

# Reversibility of Cadmium-Induced Health Effects in Rabbits

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Twenty-one male rabbits were divided into three groups: rabbits of two groups were given pelleted food containing cadmium chloride at a dose level of 300  $\mu\text{g Cd/g}$  over periods of 44 or 19 weeks. Rabbits of the last group were given ordinary commercial pelleted food and served as controls. Cadmium increased urinary protein and amino acid by week 19 and increased it to a remarkably high level by week 44. After cessation of cadmium exposure, rabbits of the first group (44 weeks exposure group) showed only little recovery from cadmium health effects: proteinuria and aminoaciduria were slightly improved. Depressed hepatic functions were also slightly improved, but did not return to the control level in 24 weeks. Fat and bone metabolism also remained depressed below the control level. Anemia did not also readily recover. On the other hand, rabbits of the second group (19 weeks exposure) recovered from the effects of cadmium: proteinuria and aminoaciduria in most animals disappeared soon after the end of cadmium exposure, plasma GPT fell after 1 week, and hemoglobin and hematocrit returned to normal in 6-11 weeks. The above results show that after cessation of cadmium exposure, mild cadmium-induced health effects were reversible in a short period, while more severe effects were not readily reversible.

High performance liquid chromatographic (HPLC) profiles of renal and hepatic cadmium-thionein (Cd-MT) during and after exposure to cadmium showed no correlation to the degree of cadmium health effects, and therefore, did not help to elucidate mechanisms of the recovery from cadmium-induced health effects, probably because cadmium not bound with metallothionein (non-MT-Cd) is responsible for inducing renal effects.

## Introduction

Since Itai-Itai disease was officially established as a cadmium-induced disease (1), more than 50,000 residents of cadmium-polluted areas in Japan have taken health checks for preventing cadmium-induced renal effects and Itai-Itai disease. Episodes of cadmium pollution have been reported in the United States and European countries as well (2,3). Furthermore, some workers exposed to cadmium have been reported to suffer from cadmium health effects (4-6). Once residents of Cd-polluted areas or workers occupationally exposed to cadmium show effects by accumulating cadmium in the renal cortex at a critical concentration (7), the adverse health changes induced by cadmium seem essentially irreversible, because cadmium in the renal cortex hardly decreases due to its long biological half-time (7,8) even long after cessation of cadmium exposure.

The present authors, therefore, studied the reversibility of cadmium health effects in animals.

## Materials and Methods

Twenty-one male rabbits of the Japanese white strain weighing 2.8 kg were divided into three groups: 14 rabbits of the first cadmium group were given pelleted food containing cadmium chloride at a dose level of 300  $\mu\text{g Cd/g}$  for a period of 44 weeks, and four rabbits of the second cadmium group were given cadmium at the same dose for a period of 19 weeks. The remaining three rabbits were then given ordinary commercial pelleted food and served as controls. Animals of all groups were given ordinary commercial pelleted food to study the reversibility of the cadmium-induced health effects.

Urine and blood specimens were collected every 6 weeks for the biochemical determinations. Urinary protein and amino acids were analyzed by the Tsuchiya-Biuret method (9) and the trinitrobenzene sulfonic acid (TNBS) method (10), respectively. Urinary protein, glucose, ketone body, oc-

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cult blood, urobilinogen and acidity were evaluated by test tapes (Ames). Creatinine clearance ( $C_{Cr}$ ) and tubular reabsorption of phosphorus (% TRP) were calculated from values of urine and plasma creatinine and phosphorus, which were determined by the Folin-Wu method (11) and the Fiske-Subbarow method (12), respectively. Plasma urea nitrogen, uric acid and glucose were determined by the diacetylmonoxime method (13) uricase-peroxidase method (14) and *o*-toluidineboric acid method (15), respectively. To evaluate renal injuries, urinary enzymes were also determined by the same methods used for as plasma enzymes mentioned below. Plasma specimens were subjected to the determinations of plasma total protein, albumin to globulin ratio (A/G), GOT, GPT, LDH, alkaline phosphatase (ALP), and cholinesterase, respectively, by using reagent Total Protein AR (Wako), electrophoresis, Japanese Society for Digestive Organs method (16), Wroblewski method (17), Kind-King method (18) and benzoylcholine method (19). Cholesterol and triglyceride were assayed by using the cholesterol-FA test (Wako) and the triglyceride G-FA test (Wako). Plasma sodium and potassium were analyzed by flame photometry, calcium by atomic absorption spectrophotometry and chloride by chloridometry.

Cadmium and copper in urine specimens were determined by Zeeman effect atomic absorption spectrophotometry (20).

