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Effect of Zinc Deficiency on the Accumulation of Metallothionein and Cadmium in the Rat Liver and Kidney

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Abstract. The effects of zinc (Zn) deficiency and repeated exposure to cadmium (Cd) on the accumulation and distribution of metallothionein (MT), Cd and Zn in the liver and kidney were studied. Male Sprague-Dawley rats were fed either a Zn-deficient (1 ppm) or a Zn-adequate (40 ppm) diet during the experiment, and the rats were injected subcutaneously with a cadmium chloride solution (1.0 mg Cd/kg of body weight, 5 days a week) for 4 weeks. Cadmium, Zn, and Cd-induced MT concentrations in the liver and kidney were lower in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd), while the content of Cd bound to high molecular weight proteins (HMWP) was greater in the Zn-deficient rats (−Zn + Cd). The Zn bound to Cd-induced MT was reduced to 30% in the liver and to 60% in the kidney of the Zn-deficient rats (−Zn + Cd) as compared with that of the Zn-adequate rats (+Zn + Cd). In the kidney of Zn-deficient rats, exposure to Cd caused a decrease in essential Zn associated with HMWP as compared with that of Zn-adequate rats (+Zn + Cd). Thus, Zn-deficiency affected the distribution of Cd in tissues, MT and HMWP and accelerated substantially Cd-induced Zn-deficiency in the kidney. Although the renal Cd concentration was lower in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd), exposure to Cd for four weeks resulted in glucosuria and an increase in liver and kidney weights in the Zn-deficient rats (−Zn + Cd), but not in the Zn-adequate rats (+Zn + Cd). These results suggest that development of Cd toxicity is related to the Zn status of the body, to the accumulation of Cd in HMWP and to the amount of essential Zn associated with HMWP.

Zinc (Zn) diminishes some toxic effects of cadmium (Cd) such as testicular damage (Parizek and Zabor 1956), lethality (Gunn *et al.* 1968), hepatotoxicity (Goering and Klaassen 1984) and lung toxicity (Petering *et al.* 1979). The reduction of toxic effects of Cd has been attributed to the induction of metallothionein (MT) (Yoshikawa and Ohta 1982) and altered hepatic subcellular distribution of Cd (Goering and Klaassen 1984). Metallothionein is a low molecular weight protein with a high affinity for metals (Kägi and Vallee 1961). The induction of MT by Cd or Zn occurs via a mechanism that involves stimulation of the transcription of MT mRNA (Shapiro *et al.* 1978). This stimulation process is catalyzed by DNA-dependent RNA polymerase which is a Zn-enzyme (Terhune and Sandstead 1972). Although the critical organ in chronic Cd toxicity is considered to be the kidney, Zn has not been shown to protect against Cd-induced renal damage in situations where renal Cd concentration chronically reaches critical levels (Bremner 1982).

We have previously reported possible mechanism of renal damage in which there is an increase in Cd bound to non-MT fractions in both the cytoplasmic and particulate fractions of the tissues following chronic exposure to Cd (Sato and Nagai 1982). Other authors have suggested that Cd toxicity results from an alteration in the normal metabolism of Zn (Petering *et al.* 1984; Wada *et al.* 1982). To clarify the interaction between Cd and Zn in the development of Cd toxicity in the kidney and liver, the present research investigated changes in MT accumulation and distribution of Cd and Zn in the liver and kidney of Zn-deficient rats following repeated exposure to Cd. Since changes in copper (Cu) content in the kidney and renal MT are correlated with renal tubular injury in rats injected with

Cd (Suzuki *et al.* 1980), Cu content in the kidney and renal MT was also analyzed.

Materials and Methods

Reagents

Bolton and Hunter reagent (N-succinimidyl 3-(4-hydroxy, 5-(^{125}I) iodophenyl)propionate) (5 mCi/ml) was obtained from Amersham Japan Ltd (Tokyo). Donkey anti-(sheep IgG) serum was obtained from the Scottish Antibody Production Unit, Lanarkshire, U.K.

