Environmental Health Perspectives
Vol. 54, pp. 21–30, 1984

Studies of Cadmium Uptake and Metabolism by the Kidney

by Kazuo T. Suzuki*

Our investigation was centered on a possible relationship between the toxicity of cadmium and changes in its chemical forms in tissues. Two models have been studied: one is the renal damage induced by a single injection of cadmium-containing metallothionein and the other is the renal damage induced by repeated injections of cadmium salt.

Parenteral loading of cadmium-containing metallothionein caused acute and transitory necrotic damage of renal tubular lining cells. This was explained by the selective and rapid uptake of metallothionein at the proximal tubules and degradation of the protein, resulting in liberation of cadmium ions.

Cadmium ions were injected repeatedly into rats, and the changes in the chemical forms of cadmium, zinc and copper in the liver and kidneys were correlated with the histological observations. The transitory necrotic damage of the proximal tubules caused during the repeated injections of cadmium was accompanied with a rapid decrease of the copper content in the kidney metallothionein. Further loading of cadmium ions induced increases in the amounts of cadmium not bound to metallothionein and its oxidation products as well as an increase of the Cd/Zn ratio in metallothionein. With these changes in the chemical forms of cadmium, persistent damage of the kidneys occurred.

The transitory renal damage caused both by a single injection of cadmium-containing metallothionein and by repeated injections of cadmium salt can be explained by a limit of the native biosynthetic capacity of metallothionein in the kidney, while the persistent damage appears to be due to a limit of the induced capacity.

Cadmium in living tissues is mostly bound to an inducible low molecular weight metal-binding protein, metallothionein, which is usually present as an intracellular soluble protein. Metallothionein is assumed to have several biological functions (*1*), the most important one being the protection of the organism from toxic effects of harmful heavy metals, especially from cadmium (*2–5*). In fact, cadmium bound to metallothionein is not toxic *in vitro,* and metallothionein does not show toxic effects as long as it remains an intracellular protein.

Although the biological half-life of cadmium is extremely long and cadmium is present as bound to metallothionein, the biological half-life of the apoprotein, thionein, is as short as those of usual proteins (*6–9*). This fact suggests that the toxicity of cadmium may be due to metal ions not bound to metallothionein and that the toxicity is possibly related to the capacity of metallothionein biosynthesis in tissues. Therefore, our approach was focused on a limit detection of the biosynthetic capacity of metallothionein and on the examination of changes in the chemical forms of cadmium after loading with cadmium in various ways.

We selected two models to clarify a possible relationship between the toxicity of cadmium and changes in its chemical forms: induction of renal damages by (a) parenteral loadings with cadmium-containing metallothioneins and (b) repeated injections of cadmium salts. The results of our investigations on the two models will be reviewed in this communication with some schematic presentations.

Renal Damage Induced by Single Injections of Cadmium-Containing Metallothioneins

One of the proposed biological roles of metallothionein is its function as a carrier protein for metals such as cadmium, zinc and copper, espe-

*National Institute for Environmental Studies, Yatabe, Tsukuba, Ibaraki 305, Japan.

cially from the liver to kidneys. Tanaka et al. (*10*) and Cherian and Shaikh (*11*) examined this biological role by administering parenterally an isolated and labeled metallothionein. In contrast to ionic cadmium, the metal bound to metallothionein was less effectively trapped by the liver and was mostly taken up by the kidney. The selective transfer of cadmium bound to metallothionein has been confirmed in many laboratories (*12–16*).

Nordberg et al. (*17*) and Cherian et al. (*18*) demonstrated that parenteral administration of metallothionein causes severe necrotic damage to the kidney. This observation contradicts one of the assumed biological roles of metallothionein, that is, the protection of the living body from harmful effects of cadmium by sequestering it as a stable complex. Two possibilities have been pointed out to explain the potent nephrotoxic action of parenterally administered metallothionein (*13,14,16–19*): one is the toxicity of metallothionein itself and the other is the toxicity of ionic cadmium liberated from metallothionein.

Our approach was to correlate the changes in the chemical forms of cadmium with the histological change of the renal tubular lining cells after administration of metallothioneins having various metal compositions. The metallothioneins used in our experiment were as follows (*14*): Cd,Zn-thionein (native rat liver metallothionein) (*20*), Cd,Cu,Zn-thionein (native rat kidney metallothionein) (*21*) and Cd-thionein (artifical rat liver metallothionein) (*22–24*). The results of our experiments can be summarized as follows.

Time-dependent changes in the chemical forms of cadmium in the kidney supernatant were traced by gel filtration chromatography after a single injection of cadmium-containing metallothioneins. Cadmium was selectively taken up by the kidney shortly after the administration, and the distribution profiles of cadmium in the supernatant fraction changed dramatically after the uptake. Cadmium was present in the metallothionein fraction at first, and then the metal changed the distribution to the high molecular weight protein fraction at the expense of the metal in the metallothionein fraction. Cadmium in the high molecular weight protein fraction started to decrease about 6 hr after injection, and the metal in the metallothionein fraction increased. Finally, most of cadmium was confined to the metallothionein fraction again. This change in the distribution of cadmium can be explained by the degradation and resynthesis of metallothionein in the kidney, because the cadmium in the high molecular weight protein fraction can be explained only by nonselective binding of the cadmium ions liberated from the degraded metallothionein (*25*).