At intervals, animals were sacrificed to determine tissue cadmium and copper. A 1-g portion of tissue was ashed with nitric acid and sulfuric acid, and metal concentration was determined by atomic absorption spectrophotometry, subtracting the background with a deuterium lamp. For the tissue metallothionein (MT) composition analysis, tissues were homogenized in five volumes of 0.25 M sucrose—0.02 M Tris-HCl buffer (pH 8.0), and the homogenate was centrifuged at 40,000*g* for 60 min. A 500  $\mu$ L portion of the cytosol was subjected to high performance liquid chromatographic (HPLC) analysis. MT composition was analyzed by Suzuki's procedure (21) with the use of a Toyo Soda HPLC instrument, Model 803, equipped with a gel permeation column (Toyo Soda TSK GEL 3000, 21.5 mm diameter  $\times$  600 mm) and 0.05 M Tris-HCl buffer (pH 8.0) containing 0.1% sodium dodecyl sulfate at a flow rate of 3.5 mL/min. The eluate was directly introduced into a Varian-Techtron atomic absorption spectrophotometer, Model AA 1100, equipped with a background corrector BC 6. Atomic absorptions of heavy metals were continuously monitored. The chart speed was 2 mm/min.

## Results

### Body Weight

Rabbits of the first cadmium group, receiving Cd for 44 weeks, showed a depressed weight gain after week 8 and a decrease in weight after week 33 (Fig. 1). The weight decrease stopped after cadmium exposure stopped, and began to reverse 25 weeks after cadmium exposure.

Rabbits of the second group, which were given cadmium for 19 weeks, also showed a slight decrease in body weight after 14 weeks of cadmium exposure. Six weeks after cadmium exposure, body weight began to increase again.

### Urinary Volume

No cadmium-induced changes were observed during cadmium exposure, but urinary volume of the first cadmium group increased significantly 21 weeks after cadmium exposure.

### Urinary Protein

Urinary protein of the first cadmium group was elevated slightly after week 18 and elevated significantly after week 31 (Fig. 1). After the cessation of cadmium exposure, urinary protein decreased by half, but still remained high even at 34 weeks after cadmium exposure.

In the second cadmium group, urinary protein increased slightly and decreased upon the cessation of cadmium exposure. However, urinary protein was elevated again after the 23rd week of the recovery period.

### Urinary Glucose

Glycosuria was detected in two of six rabbits in the first cadmium group at the end of exposure, but glycosuria disappeared by 19 weeks after cadmium exposure. On the other hand, glycosuria was found in one of four rabbits in the second cadmium group at the end of exposure, but glycosuria disappeared on the day after cessation of cadmium exposure.

### Urinary Amino Acids

Urinary amino acids increased in the first cadmium group after week 18 (Fig. 1). Immediately after cessation of cadmium exposure, urinary amino acids decreased slightly, but it took 26 weeks for the depressed urinary amino acids to recover to the control level.

