Effects of Cysteine on the Biological Actions of Cadmium in Rats

—Effects of Cadmium on the Fates of Copper and Zinc—

(Studies on the Fate of Heavy Metals in Animals. IV)

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Yukio TANAKA, Ryoichi TANAKA and Takashi KASHIMOTO

(Osaka Prefectural Institute of Public Health: 3-69, Nakamichi
1-chome, Higashinari-ku, Osaka, Japan)

The fates of cadmium, copper, and zinc in rats were examined after administrations of cadmium chloride (80 mg/kg/day) alone and cadmium chloride (80 mg/kg/day) with L-cysteine (1,500 mg/kg/day) for 20 consecutive weeks.

At this high dose of cadmium, cadmium levels in the kidney reached a plateau by the 6th week of administration in both groups, while cadmium levels in the liver reached a plateau of about 400–500 $\mu\text{g/g}$ at about 16 weeks in the cadmium chloride plus L-cysteine administration group and about 20 weeks in the cadmium chloride administration group. Urinary excretion of cadmium increased markedly in proportion to cadmium accumulation in the liver in both groups.

The fates of copper and zinc in rats during repeated oral administration of cadmium were examined by evaluating (1) intestinal absorption, (2) interactions with metallothionein and (3) urinary excretion. Copper levels in the organs (liver and kidney) and blood were found to decrease below the control values, while simultaneous administration of L-cysteine with cadmium suppressed the decrease in copper levels. The decrease of copper levels was considered to be attributable partly to cadmium-induced promotion of urinary copper excretion and mainly to cadmium-induced inhibition of intestinal copper absorption. Zinc levels in organs rapidly increased in the early period of cadmium administration and showed a gradual decrease at and after the middle period of the administration. Zinc levels in organs did not decrease below the control values.

Key words: cadmium; cysteine; metallothionein; copper; zinc; ceruloplasmin; copper deficiency; chemical form

Introduction

We showed in our previous report¹⁾ that the administration of cadmium (Cd) in a bound form, namely, the simultaneous oral administration of cadmium chloride and L-cysteine, has an effect on the intestinal absorption and accumulation of Cd and that, once taken into the body and captured as metallothionein (MT) in organs, the fate of Cd thereafter is the same as that of unbound Cd (inorganic form) on oral administration of cadmium chloride alone. Our previous report¹⁾ also showed that repeated oral administ-

ration of Cd in unbound form and bound form allowed Cd levels in the kidney to reach a plateau in 20 weeks in both groups while Cd levels in the liver did not reach a plateau.

In the present experiment, the Cd dose was increased so that Cd levels in the liver could reach a plateau, and the oral administration was continued for 20 weeks. The fates of Cd, copper (Cu) and zinc (Zn), and the effect of simultaneous administration of Cd and L-cysteine on hemobiochemical parameters were examined.

Materials and Method

1. Reagents

Cadmium chloride ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$), L-cysteine hydrochloride ($\text{Cys} \cdot \text{HCl}$) and analytical reagents used in the experiment were reagent grade chemicals or chemicals for harmful metal determination (Wako Pure Chemical Industries, Ltd.).

2. Preparation of samples for administration

Samples for repeated oral administration: The solution for oral administration of cadmium chloride was prepared by dissolving 1,600 mg of cadmium chloride in distilled water to make 100 ml. The solution for simultaneous oral administration of cadmium chloride and cysteine was prepared by dissolving 1,600 mg of cadmium chloride and 30.0 g of L-cysteine hydrochloride in distilled water to make 100 ml.

Solution for subcutaneous administration: 240 mg of cadmium chloride was dissolved in physiological saline to make 100 ml.

3. Animal experiments

Four-week-old Jcl: Sprague Dawley female rats were obtained from Clea Japan, Inc., raised at room temperature (about 23°C) and a humidity of about 50% for ten days, and then used in the experiments. MF solid feed (Oriental Yeast Industries, Ltd.) and tap water were given *ad libitum*.