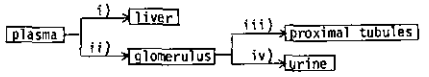
The degradation and resynthesis of metallothionein in the kidney was further examined by tracing the changes in the distribution profiles of zinc and copper after the injection of metallothionein. The distribution profiles of the two metals showed an identical or at least a similar change, even when metallothioneins of different metal compositions were injected. Although zinc was not found in the metallothionein fraction until most of the cadmium in the supernatant fraction was redistributed to the metallothionein fraction (the affinity of zing to metallothionein is less than that of cadmium), zinc was always present as a co-existing metal in the metallothionein fraction 1 day after injection. Furthermore, although copper is a major co-existing metal in the kidney metallothionein when cadmium ions are administered (*26,27*), copper was present only as a minor co-existing metal in the resynthesized metallothionein even when Cd,Cu,Zu-thionein was injected (*21*).

Parenteral administration of metallothioneins with differing cadmium/zinc ratios revealed that the extent of necrotic damage of the tubular linings depends on the amount of cadmium in the injected metallothionein but not on that of metallothionein (protein) (*24*).

The renal damage was found to be a transitory one, and the time course of the histological change was well correlated to the changes in the distribution profiles of cadmium in the kidney (*20,21,23,24*). As a result, we suggested that the toxicity of parenterally administered metallothionein is due to the cadmium ion liberated from the degraded metallothionein, the cadmium ions being found in the high molecular weight protein fraction. Furthermore, the metal composition in the resynthesized metallothionein was found to be different from that induced by loading of cadmium ions; the former contains zinc as a major co-existing metal (*20–24*), whereas in the latter copper is predominant (*26,27*).

Selective Transfer and Uptake of Cadmium Bound to Metallothionein by the Kidney

The selective transfer of cadmium to metallothionein to the kidneys is a clue to clarify the mechanisms of the renal damage caused not only by the injection of cadmium-containing metallothionein but also by exposure to cadmium salt



i) Cd-high molecular weight complexes (Cd-plasma proteins)

ii) Cd-low molecular weight complexes (MT, low molecular weight complexans) with higher stability constants than those to plasma proteins

iii) Cd-low molecular weight complexes that can be reabsorbed at the tubules such as MT

iv) Cd-low molecular weight complexes that are not reabsorbed at the tubules

FIGURE 1. Schematic presentation of cadmium distribution in tissues according to the molecular sizes and stability constants of cadmium complexes in plasma.

in chronic cadmium poisoning. For the selective transfer of cadmium to the kidney, cadmium is required to be bound in plasma to low molecular weight complexes that can be freely filtered through the glomeruli. At the same time the stability constants of the cadmium complexes need to be higher than those of cadmium-plasma high molecular weight proteins.

Metallothionein meets this prerequisite and is selectively transferred to the kidney. On the other hand, cadmium ions in plasma are mostly bound to the high molecular weight proteins and are effectively trapped by the liver (*25*). When low molecular weight chelating agents work as an effective vehicle for cadmium, the distribution of the metal between the liver and kidney is altered, and the metal is more effectively transferred to the kidney than to the liver. The distribution of cadmium between the high molecular weight proteins and low molecular weight chelating agents in plasma is certainly also affected by the molar ratio of cadmium to the chelating agents. The predominant transfer of cadmium to the kidney in the case of higher cysteine ratios (*28*) supports this assumption.

Reabsorption mechanisms of cadmium complexes at the proximal tubules will have to be investigated further; however, that of metallothionein has been studied in detail (*12*).

The tissue distribution of cadmium is schematically shown in Figure 1, based on the discussion above and our data that support this discussion (*29,30*).

Renal Damage Induced by Repeated Injections of Cadmium Salt

Repeated injections of cadmium salt resulted in the accumulation of cadmium in tissues, especially in the liver and kidney. This investigation