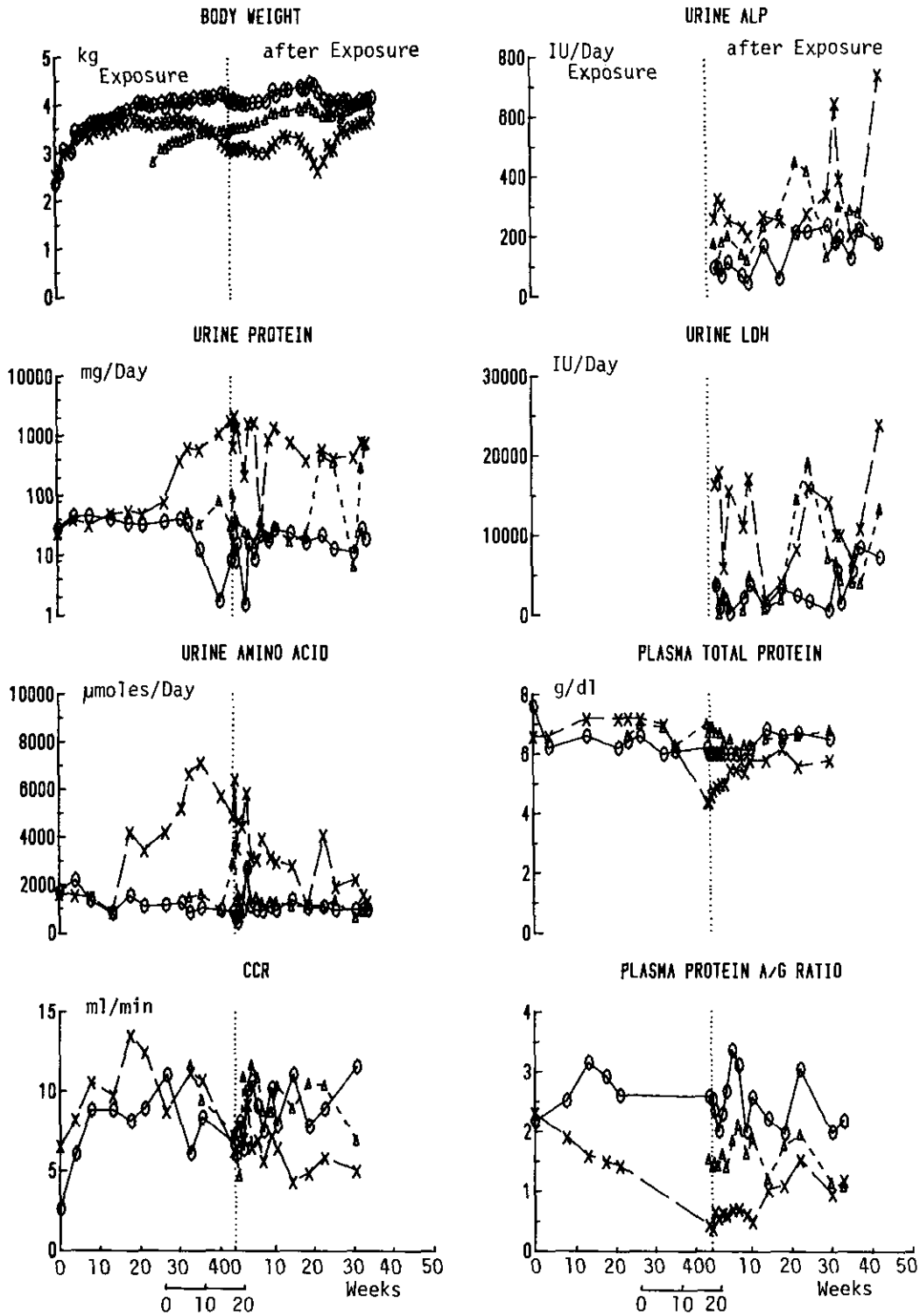


FIGURE 1. Body weight, urinary protein, urinary amino acid, creatinine clearance ( $C_{cr}$ ), urinary alkaline phosphatase (ALP), urinary LDH, plasma total protein and plasma protein albumin to globulin (A/G) ratio: (○) rabbits given cadmium for 44 weeks; (×) rabbits given cadmium for 19 weeks; (△) control rabbits.

Rabbits of the second cadmium group showed an increase in urinary amino acids after week 6, and the level decreased upon the cessation of cadmium exposure, reaching the control level by 17 weeks.

### **Creatinine Clearance ( $C_{cr}$ )**

Creatinine clearance of the first cadmium group was not affected during cadmium exposure, but was depressed after cessation of cadmium exposure (Fig. 1). Rabbits of the second cadmium group did not show any changes in creatinine clearance either during or after cadmium exposure.

### **Tubular Reabsorption of Phosphorus (%TRP)**

Cadmium had no effect on the tubular reabsorption of phosphorus in either the first or second cadmium groups.

### **Plasma Uric Acid and Urea Nitrogen**

No effects of cadmium were found.

### **Urinary Alkaline Phosphatase (ALP)**

Urinary ALP increased to two and three times as much as the control in the first and second cadmium groups, respectively, by the end of cadmium exposure (Fig. 1). Urinary ALP remained elevated after cessation of cadmium exposure in the first cadmium group.

### **Urinary GOT**

Urinary GOT was significantly elevated in the first cadmium group at week 44 of cadmium exposure and almost returned to the control level by 44 weeks after cessation of cadmium exposure. No changes were observed in the second cadmium group after cadmium exposure except for occasional elevations.

### **Urinary GPT and LDH**

Urinary GPT both in the first and second cadmium groups were elevated at the end of cadmium exposure, but recovered to the control level by 28 and 6 weeks after cadmium exposure, respectively.

The elevation and the recovery in urinary LDH in the first cadmium group was similar to urinary GOT (Fig. 1). No changes were observed in the second cadmium group.

### **Plasma Protein**

The plasma protein in the first cadmium group was 4.4 g/dL, depressed compared to the control level of 6.2 g/dL at the end of cadmium exposure (week 44) (Fig. 1). It barely recovered to the control level and remained at 5.8 g/dL 33 weeks after cadmium exposure. No changes were observed in the second cadmium group.

### **Plasma Albumin to Globulin (A/G) Ratio**

The plasma A/G ratio decreased significantly with the dose of cadmium, being 0.44 and 1.53 in the first and second cadmium groups, respectively, at the end of cadmium exposure, and 2.58 in the control group (Fig. 1). The depressed plasma A/G ratio recovered slowly, and the ratio of the first cadmium group still remained half that of the control group 36 weeks after cadmium exposure.

### **Plasma GOT, GPT and LDH**

No changes were observed in plasma GOT due to cadmium exposure. Plasma GPT was elevated in both the first and second cadmium groups at the end of cadmium exposure, but recovered to the control level 1 week after cadmium exposure (Fig. 2).

Plasma LDH of the first cadmium group increased after week 27 and was three times as high as that of the control group at the end of cadmium exposure (Fig. 2). It recovered fairly soon after cessation of cadmium exposure. No changes were observed in the second cadmium group.

### **Plasma Cholesterol and Triglyceride**

In the first cadmium group, plasma cholesterol was significantly elevated and was 10 times as high as that of the control level. It decreased slightly upon the cessation of cadmium exposure. It was still slightly higher than in the control level, even 34 weeks after the end of cadmium exposure. No changes were observed in the second cadmium group.

Similar changes were observed in plasma triglyceride (Fig. 2).

### **Plasma Sodium, Potassium and Chloride**

No changes due to cadmium exposure were observed in plasma sodium, potassium or chloride.