In the repeated oral administration experiment, a total of 100 animals was divided into four groups, the Cd administration group ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ 80 mg/kg/day), the Cd and cysteine simultaneous administration group ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ 80 mg/kg/day + $\text{Cys} \cdot \text{HCl}$ 1,500 mg/kg/day), the control group, and the cysteine administration group ($\text{Cys} \cdot \text{HCl}$ 1,500 mg/kg/day), each consisting of 25 animals. Administration via a stomach tube was performed at about 10:00 A.M. five times a week for 20 weeks. The control group animals received distilled water. The Cd administration group (Cd group) animals received 1/2 dose of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ 80 mg/kg and the Cd and cysteine simultaneous administration group (Cd+Cys group) animals received 1/4 dose of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ 80 mg/kg + $\text{Cys} \cdot \text{HCl}$ 1,500 mg/kg for the first ten days. Body weight of the animals was measured every day during the experimental period. Three animals of each group were individually put into urine-feces

separation type metabolic cages to collect 24-hour urine samples once every two weeks. Five animals of each group were anesthetized with ether at 4, 8, 12, 16 and 20 weeks after the start of the administration and killed by cardiopuncture, then the blood and organs to be studied were removed.

In the subcutaneous administration experiment, five animals each were used in the Cd administration group ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ 6.0 mg/kg) and the control group. Subcutaneous administration was performed seven times on the back of the animals once every four days. The control group animals received physiological saline. The rats were sacrificed at 24 hours after the final administration, and the blood and organs to be studied were removed.

4. Determination of metal levels in organs, blood and urine

Metal levels in the organs and blood were determined by the low temperature ashing-atomic absorption method as reported previously.^{2),3)} Metal levels in the urine were determined by the sulfuric/nitric acid wet ashing-atomic absorption method.

5. Gel filtration of liver and kidney supernatant fractions

The liver and kidney excised from rats were homogenized with 5 volumes of ice-cold 0.25 M sucrose solution. The liver and kidney supernatant layers were obtained by ultracentrifugation (Hitachi Co., Ltd., 55-P-2) at $10,500 \times g$ for 1 hour, at 4°C . Then 3 ml of the supernatant fractions was applied to a Sephadex G-75 gel filtration column ($\phi 2.0 \times 60$ cm) and eluted with 0.01 M tris-HCl buffer, pH 7.4, 30 ml/hr. Fractions of 5 ml each were collected.

6. Hemobiochemical tests

The activities of serum glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined by the method of Lippi and Guidi.⁴⁾ The activities of serum lactic dehydrogenase (LDH), alkaline phosphatase (Al-P), leucine aminopeptidase (LAP) and γ -glutamyl transpeptidase (γ -GTP) were determined by the methods of Cabaud and Wróblewski⁵⁾, Kind and King⁶⁾, Goldbarg and Rutenburg⁷⁾, and Orłowski and Szewczuk⁸⁾, respectively. N-Acetyl- β -D-glucosaminidase (NAG) activity in urine was assayed with a kit

Fig. 1. Growth curve of female rats up to 20 weeks

—○—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); —●—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg); —△—: control (H_2O); —▲—: cysteine·HCl (1,500 mg/kg)

Table 1. Relative Organ Weights of Female Rats After Repeated Oral Administration for 20 Weeks

Group	Liver	Kidney	Spleen	Heart	Lung
Cd	$4.77 \pm 0.12^*$	$0.96 \pm 0.09^*$	$0.40 \pm 0.12^*$	0.29 ± 0.01	$0.53 \pm 0.06^*$
Cd+Cys	$5.07 \pm 0.57^{**}$	$1.18 \pm 0.08^{***}$	$0.53 \pm 0.14^{**}$	0.30 ± 0.02	$0.54 \pm 0.03^{**}$
Control	2.83 ± 0.27	0.73 ± 0.07	0.20 ± 0.04	0.28 ± 0.03	0.43 ± 0.06
Cys	3.02 ± 0.32	0.76 ± 0.06	0.19 ± 0.05	0.30 ± 0.02	0.42 ± 0.04

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); Cd+Cys: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg)

Control: Distilled water; Cys: cysteine·HCl (1,500 mg/kg)

The values each represent the mean $\pm$ S.D. for 5 rats (relative organ weight, %).

* $p < 0.05$ from Control

** $p < 0.05$ from Cys

*** $p < 0.05$ from Cd

(Shionogi & Co., Ltd.). Serum inorganic phosphorus level was determined by the method of Fiske and Subbarow⁹⁾. Serum ceruloplasmin activity was assayed by the method using Bandrowski base as a standard substance^{10), 11)}.

Results

1. Repeated oral administration experiment

1-1. General appearance

Except for two or three animals that died from suffocation at the time of oral administration, no deaths were observed in any group during the experimental period (20 weeks). However, loss of hair was observed in over 30% of the rats of the Cd group and the Cd+Cys group by about three weeks after the start of the administration. The severity of the

symptom varied among individual rats. Those with severe symptoms were similar in appearance to nude mice about eight weeks after the start of the administration. Aversion to the oral administration was more evident among the Cd group animals than among the Cd+Cys group animals.