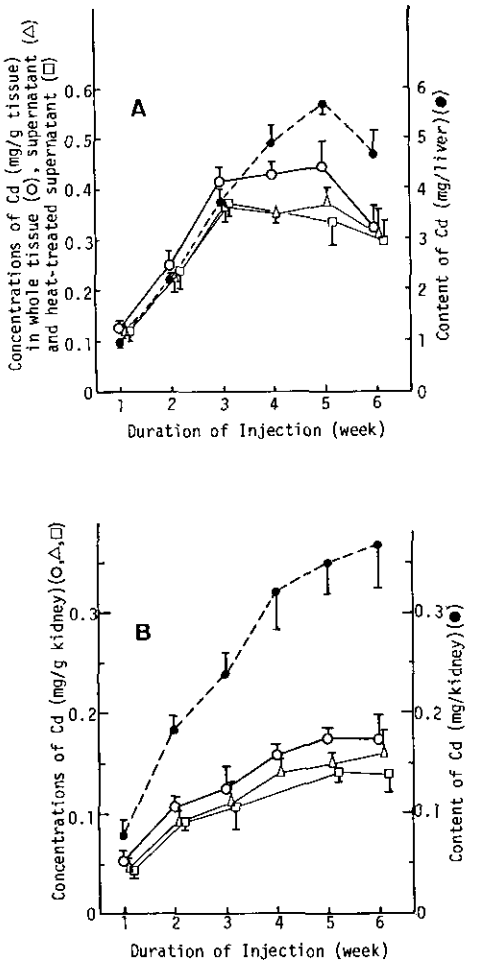


FIGURE 2. Changes in the concentrations and content of cadmium in (*A*) the liver and (*B*) kidneys of rats induced by injections of cadmium salt (*33*). Cadmium chloride was injected subcutaneously into 30 female rats of the Wistar strain (8 weeks old at the beginning of the injection) at the dose of 3.0 mg Cd/kg body weight, four injections a week up to 6 weeks. The rats (five heads/group) were killed 3 days after the last injection in each group. Portions of the liver and kidneys of each rat were homogenized in nine volumes of an extraction buffer (0.1 M Tris/HCl, pH 7.4, containing 0.25 M glucose; dissolved oxygen gas was removed before use by bubbling nitrogen gas) in an atmosphere of nitrogen gas using a Polytron homogenizer. The homogenates were centrifuged at 170,000*g* for 60 min; 2 mL portions of the supernatants were heated at 70°C for 10 min and then centrifuged at 2300*g* for 10 min after cooling in an ice-water bath. Concentrations of cadmium in the homogenates (–○–), supernatants (–△–), and heat-treated supernatants (–□–) multiplied by 10 are expressed as means ± SD of five samples and plotted versus duration of cadmium injections. The concentrations of cadmium were determined simultaneously with other metals by inductively coupled plasma-atomic emission spectrophotometry (ICP-AES). Contents of cadmium (–●–) in the liver and kidneys were also expressed as means ± SD of five samples.

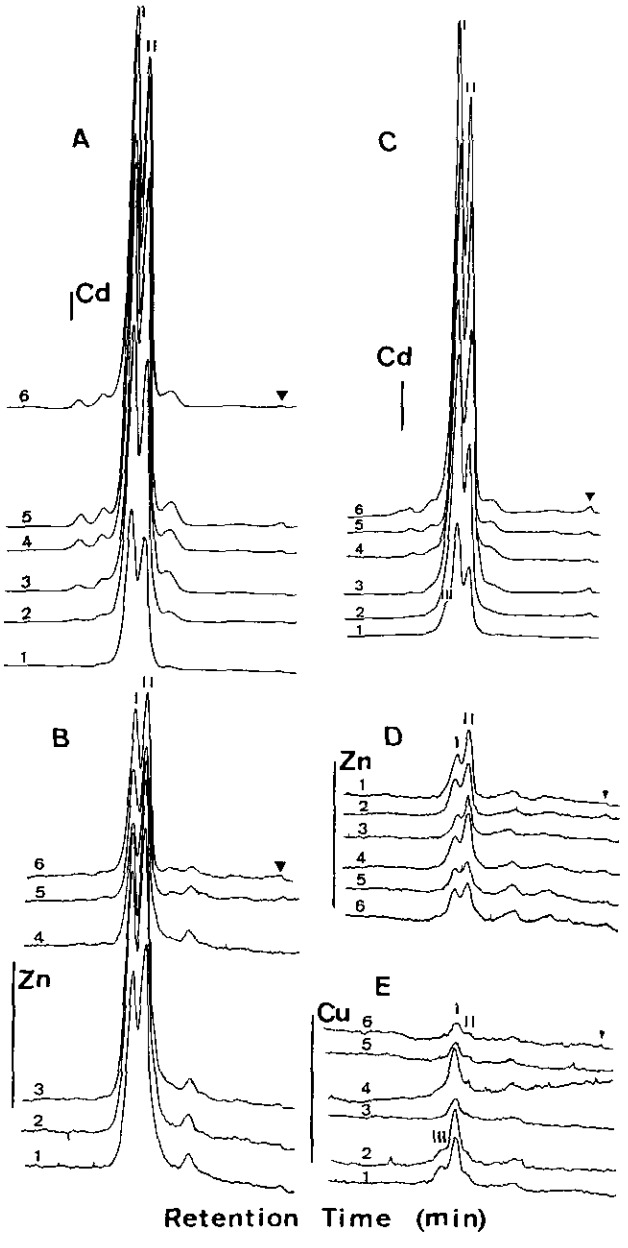


FIGURE 3. Distribution profiles of (*A*) cadmium and (*B*) zinc in the liver and (C) cadmium, (*D*) zinc and (*E*) copper in the kidney supernatants of rats injected repeatedly with cadmium salt (*33*). The distribution profiles of the three metals in the liver and kidney supernatants were determined by applying a 0.1 mL portion of the pooled supernatants in each group to an SW column (TSK gel G3000SW, 7.5 × 600 mm with a precolumn of 7.5 × 75 mm; Toyo Soda Co., Tokyo). The column attached to a high performance liquid chromatograph (HPLC, HLC 803A, Toyo Soda Co.) was eluted with 50 mM Tris/HCl buffer (pH 8.0, containing 0.1% NaN_3, dissolved gases were removed by heating at 80°C under reduced pressure) at a flow rate of 1.0 mL/min. The concentrations of the metals were determined by connecting the outlet of the column to the nebulizer tube of an atomic absorption spectrophotometer (AAS, Hitachi 170-50A, Hitachi Ltd., Tokyo) (HPLC-AAS method) (*34*). I (21.2 min) and II (20.2 min) indicate metallothionein-I and -II, respectively. Peaks at retention times of 11.2 (void volume of the column), 18.3 (metallothionein dimers) (*35*), and 22.8 and 24.3 min (cadmium-binding proteins related to metallothionein) (*32*) were identified as already reported. The vertical bar indicates the detector level of the metal by the corresponding standard metal solution. As the column was washed well with mercaptoethanol to remove metals bound nonselectively to the column, cadmium bound to the high molecular weight protein fraction was adsorbed to the column and was not observed in the present experiment.