Animals

Male Sprague-Dawley rats weighing 50 to 60 g (3 weeks old, Funabashi-nōjo Ltd., Funabashi, Japan) were housed in wire bottom, stainless-steel cages in a temperature-controlled room (20–24°C). A purified diet based on 20% egg albumin with supplementation of 1 ppm Zn as sulfate, was prepared by the method of Williams and Mills (1970). Instead of calciferol or arachis oil, vitamin D_3 (Wako Pure Chemical Industries Ltd, Osaka) or corn oil (Nakarai Chemicals Ltd, Kyoto) was used, respectively. Rats were fed a Zn-deficient (1 ppm) or a Zn-adequate (40 ppm) diet, and water *ad libitum* for 7 days before the first injection and whole experimental period. Each group was further divided into two groups, one for saline (1 ml/kg) as the control, and the other for Cd (1 mg/kg, as $CdCl_2$) injections. Five rats per group were injected subcutaneously 5 days a week for 4 weeks. The dose used in the experiment was decided on the basis of our previous report on the dose-dependent effects of Cd on biochemical indicators for renal tubular dysfunction (Sato *et al.* 1983).

Experiment

Soon after the last weekly injection, urine specimens were collected from the rats by placing them in individual stainless steel metabolic cages for 24 hr, during which time water and food were provided. Urine was frozen at −40°C before use. Glucose in urine was determined colorimetrically with a commercially available kit (Wako Pure Chemical Industries Ltd, Osaka). After urine collection in the 4th week, blood was collected by heart puncture under light ether anesthesia and then the rats were sacrificed. Blood clotting was prevented by the addition of sodium heparin (Wako Pure Chemical Industries Ltd, Osaka) to the blood, and plasma was prepared by centrifuging the whole blood at 1500 g for 15 min at 4°C. Tissues were excised, immediately frozen in liquid nitrogen and stored at −40°C before analysis. Livers and kidneys were homogenized at 4°C in 4 vols of 0.25 M sucrose containing 10 mM Tris-acetate (pH 8.0) with a Potter-Elvehjem homogenizer. For measurement of the tissue Cd, Zn and Cu concentrations, a portion of tissue homogenate was placed in an acid-washed test tube and was digested in an acid mixture as described previously (Sato and Nagai 1980a) using a dry block heater. Distilled deionized water was then added to each tube to a final volume of 10 ml. Metal content was measured on a Perkin Elmer Model 403 atomic absorption spectrophotometer. The resulting homogenate was centrifuged at 105000 g for 60 min to obtain the cytosol fraction in a Beckman L8-80 ultracentrifuge. The cytosol fraction from kidney (4.5 ml) or liver (7.5 ml) was chromatographed on Sephadex G-75 (2.5 × 65 cm) equilibrated with 10 mM Tris-acetate (pH 8.0) and 5 ml fractions were collected. The Cd, Zn, and Cu contents of each fraction were directly measured by atomic absorption spectroscopy.

Measurement of Metallothionein

Two forms of MT have been identified in rats, a metallothionein-I (MT-I) and a metallothionein-II (MT-II) (Nordberg and Kojima 1979). On the fractionation of the cytosol fraction on Sephadex G-75, MT containing both MT-I and MT-II was routinely eluted as a symmetrical peak from the column with a Ve/Vo ratio of 1.95–2.25 (where Ve = elution volume, Vo = void volume) (Figure 2). Since the Cd-induced MT contained Cd, Zn and Cu, and most of the Cd was distributed to the cytosol fraction (Sato and Nagai 1982), amount of MT was calculated by the total amount of three metals bound to MT fractions of the cytosol and was expressed as micromoles of the metals bound to MT per gram of tissue. In plasma and urine, concentrations of MT-I were measured, using a radioimmunoassay procedure as described by Mehra and Bremner (1983). Antiserum for MT-I was generously provided by Dr. Ian Bremner, Rowett Research Institute, Scotland.