1-2. Changes in body weight and organ weight

Fig. 1 shows changes in mean body weight over 20 weeks in each group.

Suppression of body weight gain was evident in the Cd group and the Cd+Cys group compared with the control group and the Cys group, respectively. However, no significant difference was observed in the suppression of body weight gain between the Cd group and the Cd+Cys group.

Table 1 shows relative organ weights. The

Fig. 2. Time course of cadmium concentration in the liver and kidney of rats during repeated oral administration for 20 weeks

(A): $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); (B): $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg)

Each point represents the mean $\pm$ S.D. for 5 rats.

Table 2. Cadmium Contents in Rat Organs After Repeated Oral Administration for 20 Weeks ($\mu\text{g/g}$)

Group	Liver	Kidney	Spleen	Heart	Lung	Blood
Cd	426.7 $\pm$ 38.4	185.7 $\pm$ 17.4	27.5 $\pm$ 5.6	12.2 $\pm$ 2.9	8.8 $\pm$ 1.3	0.77 $\pm$ 0.20
Cd+Cys	470.3 $\pm$ 42.7	189.5 $\pm$ 10.9	45.3 $\pm$ 7.0*	24.4 $\pm$ 2.9*	17.6 $\pm$ 4.5*	1.20 $\pm$ 0.11*
Control	0.04 $\pm$ 0.01	0.11 $\pm$ 0.03	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01
Cys	0.04 $\pm$ 0.02	0.12 $\pm$ 0.03	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); Cd+Cys: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + Cysteine·HCl (1,500 mg/kg)

Control: Distilled water; Cys: Cysteine·HCl (1,500 mg/kg)

The values each represent the mean $\pm$ S.D. for 5 rats.

* $p < 0.05$ from Cd

liver, kidney, spleen and lung weights were increased significantly in the Cd group and Cd+Cys group compared with the control group and the Cys group, respectively. The increase of kidney weight was more significant in the Cd+Cys group than in the Cd group.

1-3. Cd accumulation in organs

Cd accumulation curves for the liver and kidney were obtained after repeated oral administration for 20 weeks in the Cd group and the Cd+Cys group (Fig. 2). Cd contents in the organs and blood in each of the four groups were also examined after administration for 20 weeks (table 2).

The Cd accumulation curves for the liver and kidney (Fig. 2) show that the rate of Cd intake into organs was higher in the Cd+Cys group than in the Cd group. In the Cd+Cys group, Cd accumulation in the kidney reached a plateau in the 4th week of administration, while Cd accumulation in the liver reached a plateau in the 16th week of administration. In

the Cd group, the accumulation in the kidney reached a plateau in the 6th week of administration while the accumulation in the liver had almost reached a plateau in the 20th week of administration. Cd saturation levels of about 170–200 $\mu\text{g/g}$ and about 400–500 $\mu\text{g/g}$ were observed in the kidney and liver, respectively, in both groups. Similar Cd saturation levels were also observed after repeated oral administration for 28 weeks in both groups.

Cd levels in organs and blood after the administration for 20 weeks (Table 2) revealed no significant difference in the liver and kidney between the Cd group and the Cd+Cys group, whereas Cd levels in the spleen, heart, lung and blood were evidently higher in the Cd+Cys group than in the Cd group.

1-4. Changes of Cu and Zn in organs

The changes of Cu and Zn levels in the liver and kidney were examined in each of the four groups after the repeated oral administration for 20 weeks (Figs. 3 and 4), and the Cu and Zn

Fig. 3. Time course of copper concentration in the liver and kidney of rats during repeated oral administration for 20 weeks

—○—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); —●—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg); —△—: control (H_2O); —▲—: cysteine·HCl (1,500 mg/kg)

Each point represents the mean $\pm$ S.D. for 5 rats.

Fig. 4. Time course of zinc concentration in the liver and kidney of rats during repeated oral administration for 20 weeks

—○—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); —●—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg); —△—: control (H_2O); —▲—: cysteine·HCl (1,500 mg/kg)

Each point represents the mean $\pm$ S.D. for 5 rats.