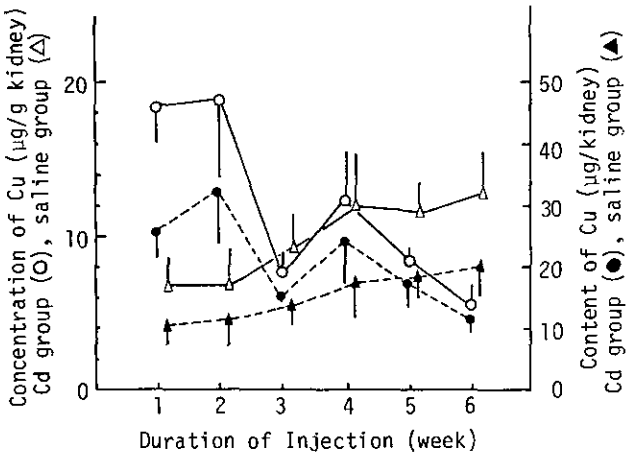


FIGURE 4. Changes in the concentration and content of copper in the kidneys induced by injections of cadmium salt (*33*). The concentration and content of copper in the kidneys of the rats in the cadmium- and saline-injected groups were determined as indicated in the legend to Fig. 2. Control rats (30 heads in total) were injected with physiological saline at a volume of 0.1 mL/rat and the concentrations of the metals were determined exactly at the same manner as mentioned for the cadmium group. The data are expressed as means ± SD of five samples.

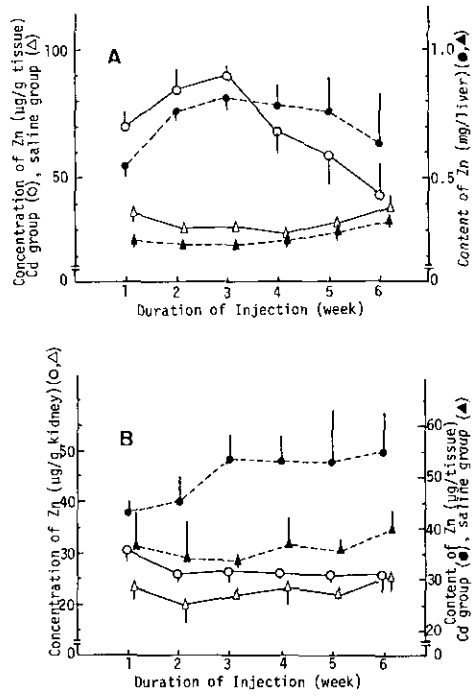


FIGURE 5. Changes in the concentrations and contents of zinc in (*A*) the liver and (*B*) kidneys of rats induced by injections of cadmium salt (*33*). The concentrations of zinc in the livers and kidneys of the rats in the cadmium- and saline-injected groups were determined as indicated in the legend to Figs. 2 and 4 and were plotted versus duration of the injection. The data are expressed as means ± SD of five samples and absence of a bar indicates that the range is less than the size of the symbol.

was selected as the second model to examine the relation between the toxicity of cadmium and the changes in its chemical forms. Although the extent of accumulation depends on the dose and method of administration, we administered a relatively high dose of cadmium salt into female rats by subcutaneous injection and studied the possible relation between the toxicity of cadmium and the changes in the chemical forms of cadmium (*14,31–33*).

During the repeated injections of cadmium salts, we have observed the transitory necrotic damage of renal tubular linings (*31,33*) that is similar to the renal lesion observed after the single injection of cadmium-containing metallothionein. This necrotic damage was transitory, and the tubular linings were restored despite further continuous injections of cadmium salt. Persistent renal damage was observed later after the transitory necrotic damage. The transitory and persistent renal damage will be discussed later in this paper in consideration of the viewpoints on the biosynthetic capacity of metallothionein.

As shown in Figure 2, the concentration and content of cadmium in the liver and kidney of rats changed with repeated injections of cadmium salt (*33*). Cadmium in the two tissues was further separated into metal extractable to the soluble fraction and also to the heat-stable soluble fraction. Cadmium in the latter two fractions was bound mostly to metallothionein and its related cadmium-binding proteins (Fig. 3) (*33*). The difference in cadmium concentration between the whole tissue and the heat-stable soluble fraction does not exactly show the concentration of cadmium not bound to metallothionein but is closely related to it.

The renal tubular linings were severely damaged after injections for two weeks and then were restored despite further continuous injections. However, no appreciable signs that may indicate transitory damage were observed from the changes in the cadmium concentrations and content in the kidney. On the other hand, the copper concentration in the kidney showed a dramatic change at this stage as shown in Figure 4 (*31,33*). The copper concentration that continued to increase with accumulation of cadmium in the kidney (*26,27*) suddenly dropped to the control level after the damage. The copper concentration in the kidney decreased to lower levels than those of the respective controls with further injections of cad-