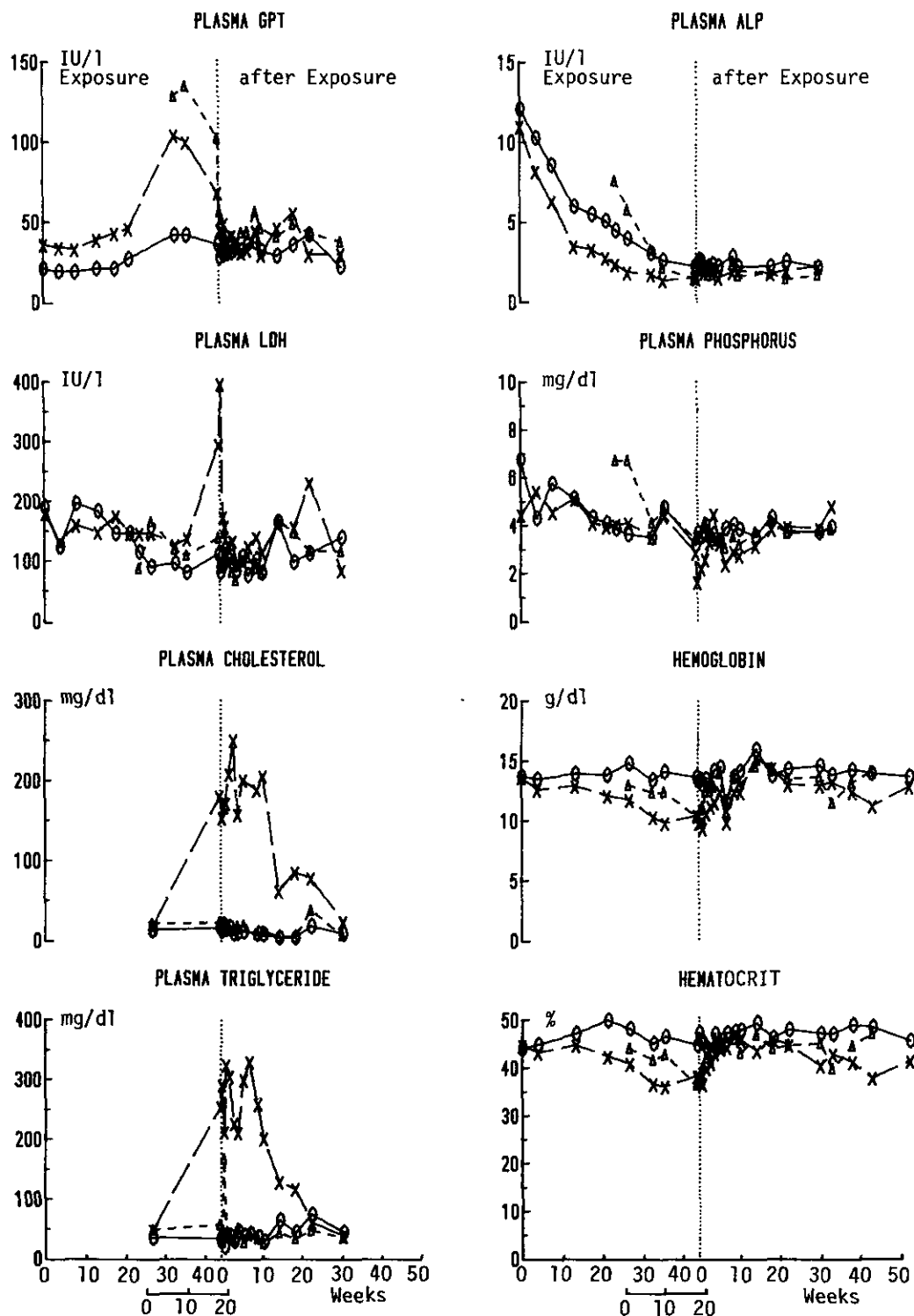


FIGURE 2. Plasma GPT, plasma LDH, plasma cholesterol, plasma triglyceride, plasma ALP, plasma phosphorus, hemoglobin and hematocrit. Symbols as in Fig. 1.

## Plasma Alkaline Phosphatase (ALP)

Plasma ALP was depressed by cadmium exposure and was half of the control level at the end of cadmium exposure (Fig. 2). It was elevated by the cessation of cadmium exposure, but still remained low for a long period. In the second group, the decreased plasma ALP recovered to the control level 1 week after cadmium exposure.

## Plasma Phosphorus and Calcium

Depressed plasma phosphorus was observed in the first cadmium group at the end of cadmium exposure (Fig. 2). It recovered slowly. No changes were seen in the second group. No specific changes due to cadmium exposure were observed in plasma calcium.

## Hemoglobin and Hematocrit

Hemoglobin of the first cadmium group decreased upon administration of Cd and was 10.5 g/dL (control, 13.7 g/dL) at the end of cadmium exposure (Fig. 2). Even though cadmium exposure ceased, hemoglobin continued to decrease for 1.5 weeks and then began to increase. It remained below the control level even 55 weeks after the end of cadmium exposure. In the second cadmium group, hemoglobin decreased to 10.4 g/dL at the end of cadmium exposure, but increased right after the cessation of cadmium exposure to reach the control level by 6 weeks after cadmium exposure.