Table 3. Copper and Zinc Contents in Rat Organs After Repeated Oral Administration for 20 Weeks

		(μg/g)					
		Liver	Kidney	Spleen	Heart	Lung	Blood
[Cu]	Cd	3.02 $\pm$ 0.47*	5.53 $\pm$ 0.74*	0.89 $\pm$ 0.24*	5.19 $\pm$ 0.37	1.28 $\pm$ 0.19*	0.32 $\pm$ 0.11*
	Cd+Cys	4.44 $\pm$ 0.93***	6.99 $\pm$ 0.71***	1.12 $\pm$ 0.18**	5.40 $\pm$ 0.42	1.36 $\pm$ 0.17**	0.60 $\pm$ 0.19***
	Control	5.91 $\pm$ 0.50	7.77 $\pm$ 0.72	1.47 $\pm$ 0.12	5.65 $\pm$ 0.37	1.86 $\pm$ 0.13	1.32 $\pm$ 0.21
	Cys	5.87 $\pm$ 0.33	8.12 $\pm$ 1.18	1.37 $\pm$ 0.13	5.55 $\pm$ 0.22	1.92 $\pm$ 0.10	1.26 $\pm$ 0.17
[Zn]	Cd	44.4 $\pm$ 5.4	27.0 $\pm$ 0.5	21.7 $\pm$ 1.0	16.9 $\pm$ 1.1	18.0 $\pm$ 0.9	4.0 $\pm$ 0.3
	Cd+Cys	41.4 $\pm$ 9.2	29.8 $\pm$ 2.6	21.5 $\pm$ 1.1	18.0 $\pm$ 0.9	19.5 $\pm$ 0.6	5.2 $\pm$ 0.5
	Control	38.5 $\pm$ 2.5	25.0 $\pm$ 1.1	19.5 $\pm$ 0.4	17.3 $\pm$ 1.0	19.1 $\pm$ 0.8	5.4 $\pm$ 0.2
	Cys	36.6 $\pm$ 3.5	26.4 $\pm$ 2.3	19.7 $\pm$ 0.5	16.4 $\pm$ 1.5	18.7 $\pm$ 0.6	5.5 $\pm$ 0.3

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); Cd+Cys: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg)

Control: Distilled water; Cys: cysteine·HCl (1,500 mg/kg)

The values each represent the mean $\pm$ S.D. for 5 rats.

* $p < 0.05$ from control

** $p < 0.05$ from Cys

*** $p < 0.05$ from Cd

Fig. 5. Sephadex G-75 gel filtration of liver and kidney supernatants after repeated oral administration for 4 weeks

(A): $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); (B): control (H_2O)

—○—: Cd; —△—: Cu; —●—: Zn

Fig. 6. Sephadex G-75 gel filtration of liver and kidney supernatants after repeated oral administration for 20 weeks

(A): $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); (B): control (H_2O)

—○—: Cd; —△—: Cu; —●—: Zn

contents in the organs and blood were also determined in each group (Table 3).

As shown in Fig. 3, the Cu level in the liver was evidently decreased in the 4th week of administration in the Cd group and the Cd+Cys group compared with the control group and the Cys group, and the decrease of Cu level was more evident in the Cd group than in the Cd+Cys group. The decrease of Cu level lasted from the 4th week until the 20th week of administration. On the other hand, Cu level in the kidney increased in the 4th week of administration in both the Cd group and the Cd+Cys

group, and then decreased gradually. As in the case of Cu in the liver, the decrease of Cu level in the kidney was also found more clearly in the Cd group than in the Cd+Cys group. At the 20th week of administration, the Cu levels in the liver, kidney, spleen, lung and blood were decreased significantly (Table 3) in the Cd group as compared with the control group, the Cu levels in the liver, spleen, lung and blood were decreased significantly in the Cd+Cys group as compared with the Cys group, and the Cu level decreases in the liver, kidney and blood were smaller in the Cd+Cys group than in the Cd

Fig. 7. Time course of cadmium in urine of rats during repeated oral administration for 20 weeks

—○—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); —●—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg) + cysteine·HCl (1,500 mg/kg)

Each point represents the mean $\pm$ S.D. for 3 rats.

group.

As regards Zn levels in the liver and kidney (Fig. 4), Zn levels rapidly increased in the 4th to 8th week of administration in both the Cd group and the Cd+Cys group, the increase being more evident in the liver than in the kidney, but the Zn levels began to decrease at and after the middle period of the administration, although they did not decrease below the levels in the control group and the Cys group even in the 20th week of administration. At the 20th week of administration (Table 3) the Zn levels in the liver, kidney and spleen were a little high in the Cd group and the Cd+Cys group as compared with the control group and the Cys group, but the Zn levels in the heart, lung and blood did not differ among the four groups.