A concentration range of rat Zn MT-I (100 − 25400 pg) was assayed for the standard.

Statistical Analysis

The significance of the differences between groups of means was determined by student's t-test. The level of significance for all tests was $p < 0.05$.

Results

Development of Zinc Deficiency

In order to assess zinc status of rats, the plasma concentration and urinary excretion of Zn were determined. At the end of the experiment for 4 weeks, plasma Zn was 2.03 ± 0.02, or 0.53 ± 0.01 μg/ml of plasma (mean ± SE, n = 5) in the Zn-adequate (+Zn) or Zn-deficient (−Zn) rats, respectively ($p < 0.001$). Urinary excretion of Zn was significantly lower in both the saline- and Cd-treated groups of Zn-deficient rats than those of Zn-adequate rats during the whole period of experiment (Figure 1A).

Urinary excretion and plasma concentration of MT-I were lower in the Zn-deficient rats (−Zn) than in the Zn-adequate rats (+Zn) (Figures 1B and 1C). Urinary excretion of MT-I increased with the increase of duration of Cd-exposure in both the Zn-adequate (+Zn + Cd) and Zn-deficient (−Zn + Cd) rats. Repeated exposure to Cd resulted in an increase of plasma MT-I in both the Zn-adequate

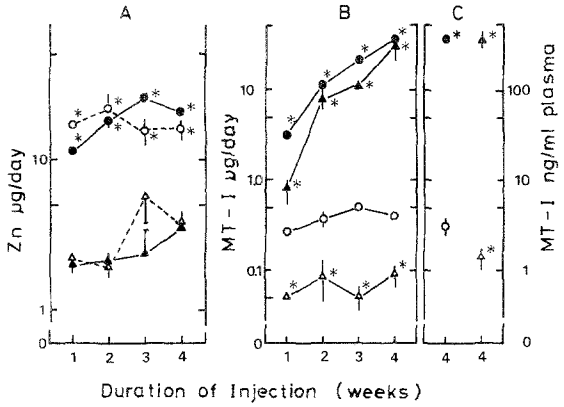

Fig. 1. Changes in the amounts of urinary (A) zinc, (B) metallothionein-I (MT-I), and in plasma concentration of MT-I (C) induced by repeated injections of cadmium chloride in the Zn-adequate or Zn-deficient rats. The amounts of Zn and MT-I excreted into urine are expressed as µg/day and the means ± SE of 5 rats. ○, Zn-adequate; ●, Zn-adequate + Cd; △, Zn-deficient; ▲, Zn-deficient + Cd. *Denotes significant difference from the values in Zn-adequate rats at $p < 0.05$

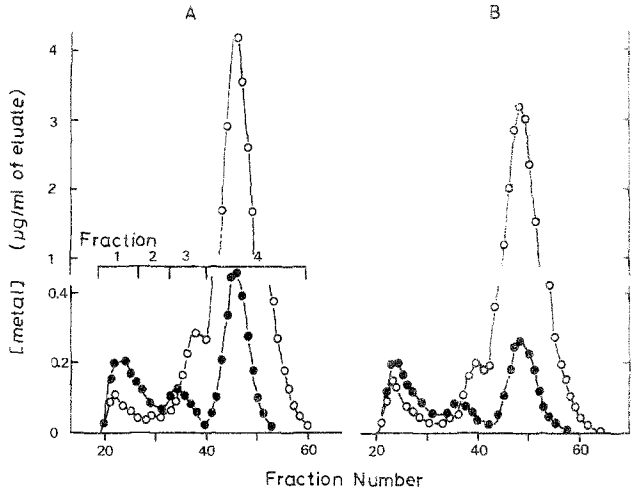

Fig. 2. Representative Sephadex G-75 gel filtration profile of cytosol of kidney homogenate from one of the rats fed a Zn-adequate (40 ppm) or Zn-deficient (1 ppm) diet and given repeated injections of Cd. A, Zn-adequate + Cd; B, Zn-deficient + Cd. ○, Cd; ●, Zn

(+Zn + Cd) and Zn-deficient (−Zn + Cd) rats at the same level.