Hematocrit was also depressed by cadmium (Fig. 2). At the end of cadmium exposure, Hct was 38% and 37% in the first and second cadmium groups, respectively, while it was 45% in the control group. It recovered to the control level in 11–17 weeks after cadmium exposure. Hematocrit in the first cadmium group was again depressed over a period of 55 weeks after the end of cadmium exposure.

## Urinary Cadmium

Urinary excretion of cadmium of the first cadmium group increased with the dose of cadmium and reached a maximum of 170  $\mu\text{g}/\text{day}$  at week 36 (Fig. 3). Immediately after the cessation of cadmium exposure, urinary excretion of cadmium decreased and reached a level of 30  $\mu\text{g}/\text{day}$  2.5 weeks after termination of cadmium exposure and then decreased slowly to a level of 2  $\mu\text{g}/\text{day}$  in 34 weeks.

Urinary excretion of cadmium of the second cadmium group (50–70)  $\mu\text{g}/\text{day}$  at week 19 de-

creased to 2–4  $\mu\text{g}/\text{day}$  level in 2.5 weeks upon the cessation of cadmium exposure.

## Urinary Copper

Urinary copper was elevated with the dose of cadmium in the first cadmium group, reaching a maximum at week 32 and then decreasing (Fig. 3). No further characteristic changes due to cadmium were observed during and after cadmium exposure.

## Organ Weight

The liver weight did not change due to cadmium exposure (Fig. 3). On the other hand, kidney weight, both the renal cortex and medulla, increased to two to three times the control level after 27 weeks of cadmium exposure. It did not recover to the control level even at 77 weeks after the cessation of cadmium exposure. The spleen weight increased far more markedly with the dose of cadmium, reaching a level five times the control level, but decreased upon cessation of cadmium exposure.

## Tissue Cadmium

Cadmium in the liver increased with the dose of cadmium, attaining a maximum level of 150  $\mu\text{g}/\text{g}$  in week 27 of cadmium exposure and remaining at this level until week 44 (Fig. 3). After the cessation of cadmium exposure, cadmium in the liver decreased very slowly. Cadmium in the renal cortex increased more markedly with the dose of cadmium to reach a maximum level of 300  $\mu\text{g}/\text{g}$  at week 9, and then it decreased gradually to 190  $\mu\text{g}/\text{g}$  by week 44. Upon cessation of cadmium exposure, cadmium in the renal cortex decreased to 20  $\mu\text{g}/\text{g}$  by week 27. Spleen cadmium was also elevated with the dose of cadmium to reach a maximum of 9  $\mu\text{g}/\text{g}$  at 18 weeks. Cadmium in the spleen decreased upon the cessation of cadmium exposure to half of the maximum level.

## Tissue Copper

Copper in the liver increased to 1.5 times that of the controls by week 18 and then decreased to a little below the control level (Fig. 3). Copper in the liver was maintained at the same level after cadmium exposure. Copper in the renal cortex was also increased by cadmium exposure to a maximum of 7  $\mu\text{g}/\text{g}$ , 1.5 times the control level, at week 18, and then it decreased by half by week 44. Upon the cessation of cadmium exposure, copper in the renal cortex continued to decrease.