1-5. Gel filtration of liver and kidney supernatant fractions

The liver and kidney supernatant fractions were subjected to Sephadex G-75 gel filtration every four weeks during the repeated oral administration to examine the distributions of Cd, Cu and Zn. Typical elution patterns obtained in the 4th and 20th weeks of administration in the Cd group rats are shown in Fig. 5 and 6, respectively.

The results of gel filtration at the 4th week of

administration (Fig. 5) show that, as described in the previous report¹⁾, most of the Cd supernatant fraction was present in the metallothionein (MT) fraction in the liver and kidney, that Cu in the liver was not as much distributed in the MT fraction as in the case of the control group, that Cu in the liver was rather decreased in a second fraction that was eluted earlier than the MT fraction, that Cu in the kidney was largely distributed in the MT fraction, and that Zn in the liver and kidney was increased in the MT fraction, the increase being particularly evident in the liver.

The results of gel filtration performed at the 20th week of administration (Fig. 6) show that most of the Cd supernatant fraction was present in the MT fraction of the liver and kidney, that Cd was also present in fractions in front of and behind the MT fraction, that Cu in the liver showed patterns similar to those found at the 4th week, that Cu distributed in the MT fraction of the kidney was decreased and showed a pattern similar to that in the control group, and that Zn was decreased in the MT fraction in both the liver and kidney as compared with results at the 4th week, though some of the supernatant fraction was still found in the MT fraction even in the 20th week of administration.

1-6. Changes in urinary excretion of Cd, Cu and Zn

Figs. 7 and 8 show the time courses of urinary excretion of Cd, Cu and Zn in the rats of each group.

The urinary excretion of Cd (Fig. 7) increased to levels ranging from about 0.1 $\mu\text{g/day}$ to about 1 $\mu\text{g/day}$ at 6 weeks in the Cd group and at 4 weeks in the Cd+Cys group, and rapidly increased to levels ranging from several $\mu\text{g/day}$ to about 20–40 $\mu\text{g/day}$ at 16 weeks in the Cd group and at 10 weeks in the Cd+Cys group.

The changes in urinary excretion of Cu and Zn (Fig. 8) show that the excretion of Cu started to increase gradually to levels ranging from about 4 $\mu\text{g/day}$ to about 8–10 $\mu\text{g/day}$ at the 14th week in the Cd group and in the 8th week in the Cd+Cys group, that the excretion of Zn in the Cd group remained almost the same as in the control group until the 14th week and started to increase in the 16th week, and that the excretion of Zn in the Cd+Cys group and the Cys

Fig. 8. Time course of copper and zinc in urine of rats during repeated oral administration for 20 weeks

—○—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); —●—: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg)+cysteine·HCl (1,500 mg/kg); —△—: control (H_2O); —▲—: cysteine·HCl (1,500 mg/kg)

Each point represents the mean $\pm$ S.D. for 3 rats.

Table 4. Biochemical Findings in Female Rats After Repeated Oral Administration for 20 Weeks

	Cd	Cd+Cys	Control	Cys
GOT (Karmen U)	177.8 $\pm$ 37.0*	206.2 $\pm$ 34.4**	76.0 $\pm$ 10.5	68.3 $\pm$ 11.7
GPT (Karmen U)	76.5 $\pm$ 16.5*	57.1 $\pm$ 8.9**	37.6 $\pm$ 7.7	35.9 $\pm$ 10.8
LDH (I.U/L)	589.1 $\pm$ 169.4*	753.1 $\pm$ 184.1**	110.1 $\pm$ 20.4	138.3 $\pm$ 25.8
Al-P (K.A.U.)	14.6 $\pm$ 4.1*	15.1 $\pm$ 6.2**	7.5 $\pm$ 2.1	8.8 $\pm$ 1.9
LAP (G.R.U.)	167.0 $\pm$ 14.5*	163.2 $\pm$ 27.6**	98.0 $\pm$ 27.4	89.7 $\pm$ 24.3
γ -GTP (mU/ml)	3.19 $\pm$ 0.61*	3.33 $\pm$ 0.94**	1.29 $\pm$ 0.30	1.31 $\pm$ 0.26
NAG (U/l)	20.7 $\pm$ 6.9*	25.7 $\pm$ 9.9**	8.5 $\pm$ 4.2	9.4 $\pm$ 5.1
Inorg. P (mg/dl)	7.7 $\pm$ 0.7*	8.4 $\pm$ 1.0**	5.0 $\pm$ 0.8	5.4 $\pm$ 0.9
Ceruloplasmin (mU/ml)	31.9 $\pm$ 9.9*	69.4 $\pm$ 15.4***	88.1 $\pm$ 12.3	92.5 $\pm$ 14.5

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg); Cd+Cys: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (80 mg/kg)+cysteine·HCl (1,500 mg/kg)

Control: distilled water; Cys: cysteine·HCl (1,500 mg/kg)

The values each represent the mean $\pm$ S.D. for 5 rats.