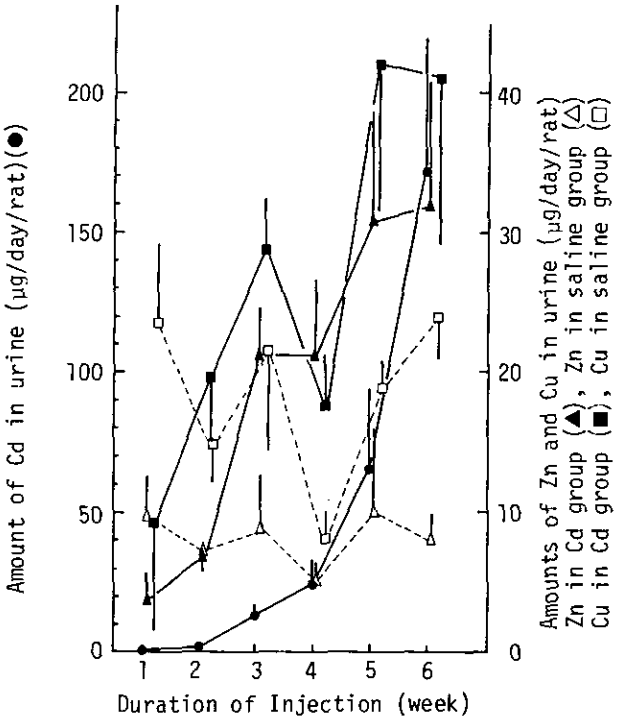


FIGURE 6. Changes in the amounts of urinary (*A*) cadmium, (*B*) zinc and (*C*) copper induced by repeated injections of cadmium salt (*33*). Urine of the rats repeatedly injected either with cadmium or saline as indicated in the legends to Figs. 2 and 4 was collected for 24 hr, 3 days after the last injection for the respective injection periods using a metabolic cage. The amounts of the three metals excreted into urine are expressed as μg/day/rat and means ± SD of five samples. The concentrations were determined by atomic absorption spectrophotometry after wet-ashing.

mium. However, this transitory damage was not characterized by another co-existing metal, zinc, in metallothionein (Fig. 5) (*33*). On the other hand, increases in the amounts of the three metals in the urine were observed after this transitory damage (Fig. 6).

The transitory renal damage caused by repeated injections of cadmium salt can thus be characterized by the changes in metal concentration as follows: (1) the damage occurs after accumulation of cadmium to about 100 μg/g kidney. (2) The concentration of copper in the kidney decreases dramatically after the damage, and this change is characterized by a sudden decrease of copper content in the kidney metallothionein. (3) Cadmium is bound to copper-rich metallothionein before the damage, while it is bound to metallothionein containing far smaller amounts of co-existing metals (copper and zinc), especially copper, after the damage (Fig. 3).

Cadmium in the kidney continued to increase both in concentration and content with the injections of cadmium even after the transitory damage, and the concentration reached almost a plateau after injections for 5 weeks (Fig. 2*B*). Although the extent and rate of cadmium accumulation in the kidney were far less than those in the liver (Fig. 2*A*), cadmium in the kidney seems to accumulate in a similar manner to that in the liver; the concentration attained a plateau faster than the content, and the difference of cadmium concentrations between the whole tissue and the heat-treated supernatant fraction was wide during the plateau. Histological examination of the livers and the kidneys under a light microscope revealed necrosis of hepatocytes after injection for 5 weeks, while necrotic damage was not observed after the transitory necrosis and the restoration (*33*). These observations suggest that persistent damage can be induced after the concentration of cadmium attained a maximum and maintained a plateau.

We observed changes in the distribution profiles of cadmium and zinc in the liver supernatants with injections of cadmium salt and characterized the changes as follows: increase in the relative ratio of cadmium to zinc in metallothionein; increase in the oxidation products of metallothionein; and increase in the amount of cadmium not bound to metallothionein and the related cadmium-binding proteins (*32*). These observations have also been reported by Sato and Nagai (*36,37*).

As the dose of cadmium injected in this experiment was high, the liver was damaged earlier than the kidneys. The concentration and content of cadmium in the liver also attained maxima faster than those in the kidneys. The changes in the distribution profiles of the metals in the kidney supernatants after the transitory damage were similar to those in the liver supernatants (Fig. 3). Therefore, the persistent renal damage may result after the three changes in the distributions of cadmium and zinc (copper) in the kidney supernatants are observed.

Capacity of Metallothionein Synthesis and Its Relation to the Toxicity of Cadmium

The renal damage induced by the injection of cadmium-containing metallothionein was suggested to be caused by free cadmium ions liberated from the degraded metallothionein. The changes in the distribution profiles of cadmium and zinc after the degradation of metallothionein

(distribution of cadmium from the high molecular weight protein fraction to the metallothionein fraction) are similar to those in the liver supernatant after the injection of cadmium salt, and this suggests that the biosynthesis of metallothionein is induced by the cadmium ions liberated from the degraded metallothionein. The metallothionein induced by the injection of cadmium-containing metallothionein is not rich in copper and is different in metal composition from metallothionein induced by exposure to cadmium salt. The copper-rich metallothionein induced by loadings with cadmium salt has been explained by the sequestration of cadmium ions to the kidney metallothionein within the capacity of native Cu,Zn-thionein biosynthesis (*31*). The slow transfer of cadmium to the kidney in the case of loadings with cadmium salt is probably sufficient to sequester the metal within the native capacity. On the other hand, a rapid transfer and degradation of the injected metallothionein in the kidney would supply too much ionic cadmium to be sequestered by the native capacity resulting in the induction of a new biosynthetic system for metallothionein. The metallothionein resynthesized by the new biosynthetic system is different in metal composition from that induced within the native capacity; poor in copper and similar to that of the liver metallothionein (Cd,Zn-thionein) (*20–24*).