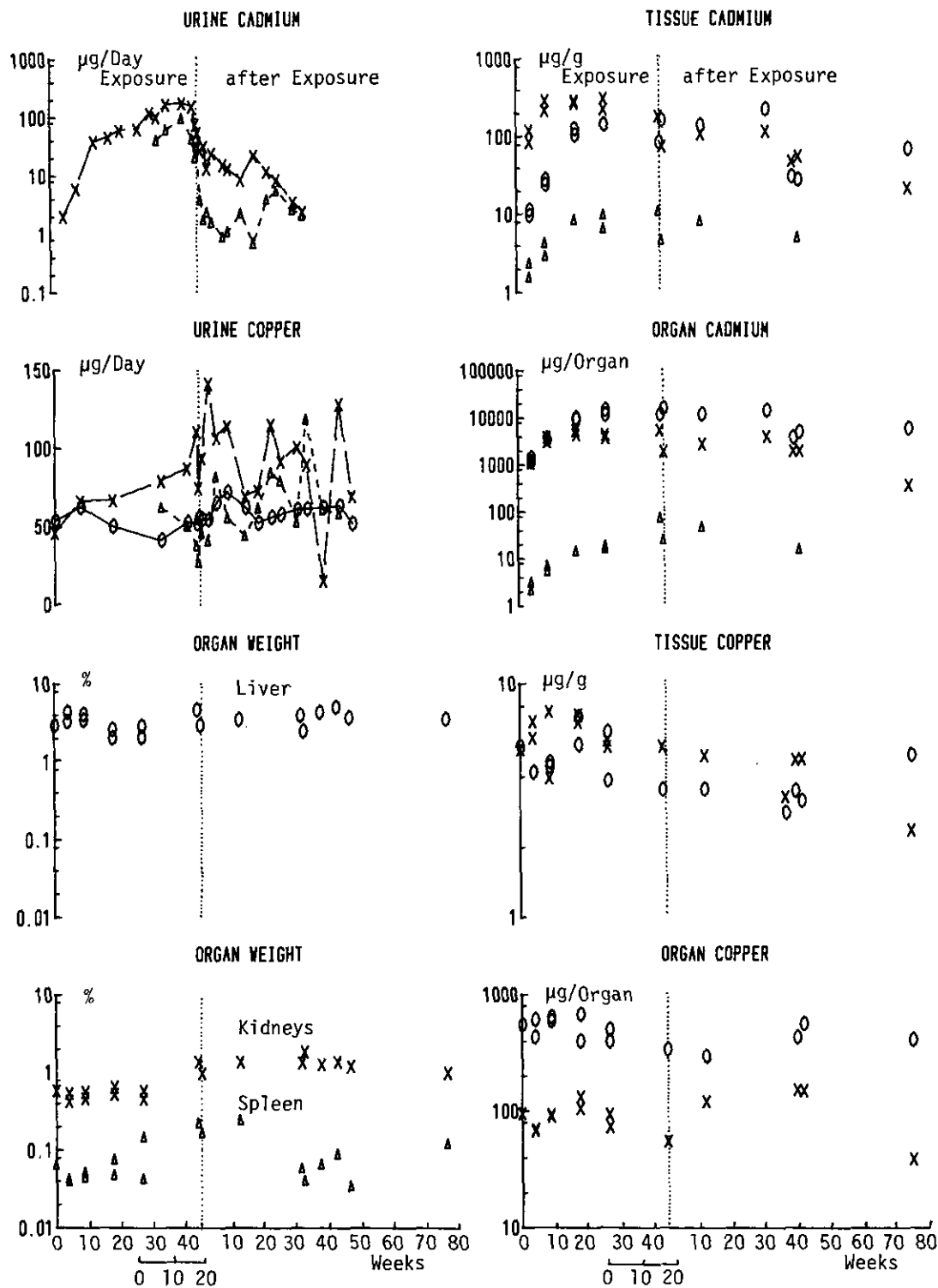


FIGURE 3. Urinary cadmium, urinary copper, organ weight, tissue cadmium, organ cadmium, tissue copper and organ copper: (○) liver; (×) kidneys (the renal cortex for tissue); (△) spleen.