* $p < 0.05$ from control

** $p < 0.05$ from Cys

*** $p < 0.05$ from Cd

Table 5. Cadmium, Copper and Zinc Contents in Rats After Seven Subcutaneous Injections

($\mu\text{g/g}$)

	Cd		Cu		Zn	
Group	Liver	Kidney	Liver	Kidney	Liver	Kidney
Cd	356.7 $\pm$ 39.6	144.9 $\pm$ 30.9	9.10 $\pm$ 0.73*	27.96 $\pm$ 5.47*	91.9 $\pm$ 9.7*	34.0 $\pm$ 2.5*
Control	< 0.01	0.01	5.42 $\pm$ 0.57	5.16 $\pm$ 0.24	37.3 $\pm$ 3.8	23.0 $\pm$ 0.9

Cd: $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (6.0 mg/kg) $\times$ 7 times

Control: saline solution

The values each represent the mean $\pm$ S.D. for 5 rats.

* $p < 0.05$ from control

group increased from the 2nd week of administration and remained higher thereafter.

1-7. Hemobiochemical tests

Hemobiochemical tests were performed in each of the four groups after repeated oral administrations for 20 weeks. The results are shown in Table 4.

Significant declines in GOT, GPT, LDH, Al-P, LAP, γ -GTP, NAG and Inorg. P were observed in both the Cd group and the Cd+Cys group as compared with the control group and the Cys group. However, no significant difference was observed in these parameters between the Cd group and the Cd+Cys group. Ceruloplasmin activity was decreased significantly in the Cd group as compared with the control group. Ceruloplasmin activity in the Cd+Cys group was decreased a little, but not significantly, as compared with the Cys group.

2. Subcutaneous administration experiment

Cd was subcutaneously administered seven times to rats and then Cd, Cu and Zn levels in the liver and kidney were examined (Table 5).

Cd levels in the liver and kidney were a little lower than the saturation levels obtained after the repeated oral administration. Cu and Zn levels rose significantly in the liver and kidney. In particular, Cu levels in the kidney and Zn levels in the liver increased remarkably.

Discussion

We previously reported that the LD₅₀ values of cadmium chloride are 204.2 mg/kg for oral administration of Cd alone and 58.3 mg/kg for oral administration of Cd with cysteine simultaneously¹²⁾. The Cd dose used in the present experiment was about 2/5 of the LD₅₀ value for oral administration of Cd and exceeded the LD₅₀ value for oral administration of Cd with cysteine simultaneously. With the preliminary administration of a decreased dosage of Cd for the first 10 days in the present experiment (see Materials and Method), few deaths were observed during the repeated administrations for 20 weeks. It has been reported that the acute toxicity of Cd can be mitigated by preli-

minary Cd administration¹³⁾. A preliminary low dose of Cd to allow sufficient formation of metallothionein (MT) in advance may prevent immediate death from the administration of Cd at doses exceeding the LD₅₀ value. The results obtained in the present experiment indicate that MT has reducing effect on Cd toxicity¹⁴⁾.

In the 8-week repeated oral administration experiment in the previous report¹²⁾, significant differences in the body weight and organ weight could not be confirmed definitely because the dose of Cd was only 1/8 of that in the present experiment. The Cd dose in the present experiment produced clear suppression of body weight gain and organ hypertrophy (Fig. 1, Table 1). However, the presence or absence of the simultaneous administration of cysteine had little effects, and no influence of cysteine on the body weight gain or organ weight could be observed.