The transitory renal damage induced by the repeated injections of cadmium salt can also be explained by the limit of the native biosynthetic capacity of metallothionein in the kidney. The slow transfer of cadmium to the kidney in the case of the injection of cadmium salt is probably sufficient for cadmium to be sequestered to metallothionein within the native biosynthetic capacity, and this will result in the induction of copper-rich metallothionein. The native biosynthetic capacity seems to trap cadmium up to a concentration of about 100 μg/g kidney (*31,33*). When the concentration of cadmium in the kidney becomes higher than this limit, cadmium ions not bound to metallothionein may increase and cause the transitory damage of the renal tubules. At the same time, the cadmium ion stimulates the new induction of metallothionein synthesis to sequester the cadmium ion as metallothionein poor in copper (*31,33*).

The transitory damage of the renal tubular linings accompanied by the change in the copper content of metallothionein after the single injection of cadmium-containing metallothionein and also after the repeated injections of cadmium salt can thus be explained by the limit of native capacity of metallothionein biosynthesis followed by the new induction of metallothionein biosynthesis in the kidney.

The persistent renal damage caused by repeated injections of cadmium salt can be observed after the concentration of cadmium attains a maximum plateau level. At this level, the relative ratio of cadmium to the co-existing metals is higher than that before the plateau, and the intra- and intermolecular oxidation products are more frequently observed in the distribution profiles of cadmium. Furthermore, the difference in cadmium concentration between the whole tissue and heat-stable soluble fraction is wider than that before the plateau. As the latter observation is closely related to the increase in cadmium concentration not bound to metallothionein, the persistent renal damage is probably caused also by the metal not bound to metallothionein. Therefore, the persistent damage can probably be caused only in the presence of persistent cadmium ion at a certain concentration beyond the capacity of metallothionein biosynthesis in tissues.

The concentration of the free cadmium ion that may cause the renal damage probably depends on species, sex, age and other physiological factors. Although it is relatively easy to estimate the concentration of free cadmium ion extractable into the supernatant fraction, it might be difficult to estimate the concentration in other subcellular fractions. Nomiyama has estimated the concentration to be 35 and 60 μg/g in the kidney supernatant fractions of monkey and rabbit (*38,39*).

Schematic Presentation of the Metabolic Pathways of Absorbed Cadmium in Rats

Metabolic pathways for cadmium absorbed in rats schematically presented in Figure 7 are based on the assumed controlling factors for the tissue distribution of cadmium shown in Figure 1 and also on the metal compositions related to the capacity of metallothionein synthesis. The metal compositions in plasma and urinary metallothioneins were obtained mainly from data reported previously (*14, 20, 33, 40–42*).

The chemical forms of cadmium in plasma are the main controlling factors that determine the distribution of cadmium either to the liver or kidney; cadmium bound to the high molecular weight plasma constituents is mostly trapped by the liver, while the metal bound to the lower molecular weight components is either selectively transferred to and taken up by the kidney or

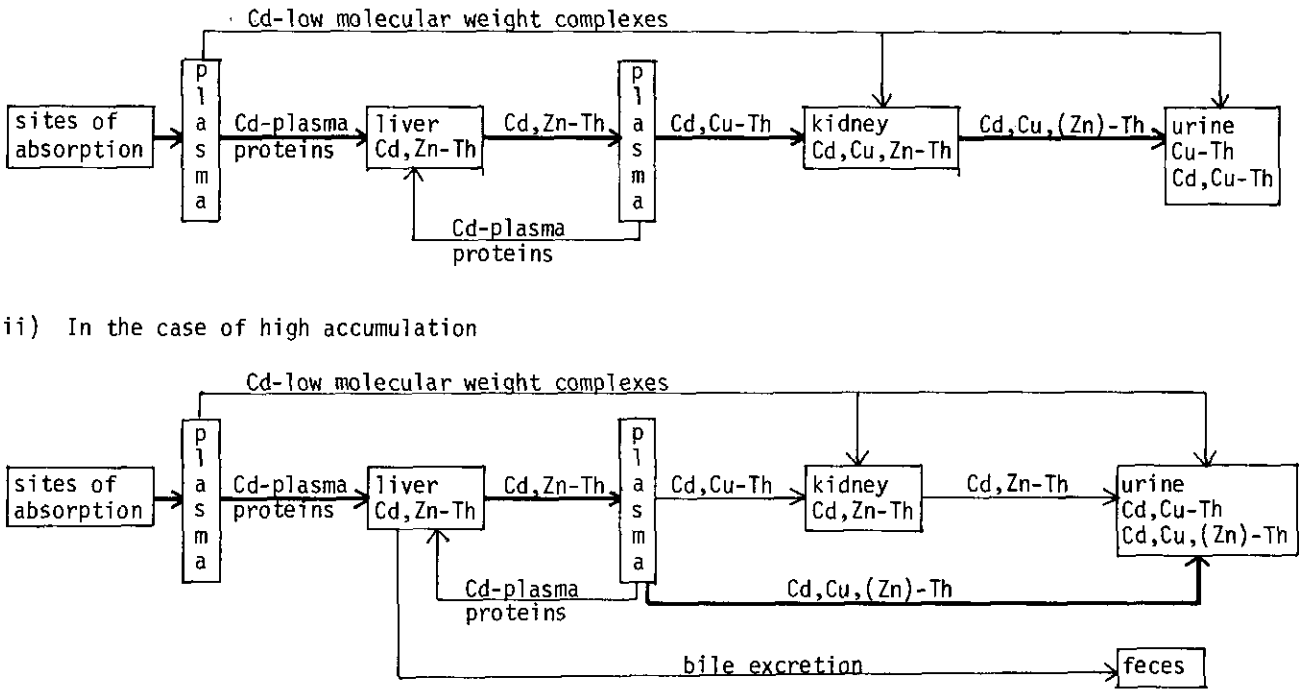


FIGURE 7. Schematic presentation of the metabolic pathways of absorbed cadmium in rats.

directly excreted into urine. Although it is not known whether the decrease of cadmium from the liver is due to a passive mechanism (like enzymes from damaged cells) or due to an active (secretory) mechanism, Cd,Zn-thionein is excreted into plasma. Zinc and cadmium in the metallothionein coming out of the liver are partly replaced by copper in plasma. Cadmium is considered to be either selectively transferred to the kidney as Cd,Cu-thionein or taken up by the liver as cadmium-high molecular weight protein complexes in plasma.