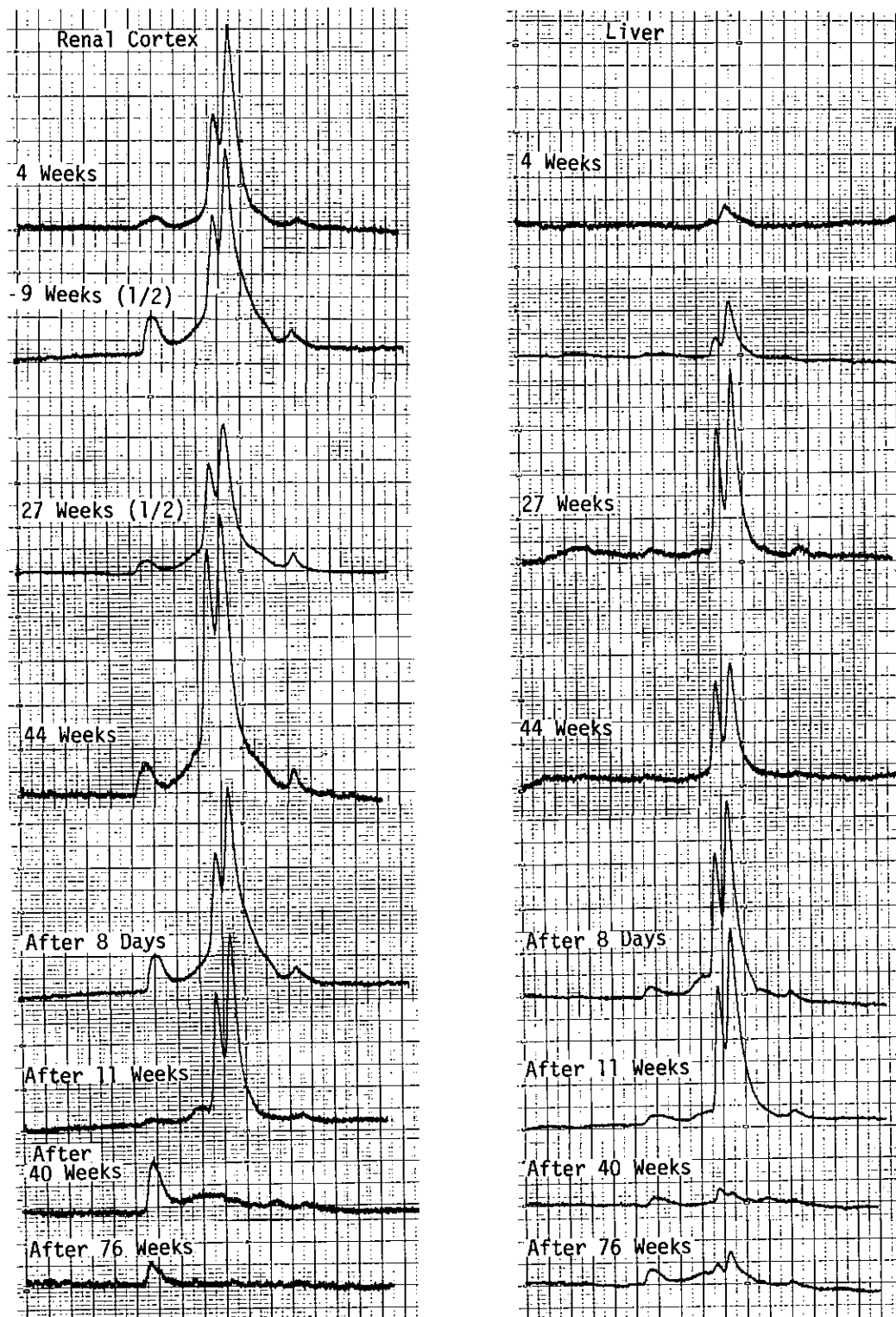


FIGURE 4. High performance liquid chromatographic (HPLC) profiles of cadmium in the liver and the renal cortex. See text for detail.



## HPLC Profiles of Tissue Cadmium-Thionein (MT)

HPLC analysis was performed only for tissue MTs of the first cadmium group (Fig. 4). Two apo-MTs, MT1 and MT2, were detected both in the renal cortex and the liver. High molecular weight cadmium -MT (HMW-Cd) and low molecular weight cadmium -MT (LMW-Cd) were found as well. Both MT1 and MT2 in the renal cortex increased with the dose of cadmium by 9 weeks and then began to decrease, regardless of continued cadmium administration. After exposure to cadmium, MTs were detected until week 11 but not detected in at 40 or 76 weeks. Almost the same concentration of HMW-Cd was detected in the renal cortex regardless of the stage of cadmium intoxication. LMW-Cd was detected during cadmium exposure and in the 1st week after cadmium exposure.

MT1 and MT2 in the liver increased with the dose of cadmium gradually. Even at 76 weeks after cadmium exposure, MTs were still detectable, though in small amounts. Small amounts of HMW-Cd and LMW-Cd were detected in the later stage of cadmium intoxication and in the period of recovery from cadmium intoxication.

## Discussion

### Renal Effects

Rabbits of the first cadmium group, which were exposed to cadmium over a period of 44 weeks, did not readily recover from renal effects following the cessation of cadmium exposure; proteinuria and aminoaciduria were slightly improved after cadmium exposure, but were still detected at week 34 after cadmium exposure. Glycosuria, which was detectable in some rabbits at the end of cadmium exposure, disappeared by week 19 after cadmium exposure. Urinary enzymes also did not readily recover to the control level long after cadmium exposure. On the other hand, the second cadmium group, which was exposed to cadmium over a period of 19 weeks, recovered from renal effects following the cessation of cadmium exposure: proteinuria and aminoaciduria disappeared soon after cadmium exposure in most animals. Enzymuria also disappeared soon after cadmium exposure.

Some of our previous papers (22-24) reported findings similar to those we described above. Rabbits that were given subcutaneous injections of cadmium chloride at a dose level of 1.5 mg Cd/kg/day over a period of 21 days recovered from renal effects after the end of cadmium exposure with a biological half-time of 13.5 days for urinary pro-

tein, 6.4 days for urinary amino acid, and less than 6 days for urinary glucose (22). In another group of rabbits that were given Cd Cl<sub>2</sub> subcutaneously at a dose level of 1.5 mg Cd/kg/day for 21 days, proteinuria disappeared 6-9 weeks after the end of cadmium exposure (22). Another group of rabbits was given pelleted feed containing cadmium chloride at a dose level of 300 µg Cd/g over a period of 18 months and then given ordinary pelleted feed in the following 7 months. After the end of cadmium exposure, glycosuria disappeared in 2 months and proteinuria in 5 months (23). Akahori et al. (24) also reported the reversibility of cadmium-induced renal effects in monkeys. Animals were given pelleted feed containing cadmium chloride at dose levels of 3, 10 and 30 µg Cd/g for 5 years and then given ordinary food for the following 6 months; no improvement in cadmium-induced nephropathy was observed, except that glycosuria disappeared after 3 months in a monkey given cadmium chloride at a dose level of 30 µg Cd/g.

These results may suggest that mild renal effects are reversible, but severe renal effects are irreversible. Results of the present experiment may help clearly to evaluate the different claims of Piscator (25) and Tsuchiya (26) on the reversibility of cadmium health effects in workers. Piscator (25) reported that severe renal effects in Swedish cadmium workers were irreversible or even aggravated after cessation of cadmium exposure. On the other hand, Tsuchiya (26) reported the contradictory observation that mild renal effects in Japanese cadmium workers were reversible when they were removed from exposure to cadmium. Both claims seem quite acceptable to us because our experimental results clearly indicated that severe renal effects were hardly reversible or aggravated after cadmium exposure, while slight renal effects were reversible.