With the Cd dose in the previous experiment of 20 weeks¹¹⁾, Cd levels in the kidney reached a plateau but those in the liver did not. In the present experiment, the Cd dose was increased so that Cd levels in the liver could reach a plateau. In fact, Cd levels in the liver almost reached a plateau of about 400-500 $\mu\text{g/g}$ at 16 weeks in the Cd+Cys group and at 20 weeks in the Cd group (Fig. 2). The Cd saturation levels in the liver obtained after oral administration were a little higher than the levels of about 360-390 $\mu\text{g/g}$ ¹⁵⁾ obtained after subcutaneous administration. The Cd levels in the kidney and liver after administration for 20 weeks were saturated, while the gel filtration results (Fig. 6) showed the presence of most of the supernatant Cd in the MT fraction, and also in the fractions in front of and behind the MT fraction (not observed at the 4th week) (Fig. 5). Sato *et al.*^{15), 16)} found that the increase of Cd level is high in precipitate fractions and high molecular weight fractions other than MT fractions at the time of renal lesion development and they suggested that this condition may have a role in the development of renal lesion.

Cd toxicity cannot be discussed without taking into account many factors in addition to Cd. Therefore, the fates of Cd itself and also those of Cu and Zn, essential metals closely related to Cd toxicity, were examined in the present experiment. Nomiyama *et al.** examin-

* Nomiyama, H., Nomiyama, K.: Ind. Hyg. Soc. Japan, Abstracts of the 55th meeting, p. 614-615 (1982) (in Japanese).

ed in detail the changes in Cd, Cu and Zn levels in plasma, urine and organs in a subcutaneous Cd administration experiment for 21 weeks in rabbits. They found that urinary excretion of Cd and Cu increased in proportion to Cd levels up to saturation in organs and that the Cu levels in organs rose slightly at the early stage of administration and dropped thereafter to 1/3–1/2 of the control levels. They found that changes such as renal lesion developed at the time when urinary excretion of Cu increased and the Cu levels in organs decreased. As a result, they concluded that Cd intoxication might be due to the increase of Cu excretion caused by Cd administration, and the consequent Cu deficiency. A discussion of the fates of Cd, Cu and Zn may require consideration of the following factors: (1) intestinal absorption, which may be the rate-determining step in the body, (2) interaction after intake into the body (interaction with MT), and (3) urinary excretion. Changes in the urinary excretion of heavy metals (Figs. 7 and 8) observed after oral administration of Cd in the present experiment were an increase in urinary excretions of Cd and Cu in both the Cd group and the Cd+Cys group in proportion to the Cd levels in the liver up to saturation. The urinary excretion of Zn in the Cd+Cys group and the Cys group increased considerably at the early stage of administration because of the chelating action of cysteine, though the excretion of Zn in the Cd group remained the same as in the control group until the 14th week. As for heavy metal levels in organs, Cd levels in the liver (Fig. 2) reached a plateau earlier in the Cd+Cys group than in the Cd group, and a decrease of Cd levels in the liver and kidney, as reported by Nomiyama *et al.*^{*}, was not observed. This may be due to the fact that Cd was administered by the oral route in the present experiment. It is well known that Cu and Zn levels increase in proportion to Cd accumulation in organs of Cd-administered animals^{(17)–(20)}. In the present experiment (Fig. 3), Cu levels in the liver were decreased below the control in the 4th week of administration in both the Cd group and the Cd+Cys group. Nomiyama *et al.*^{*} considered that the decrease of Cu levels in organs observed in their subcutaneous administration experiment might be

due to the Cd-induced increase in the excretion of Cu. In the authors' oral administration experiment, the excretion of Cu increased at and after the 8th to 12th week of administration. Though Cd may be considered as one of the causes of the increase of Cu excretion, the excretion of Cu alone cannot be a sufficient explanation of the decrease in Cu levels in the liver at the 4th week of administration. Therefore, the possible inhibition of intestinal Cu absorption by Cd must be considered. This hypothesis was supported by the results obtained in the subcutaneous Cd administration experiment (Table 5). The subcutaneous Cd administration gave almost saturated Cd levels and nearly doubled Cu levels in the liver. Cu levels in the kidney (Fig. 3) slightly rose in the 4th week of oral administration, while the Cu levels in the kidney in the subcutaneous Cd administration experiment (Table 5) increased about fivefold. The difference was considered to be attributable to the inhibition of intestinal Cu absorption. Thus, as discussed above, Cu levels in organs are considered to be influenced by a balance of inhibition of intestinal Cu absorption by Cd (negative factor), Cu binding to and dissociation from thionein induced by Cd (positive and negative factors), and Cd-induced increase in the excretion of Cu (negative factor). Zn levels in organs are also considered to be influenced by a balance similar to that in the case of Cu levels. Therefore, Zn levels in organs were also investigated in the present experiment. It was found in both the Cd group and the Cd+Cys group that inhibition of intestinal Zn absorption by Cd was weak, that the affinity of thionein for Zn was higher in the liver than in the kidney (Fig. 5), and that the Cd-induced increase was weaker in the excretion of Zn than in the excretion of Cu (Fig. 8). (The remarkable increases in the excretion of Zn in the Cd+Cys group and the Cys group are attributable to the chelating action of cysteine.)