If the amount of liver metallothionein excreted into plasma is small and the concentration of cadmium in the kidney is low, the metallothionein will be efficiently reabsorbed by the renal tubules. The reabsorbed metallothionein is degraded, and the cadmium liberated from the degraded metallothionein is sequestered as the copper-rich metallothionein within the native biosynthetic capacity. On the other hand, when the amount of liver metallothionein excreted into plasma is high and the concentration of cadmium in the kidney is high, the metallothionein may mostly be not reabsorbed by the tubules and excreted directly into urine. Thus, the origin of urinary metallothionein may depend on the extent of cadmium accumulation in the kidney and also on the renal damage (reabsorption rate).

The high copper content in the urinary metallothionein is partly explainable by the replacement of zinc and cadmium in metallothionein by urinary copper according to the higher affinity of copper to metallothionein (*20,26*). The urinary metallothionein contains copper in high content irrespective of its origin (plasma and/or urinary copper) and can be easily oxidized both by divalent copper (*43*) and by dissolved oxygen. Therefore, the amount of urinary metallothionein has to be estimated carefully when it is quantified from the amount of metals in metallothionein. The radioimmunoassay (*44–46*) is probably superior in its sensitivity in comparison with the conventional method based on metal analysis provided that the radioimmunoassay has the same detectability for metallothionein with different metal compositions and oxidation states.

The author expresses his thanks to Dr. K. Kubota for his continuous encouragement.

REFERENCES

1. Kägi, J. H. R., and Vallee, B. L. Metallothionein: a cadmium- and zinc-containing protein from equine renal cortex. J. Biol. Chem. 235: 3460–3465 (1960).