Distribution of Cadmium, Zinc, and Copper and Accumulation of Metallothionein in the Liver and Kidney

Cadmium: The Cd concentrations in the kidney and liver were lower in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd) (Table 1). Fractionation on Sephadex G-75 of the cytosol fraction from the Cd-treated rats fed the Zn-deficient (−Zn + Cd) or Zn-adequate (+Zn + Cd) diet has revealed 4 Cd-containing fraction (Figure 2). The first (F-1) and the second (F-2) fractions represented Cd bound to high molecular weight proteins (HMWP). The third fraction (F-3) corresponded to dimer of MT (Sato and Nagai 1980b; Suzuki and Yamamura 1980), and the fourth fraction (F-4) was MT. Most of the Cd in the kidney (Figure 2) and liver (result not shown) supernatants was bound to MT. The percentage of Cd bound to HMWP (F-1 and F-2) in the kidney and liver supernatants was greater in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd) (Figure 3A), although the tissue concentration of Cd was lower in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd) (Table 1).

Figure 5 shows metal contents in MT of the liver and kidney. Cadmium bound to renal and hepatic MTs was smaller in the Zn-deficient rats (−Zn + Cd) than in the Zn-adequate rats (+Zn + Cd).

Zinc: As shown in Table 1, the Zn concentration in the liver and kidney was significantly lower in the Zn-deficient rats (−Zn) than in the Zn-adequate rats (+Zn). Exposure to Cd caused an increase in the Zn concentrations of the liver and kidney in both the Zn-adequate (+Zn + Cd) and the Zn-deficient (−Zn + Cd) rats.

Four Zn-containing component were present in the renal supernatant (Figure 2). In F-3, the position of the highest tube of Zn was different from that of Cd. Figure 3B shows the amount of Zn bound to non-MT fractions (F-1, 2 and 3). Zinc in these fractions is thought to be essential for cell function. Zinc deficiency decreased the amount of Zn bound to non-MT fractions, and caused a further decrease of Zn bound to non-MT fractions in Cd-treated rats (−Zn + Cd).

In the Cd-treated rats, the Zn bound to hepatic MT was reduced to 30% in the Zn-deficient rats (−Zn + Cd) as compared with that in the Zn-adequate rats (+Zn + Cd) (Figure 5). In the kidney, the Zn bound to MT was 61 ± 1, or 7 ± 2 nmol/g kidney (mean ± SE, n = 4) in the control of Zn-adequate rats (+Zn) or of Zn-deficient rats (−Zn), respectively (Figure 5), and 148 ± 6 or 92 ± 5 in the Cd-treated group of Zn-adequate rats (−Zn + Cd), or of Zn-deficient rats (−Zn + Cd), respectively. Thus, Zn bound to renal MT was reduced to 60% in the Zn-deficient rats (−Zn + Cd) as compared with that in the Zn-adequate rats (+Zn + Cd). The net increase in Zn bound to renal MT induced by Cd was 87 nmol/g tissue in the Zn-ade-

Table 1. Concentration of cadmium, zinc and copper in the liver and kidney of zinc-deficient and zinc-adequate rats at the end of four weeks treatment

Treatment[a]	Kidney[b]			Liver[b]		
	Cd	Zn	Cu	Cd	Zn	Cu
		μg/g			μg/g	
+Zn	—	24.5 ± 0.5	10.4 ± 0.7	—	26.7 ± 0.7	4.5 ± 0.3
+Zn + Cd	152.8 ± 5.0	32.1 ± 1.0*	21.1 ± 1.6*	268.0 ± 14.0	73.1 ± 2.4*	8.0 ± 1.0*
−Zn	—	19.3 ± 0.4*	11.5 ± 1.0	—	23.2 ± 0.5*	3.7 ± 0.2
−Zn + Cd	117.9 ± 4.7**	24.4 ± 0.8**,#	16.7 ± 1.5*,**,#	219.0 ± 17.8	33.3 ± 1.0*,**,#	12.7 ± 2.1*,#