A noteworthy feature of the results obtained on the fates of Cu and Zn during oral administration of Cd is that the Cu levels in organs decreased significantly below the control levels as a result of a Cd-induced inhibition of intestinal Cu absorption and an increase in the excretion of Cu. It has been reported already

that Cd suppresses the absorption of Cu. Van Campen²¹⁾ found that Cd depressed ⁶⁴Cu uptake severely in tissues by means of dosing Cd : Cu at molar ratios of 150–600:1 via the duodenum in rats. Davies and Campbell²²⁾ showed that when Cd was added to the diet containing 2.6 mg Cu/kg to give a Cd:Cu molar ratio of 4:1, the absorption of ⁶⁴Cu into the whole body of rats was reduced. Starcher²³⁾ reported that when ^{115m}Cd and ⁶⁴Cu were given orally to chicks, the incorporation of ⁶⁴Cu into the liver was inhibited. However, it has not previously been reported that Cu levels in the liver and kidney decrease below the control during oral administration of Cd. In view of the fact that exposure of the human body to Cd mostly takes place by the oral route and that many humans, particularly the Japanese people, receive the majority of their Cd intake from rice in the diet, the inhibition of intestinal Cu absorption by Cd cannot be ignored in relation to Cd toxicity.

The effect of administration of Cd (unbound form) and simultaneous administration of Cd with cysteine (bound form) on the fates of Cd, Cu and Zn was examined. Cu levels in organs (Fig. 3) indicated that the inhibition of intestinal Cu absorption was suppressed in the Cd+Cys group. The suppression was considered to be attributable to the simultaneously administered cysteine. Though urinary excretion of Zn (Fig. 8) reached several times higher levels in the Cd+Cys group and the Cys group than in the Cd group and the control group at the early stage of administration, no difference in Zn levels in organs (Fig. 4) was observed among the four groups. This may indicate that, while Cd more or less inhibits the absorption of Zn and cysteine increases the excretion of Zn, administered cysteine accelerates the absorption of Zn. These results suggest that cysteine simultaneously administered with Cd may influence intestinal Cd absorption and accumulation in the body^{2),12)} as well as the absorptions of Cu and Zn from feed and their fates. Therefore, the model of Cd binding with cysteine may be useful for studies on the variations in toxicity due to differences in the chemical form of Cd in foods and in the foodstuff components.

The decrease below the control in Cu levels

in organs (copper deficiency) induced by the repeated oral administration of Cd may be an important factor in the study of the true character of Cd toxicity. Ceruloplasmin (CP), a copper enzyme synthesized in the liver, takes part in iron metabolism and catalyzes the oxidation of Fe (II) to Fe (III)²⁴⁾, so Cu deficiency causes anemia^{24),25)}. Cu deficiency is known to cause abnormal bone metabolism in animals through its effects on the copper enzymes lysyl oxidase²⁶⁾ and amine oxidase²⁷⁾. Other important copper enzymes are cytochrome oxidase^{28),29)} that takes part in the energy metabolism in mitochondria, and superoxide dismutase (SOD)³⁰⁾ that plays a role in superoxide radical metabolism. The activity of these enzymes may be affected to some extent by Cu deficiency. In the gel filtration (Fig. 6) of supernatant fractions of Cu-deficient liver and kidney, the first Cu peak was reported to be in fractions including CP, and the second Cu peak in fractions including SOD³¹⁾. The result of CP activity determination (Table 4) revealed that the activity was decreased significantly in the Cd group compared with the control group, corresponding with the results obtained in the gel filtration, where the first Cu peak tended to be low.

A decrease in Cu levels in organs was observed as a result of repeated oral administrations of high-dose Cd (almost LD₅₀) for 20 weeks and investigation of the fates of Cd, Cu and Zn, and suppression of the Cu level decrease was observed upon simultaneous administration of cysteine with Cd. The decrease in Cu levels was considered to be attributable mainly to the inhibition of intestinal Cu absorption by Cd. The mechanism of Cd-induced Cu deficiency and the toxicological and physiological significance of Cu deficiency may require further studies.

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