2. Piscator, M. On cadmium in normal human kidneys together with a report on the isolation of metallothionein from livers of cadmium-exposed rabbits. Nord. Hyg. Tidskr. 45: 76–82 (1964).
3. Friberg, L., Piscator, M., Nordberg, G. F., and Kjellström, T. Cadmium in the Environment, 2nd Ed. CRC Press, Cleveland, 1974.
4. Kägi, J. H. R., and Nordberg, M, Eds. Metallothionein. Birkhäuser Verlag, Basel, 1979.
5. Foulkes, E. C., Ed. Biological Roles of Metallothionein. Elsevier/North-Holland, New York, 1982.
6. Shaikh, Z. A., and Smith, J. C. The biosynthesis of metallothionein in rat liver and kidney after administration of cadmium. Chem.-Biol. Interact. 15: 327–336 (1976).
7. Shaikh, Z. A., and Smith, J. C. The mechanism of hepatic and renal metallothionein biosynthesis in cadmium-exposed rats. Chem.-Biol. Interact. 16: 161–171 (1977).
8. Feldman, S. L., and Cousins, R. J. Degradation of hepatic zinc-thionein after parenteral zinc administration. Biochem. J. 160: 583–588 (1976).
9. Kobayashi, S., Imano, M., and Kimura, M. Turnover of metallothionein in mammalian cells. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 305–322.
10. Tanaka, K., Sueda, K., Onosaka, S., and Okahara, K. Fate of ^{109}Cd-labeled metallothionein in rats. Toxicol. Appl. Pharmacol. 33: 258–266 (1975).
11. Cherian, M. G., and Shaikh, Z. A. Metabolism of intravenously injected cadmium-binding protein. Biochem. Biophys. Res. Commun. 65: 863–869 (1975).
12. Foulkes, E. C. Role of metallothionein in transport of heavy metals. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 131–140.
13. Squibb, K. S., Pritchard, J. B., and Fowler, B. A. Renal metabolism and toxicity of metallothionein. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 181–192.
14. Suzuki, K. T. Induction and degradation of metallothionein and their relation to the toxicity of cadmium. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 215–236.
15. Tanaka, K. Effects of hepatic disorder on the fate of cadmium in rats. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 237–250.
16. Webb, M., and Etienne, A. T. Studies on the toxicity and metabolism of cadmium-thionein. Biochem. Pharmacol. 26: 25–30 (1977).
17. Nordberg, G. F., Goyer, R. A., and Nordberg, M. Comparative toxicity of cadmium-metallothionein and cadmium chloride on mouse kidney. Arch. Pathol. 99: 192–197 (1975).
18. Cherian, M. G., Goyer, R. A., and Richardson, L. D. Cadmium-metallothionein-induced nephropathy. Toxicol. Appl. Pharmacol. 38: 399–408 (1976).
19. Cherian, M. G. Studies on toxicity of metallothionein in rat kidney epithelial cell culture. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 193–202.
20. Suzuki, K. T., Maitani, T., and Takenaka, S. Fate of intraperitoneally injected liver metallothionein in rat kidney. Chem. Pharm. Bull. 27: 647–653 (1979).
21. Suzuki, K. T., and Takenaka, S. Fate of kidney metallothionein intraperitoneally injected into the rat. Chem. Pharm. Bull. 27: 1753–1758 (1979).
22. Suzuki, K. T., and Yamamura, M. Degradation and resynthesis of injected liver cadmium-thioneins in rat kidney. Biochem. Pharmacol. 28: 1806–1807 (1979).
23. Suzuki, K. T., and Yamamura, M. Rat kidney metallothionein induced by injection of Cd-thionein: changes of chromatographic properties with time and their relation to copper content and kidney dysfunction. Toxicol. Letters 5: 131–138 (1980).
24. Suzuki, K. T., Takenaka, S., and Kubota, K. Fate and comparative toxicity of metallothioneins with differing Cd/Zn ratios in rat kidney. Arch. Environ. Contam. Toxicol. 8: 85–95 (1979).
25. Frazier, J. M. The role of metallothionein in the systemic distribution of cadmium. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 141–154.
26. Suzuki, K. T., Kubota, K., and Takenaka, S. Copper in cadmium-exposed rat kidney metallothionein. Chem. Pharm. Bull. 25: 2792–2794 (1977).
27. Suzuki, K. T. Copper content in cadmium-exposed animal kidney metallothioneins. Arch. Environ. Contam. Toxicol. 8: 255–268 (1979).
28. Murakami, M., and Webb, M. A morphological and biochemical study of the effects of L-cysteine on the renal uptake and nephrotoxicity of cadmium. Brit. J. Exptl. Pathol. 62: 115–130 (1981).
29. Suzuki, K. T., and Yamamura, M. Distribution of cadmium in liver and kidney by loadings of various Cd-complexes and relative metal ratios in the induced metallothioneins. Biochem. Pharmacol. 28: 3643–3649 (1979).
30. Suzuki, K. T., Yamamura, M., and Mori, T. Metabolic fate of earthworm cadmium-binding proteins in rats. Arch. Environ. Contam. Toxicol. 9: 519–531 (1980).
31. Suzuki, K. T., Yamamura, M., Yamada, Y. K., and Shimizu, F. Decreased copper content in rat kidney metallothionein and its relation to acute cadmium nephrophathy. Toxicol. Letters 7: 137–142 (1980).
32. Suzuki, K. T., Yamamura, M., Yamada, Y. K., and Shimizu, F. Distribution of cadmium in heavily cadmium-accumulated rat liver cytosols: metallothionein and related cadmium-binding proteins. Toxicol. Letters 8: 105–114 (1981).
33. Suzuki, K. T., Yaguchi, K., Ohnuki, R., Nishikawa, M., and Yamada, Y. K. Extent of cadmium accumulation and its effect on essential metals in liver, kidney and body fluids. J. Toxicol. Environ. Health 11: 713–726 (1982).
34. Suzuki, K. T. Direct connection of high-speed liquid chromatograph (equipped with gel permeation column) to atomic absorption spectrophotometer for metalloprotein analysis: metallothionein. Anal. Biochem. 102: 31–34 (1980).
35. Suzuki, K. T., and Yamamura, M. Isolation and characterization of metallothionein dimers. Biochem. Pharmacol. 29: 689–692 (1980).
36. Sato, M., and Nagai, Y. Mode of existence of cadmium in rat liver and kidney after prolonged subcutaneous administration. Toxicol. Appl. Pharmacol. 54: 90–99 (1980).
37. Sato, M., and Nagai, Y. Renal damage and form of cadmium in subcellular fractions. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 163–179.
38. Nomiyama, K. Recent progresses and perspectives in cadmium health effect studies. Sci. Total Environ. 14: 199–232 (1980).
39. Nomiyama, K., and Nomiyama, H. Tissue metallothioneins in rabbits chronically exposed to cadmium, with special reference to the critical concentration of cadmium in the renal cortex. In: Biological Roles of Metallothionein (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 47–67.
40. Suzuki, Y. Cadmium metabolism and toxicity in rats after long-term subcutaneous administration. J. Toxicol. Environ. Health 6: 469–482 (1980).

41. Suzuki, Y. Cadmium, copper and zinc distribution in blood of rats after long-term cadmium administration. J. Toxicol. Environ. Health 7: 251–262 (1981).
42. Suzuki, Y. Metal-binding properties of metallothionein in extracellular fluids and its role in cadmium-exposed rats. In: Biological Roles of Metallothionein, (E. C. Foulkes, Ed.), Elsevier/North Holland, New York, 1982, pp. 25–35.
43. Suzuki, K. T., and Maitani, T. Metal dependent properties of metallothionein: Replacement *in vitro* of zinc in zinc-thionein with copper. Biochem. J. 199: 289–295 (1981).
44. Tohyama, C., and Shaikh, Z. A. Metallothionein in plasma and urine of cadmium-exposed rats determined by a single-antibody radioimmunoassay. Fund. Appl. Toxicol. 1: 1–7 (1981).
45. Tohyama, C., Shaikh, Z. A., Nogawa, K., Kobayashi, E., and Honda, R. Elevated urinary excretion of metallothionein due to environmental cadmium exposure. Toxicology 20: 289–297 (1981).
46. Nordberg, G. F., Garvey, J. S., and Chang, C. C. Metallothionein in plasma and urine of cadmium workers. Environ. Res. 28: 179–182 (1982).