### Localization of Cadmium-Induced Nephropathy

As mentioned before (27), since ALP is located in the renal proximal tubules and GOT and GPT are found in the renal distal tubules of rabbits, enzymuria is a good indicator for detecting the localization of renal injury in an early stage of intoxication. As urinary ALP increased more markedly than GPT and GOT after cadmium administration, cadmium was thought to injure proximal tubular cells more severely than distal tubular cells. Recovery from slight injury at the distal tubular cells seemed to occur earlier than recovery from severe injury at the proximal tubu-

lar cells, as urinary GPT recovered to the control level earlier than urine ALP did.

## Hepatic Functions

Except for our previous reports (23,24), no data are available on the recovery from cadmium-induced hepatic dysfunction. Rabbits were given pelleted feed containing cadmium chloride over a period of 18 months and then were given ordinary feed for the following 7 months. Cadmium elevated plasma GOT, GPT and LDH and depressed blood glucose and the plasma protein A/G ratio after 18 months. Upon the cessation of cadmium exposure, plasma GPT and LDH recovered to the control level in 1 month, and plasma GOT recovered in 5.5 months. No improvement was observed in the plasma protein A/G ratio or in plasma protein (23). Monkeys that received cadmium chloride orally for 5 years did not show any improvement in hepatic dysfunction 6 months after removal from cadmium exposure (24).

In the present experiment on rabbits, in the first cadmium group, plasma protein, protein A/G ratio, cholesterol and triglyceride did not readily recover, even at 34 weeks after cadmium exposure. Only plasma LDH returned to the control level fairly soon after the end of cadmium exposure. On the other hand, hepatic dysfunction in the second cadmium group readily returned to the control level; for example, plasma GPT recovered in 1 week, except that the plasma protein A/G ratio remained low even 36 weeks after cadmium exposure.

## Anemia

Except for our previous report (23), no information is available on the recovery from cadmium-induced anemia. Rabbits were given pelleted food containing cadmium chloride at a dose level of 300  $\mu\text{g Cd/g}$  over a period of 17 months and then given ordinary food for the following 7 months. Depressed hemoglobin and hematocrit were not improved even 7 months after cadmium exposure. Results of the present experiment indicate that some recovery from anemia and return of hemoglobin and hematocrit towards control levels occur immediately after cadmium exposure, but the levels remained depressed even at week 56 after cadmium exposure, especially in the first cadmium group. This might suggest that severe anemia due to cadmium is not readily reversible, even after the end of cadmium exposure.

## Tissue Metals

As shown previously (29), tissue metals such as

copper, zinc and cadmium increased in rabbits given cadmium daily by subcutaneous injections of cadmium chloride and then decreased a little earlier or at the time at which renal and hepatic dysfunction appeared in the animals. Similar changes were observed in tissue metals during cadmium exposure in the present experiment. However, no proof was obtained that tissue metals decreased as a result of renal and hepatic dysfunction or as a result of cessation of cadmium exposure. Tissue copper seemed not to decrease after the end of cadmium exposure. Owing to the recovery from renal and hepatic dysfunction, production of tissue metallothionein (MT) may increase again. Tissue MT may bind with metals in the liver and the kidney and may retain tissue cadmium and copper.

## Tissue Metallothioneins (MTs)

High-performance liquid chromatographic (HPLC) profiles of renal and hepatic cadmium-thioneins (Cd-MTs) during cadmium exposure was the same as those in our previous report (29) on rabbits given cadmium chloride subcutaneously on the back at a dose level of 0.5 mg Cd/kg/day over a period of 21 weeks: MT1 and MT2 increased the dose of cadmium until renal and hepatic dysfunction appeared. At 40 and 76 weeks after the end of cadmium exposure, MTs were barely detectable in the renal cortex and the liver. Cessation of cadmium exposure restores the ability of the kidneys and the liver to produce MTs which bind with cadmium. The loss of MTs at 40 and 76 weeks after the end of cadmium exposure, therefore, may be associated with the loss of this ability, but not with the recovery from cadmium intoxication. Cadmium may not exist as Cd-MT, but probably as high molecular weight cadmium (HMW-Cd) or other chemical forms of cadmium. Low molecular weight cadmium (LMW-Cd) was suggested in our previous paper (29) to be a causative agent for renal and hepatic dysfunctions. The present experiment might support the above suggestion, because the amount of LMW-Cd in the renal cortex is related to appearance and disappearance of renal dysfunction during and after the end of cadmium exposure. On the other hand, the amount of HMW-Cd was not associated with the degree of renal effects, even though HMW-Cd has been also suggested as a causative agent for cadmium renal effects (30,31).

The present HPLC studies on Cd-MTs did not help to elucidate mechanisms of cadmium-induced renal effects. Therefore, it might be necessary to study cadmium not bound with MT (non-

MT-Cd) (active cadmium), which might be responsible for inducing cadmium nephropathy (29), in future studies.

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