30.94 (28.31—37.19) (3.09)	0.43 (0.28—0.59) (0.04)
10	110.00 (102.00—118.00) (5.79)	52.75 (51.00—54.50) (2.78)	0.55 (0.50—0.60) (0.03)

Bile and luminal solutions were collected 2 h after the administration of manganese.
a) Each value is the mean (for 2 or 3 animals) with the range in parentheses.
b) Each value is the mean for 2 or 3 animals.

Table IV. Tissue Distribution of Manganese after Continuous Oral Administration of Manganese Chloride for Three Months

Tissue	Manganese (μg/g wet tissue)[a]		
	Control	3 h[b]	48 h[b]
Blood	0.03±0.01	0.22±0.01	0.08±0.01
Liver	2.11±0.27	15.74±4.10	2.01±0.21
Kidney	0.61±0.11	4.32±0.24	0.65±0.19
Pancreas	1.11±0.21	5.86±0.83	1.61±0.13
Bone	0.38±0.06	0.41±0.03	0.34±0.10
Heart	0.35±0.03	1.20±0.06	0.21±0.08
Lung	0.21±0.03	1.29±0.50	0.27±0.15
Brain	0.55±0.10	0.61±0.06	0.49±0.03
Testis	0.33±0.01	0.58±0.05	0.37±0.04
Spleen	0.25±0.02	0.57±0.14	0.20±0.10
Carcass	0.14±0.03	0.56±0.08	0.86±0.17

a) Each value is the mean ± standard deviation for animals.
b) Rats were killed 3 or 48 h after the final dose (100 mg Mn/kg).

Continuous Oral Administration of Manganese

As mentioned above, considerably increased levels of manganese were observed in some tissues of rats after a single oral administration of excessive manganese. We examined the tissue levels of manganese after continuous oral administration of excessive manganese chloride (100 mg Mn/kg) every other day for three months in rats. Table IV shows the tissue levels of manganese at 3 and 48 h after the final administration. At 3 h after the final administration, the manganese levels in the various tissues were considerably lower in the continuous administration group than in the single administration group (Fig. 1), except for the manganese level in the brain. At 48 h after the final administration, the tissue levels of manganese in the continuous administration group were nearly identical with those in the single administration group, except for the tissue levels in the kidney, pancreas and bone, which were somewhat lower in the single administration group. Since the final administration

TABLE V. Effect of Fasting on Tissue Distribution of Manganese 3 h after a Single Oral Administration of Manganese Chloride

Tissue	Manganese (μg/g wet tissue)[a]	
	Nonfasted	Fasted
Blood	0.18±0.02	0.19±0.05
Liver	4.43±0.88	31.00±3.69[b]
Kidney	4.74±0.43	12.81±4.58[b]
Pancreas	9.04±2.42	14.34±4.05
Bone	1.50±0.12	5.21±1.90
Heart	1.90±0.28	3.83±0.86
Lung	0.72±0.13	1.36±0.75
Brain	0.52±0.04	0.36±0.09
Testis	0.55±0.09	0.65±0.13
Spleen	0.54±0.02	0.52±0.15
Carcass	0.71±0.06	1.25±0.28

a) Each value is the mean±standard deviation for 3 to 6 animals.
b) Significantly different from nonfasted, $p<0.05$.

of the manganese in the continuous administration experiment was given to nonfasted rats, the difference in the tissue levels of manganese between fasted and nonfasted rats was examined. As shown in Table V, the manganese levels in various tissues, such as the liver, kidney, pancreas, bone, heart and lung were considerably lower in the nonfasted rats than in the fasted ones. Calcium and phosphorus[27] are known to inhibit the gastrointestinal absorption of manganese. In addition, it has been reported that dietary fibers can interfere with the absorption of metals, such as zinc, calcium and magnesium.[28] Accordingly, the lower tissue levels of manganese in the continuous administration group are considered to be due to the inhibition of gastrointestinal absorption of manganese by such components in the food. These results show that even when excessive manganese are given for a long period in rats, the tissue accumulation of manganese is extremely small. Further, we found no changes in the behavior or body weight gain in rats after continuous oral administration of excessive manganese chloride for three months (data not shown).

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