[a] Cadmium chloride was injected subcutaneously into male rats at the dose of 1.0 mg Cd/kg body weight, 5 days a week for 4 weeks, while the remaining two groups received saline (1 ml/kg)
[b] The results represent the mean ± SEM for 4 to 5 rats
Significantly different from the values in Zn-adequate rat: *$p < 0.05$
Significantly different from the values in Zn-adequate + Cd rat: **$P < 0.05$
Significantly different from the values in Zn-deficient rat: #$P < 0.05$

quate rats (+Zn + Cd) and 84 nmol/g tissue in the Zn-deficient rats (−Zn + Cd).

Copper: Exposure to Cd caused an increase in liver and kidney Cd concentration (Table 1) and in renal MT (Figure 5) in both the Zn-adequate (+Zn + Cd) and the Zn-deficient (−Zn + Cd) rats.

Metallothionein: Figure 4 shows an amount of MT evaluated by total amount of Cd, Zn and Cu bound to MT fractions. Metallothionein contents in the liver and kidney were lower in the Cd-treated groups of Zn-deficient rats (−Zn + Cd) than those of Zn-adequate rats (+Zn + Cd).

Development of Cadmium Toxicity in the Zn-Deficient Rat

Table 2 shows the effect of Zn-deficiency and Cd administration on the body and tissue weights at the end of 4 weeks of treatment. There were no differences in the relative tissue weights of the liver and kidney between Zn-adequate (+Zn) and Zn-deficient (−Zn) rats. Repeated injections of Cd caused significant increases in the tissue weights of the liver, kidney and spleen in the Zn-deficient rats (−Zn + Cd), while there were no changes in the Zn-adequate rats (+Zn + Cd).

There were no differences in amount of urinary excretion of glucose between the control (+Zn) and the Cd-treated group of the Zn-adequate (+Zn + Cd) rats during the experiment (Figure 6). In the Cd-treated group of Zn-deficient rats (−Zn + Cd), excretion of glucose began to increase at 4 weeks, that is, increased glucose excretion was observed in 3 of 5 rats. Glucose excretion was 37.5 ± 20.9 mg/day (mean ± SE) in 3 of 5 rats and 2.0 ± 0.2 mg/day in 2 of 5 rats of Cd-treated rats fed Zn-deficient diet (−Zn + Cd), while it was 1.6 ± 0.4 mg/day in the 5 control rats (−Zn).

Discussion

The metallothionein concentrations in the liver and kidney were lower in the Cd-treated group of Zn deficient rats (−Zn + Cd) than in that of Zn-adequate rats (+Zn + Cd) (Figure 4). Differences in MT concentration between these two groups of rats may be caused by the differences in (a) synthesis rates of MT, (b) degradation rates of MT, (c) excretion rates of MT into plasma and urine by tissue damage and/or (d) amount of incorporated Zn into the tissue (if the incorporated Zn itself induces thionein synthesis).

Zinc is an essential nutrient that appears necessary for the synthesis of nucleic acid and protein. Terhune and Sandstead (1972) showed a Zn requirement for an activity of nucleas DNA-dependent RNA polymerase in mammalian liver. Metallothionein synthesis, therefore, is expected to decrease in Zn-deficient rat liver. Onosaka *et al.* (1984) reported that MT content in the liver of Zn-deficient rats following three daily injections of Cd was not reduced compared with that of Zn-deficient rats. In addition, a single injection of Cu or Cd into Zn-deficient of Zn-adequate rats stimulated a comparable synthesis of MT (Bremner and Davies 1976; Winge *et al.* 1978). However, accumulation of MT in the liver was lower in the Cd-treated group of Zn-deficient rats (−Zn + Cd) than in that of Zn-adequate rats (+Zn + Cd) following repeated exposure to Cd (Figure 4). Furthermore, post-Cd injection measurements after a short interval showed that MT-I synthesis in the liver was smaller in Zn-deficient

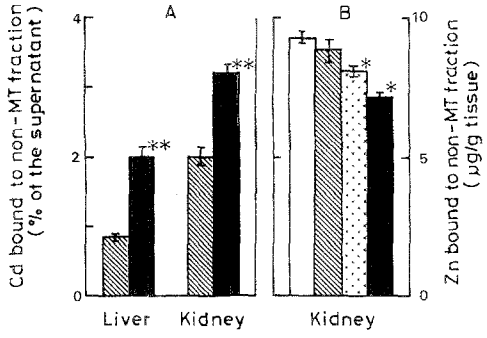

Fig. 3. Distribution of Cd and Zn associated with non-metallothionein fractions in the cytosol of liver and kidney homogenates. Results are expressed as (A) percentage of total Cd in the cytosol and (B) μg of Zn per g of tissue. □, Zn-adequate; ▨, Zn-adequate + Cd; ⊠, Zn-deficient; ■, Zn-deficient + Cd. Single or double asterisks denote significant difference from the values in Zn-adequate rats at $p < 0.05$, and the values in the Zn-adequate + Cd rats at $p < 0.05$, respectively

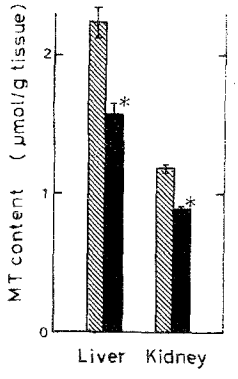

Fig. 4. Effects of repeated administration of Cd on the accumulation of metallothionein in the liver and kidney. Concentration of MT was evaluated by the sum in μmoles of Cd, Zn and Cu in the MT fractions obtained from gel filtration of Sephadex G-75 (see Fig. 2). ▨, Zn-adequate + Cd; ■, Zn-deficient + Cd. *Denotes significant difference at $p < 0.05$ from the Zn-adequate + Cd rats

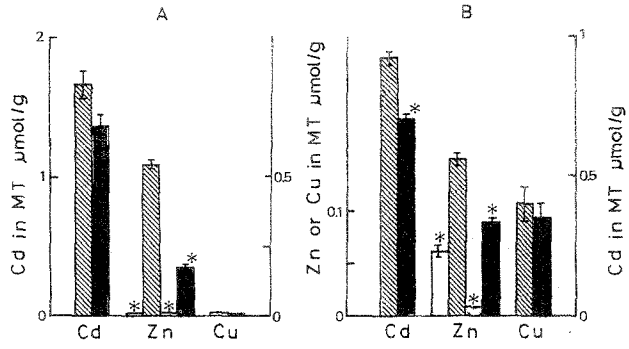

Fig. 5. Effects of Zn-deficiency and Cd administration on metal content bound to MT in the cytosol of kidney and liver. □, Zn-adequate; ▨, Zn-adequate + Cd; ⊠ Zn-deficient; ■, Zn-deficient + Cd. Results are expressed as the mean ± SE for 4–5 rats. *Denotes significant difference at $p < 0.05$ from the value in Zn-adequate + Cd rats

rats than in Zn-adequate rats (unpublished result). One of the reasons for the decreased accumulation of MT in the liver may be the decreased rate of MT synthesis.

Since degradation of MT plays a role in determining the level of MT, the effect of Zn deficiency on the turnover of Cd-induced MT in rat liver was investigated by Held and Hoekstra (1984). They observed that the half-life of hepatic MT in the Zn-deficient rats was significantly shorter than in the Zn-adequate rats following Cd exposure. The shorter half-life of MT in the Zn-deficient rats may also be one of the contributing factors to the reduced amount of MT in the Zn-deficient rat liver.

Since there were no significant differences in amounts of urinary excretion of MT-I between Cd-treated groups of Zn-adequate (+Zn + Cd) and Zn-deficient (−Zn + Cd) rats (Figure 1B), the lower concentration of MT in the tissues in Cd-treated group of Zn-deficient rats (−Zn + Cd) as compared with that of Zn-adequate rats (+Zn + Cd) (Figure 4) can not be explained by the differences in amount of urinary excretion of MT.

Winge *et al.* (1978) showed that since the MT fractions were virtually devoid of Zn in the rats injected with Cd 2 hr prior to killing, mobilization of Zn and incorporation of Zn were not essential for MT induction. They also observed, however, that the hepatic Zn-enzyme (alcohol dehydrogenase) activity in Cd-treated rats which were fed Zn-deficient diet was significantly higher than that in the Zn-deficient rats, and suggested that the mobilized Zn induced by Cd could be utilized for tissue Zn demands in addition to being incorporated into MT. Onosaka *et al.* (1984) stated the possibility that the increase in hepatic MT following injection of nickel and manganese may be due to an indirect effect of increased hepatic Zn concentration which may increase MT synthesis. Whether increased Zn induced by Cd injection in the tissue (Table 1) induces MT synthesis or not is still unclear.

Axelsson and Piscator (1966) found glucosuria and a decreased average capacity of the kidney to reabsorb glucose in rabbits given daily injections of Cd. The degree of toxic response was defined by two levels of tissue weights and the urinary excretion of glucose (Figure 6 and Table 2). The present study showed a possibility that Zn deficiency potentiated the development of Cd toxicity. Zinc deficiency reduced the accumulation of MT and Cd in the liver and kidney following chronic exposure to

Table 2. Effect of zinc-deficiency and cadmium administration on body and tissue weights at the end of four weeks of treatment

Treatment group[a]	Body weight[b]	Tissue weights (g/100g body weight)			
		Liver	Kidney	Spleen	Lung
Zn-adequate	254 ± 15	4.64 ± 0.20	0.82 ± 0.03	0.20 ± 0.01	0.48 ± 0.01
Zn-adequate + Cd	219 ± 7*	4.76 ± 0.20	0.89 ± 0.03	0.35 ± 0.01*	0.51 ± 0.01
Zn-deficient	130 ± 4*	4.47 ± 0.40	0.87 ± 0.02	0.22 ± 0.01	0.59 ± 0.02*
Zn-deficient + Cd	103 ± 6*,**,#	6.69 ± 0.29**,#	1.35 ± 0.08**,#	0.51 ± 0.03*,**,#	0.66 ± 0.02*,**,#

[a] During the whole experiment period including a week before start of injection of Cd or saline, animals were fed zinc-adequate or zinc-deficient diet. Rats were given Cd (1 mg Cd/kg, sc, 5 days a weeks for 4 weeks) or saline (1 ml/kg)
[b] Each value represents the mean ± SEM for 5 rats
Significantly different from the values in Zn-adequate rat: *P < 0.05
Significantly different from the values in Zn-adequate + Cd: **P < 0.01
Significantly different from the values in Zn-deficient rat: #P < 0.01

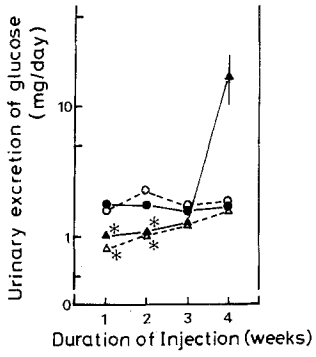

Fig. 6. Effects of Zn-deficiency and Cd administration on urinary excretion of glucose in the rats. ○, Zn-adequate; ●, Zn-adequate + Cd; △, Zn-deficient; ▲, Zn-deficient + Cd. Results are expressed as the mean ± SE for 5 rats. *Denotes significant difference from the Zn-adequate rats at $p < 0.05$

Cd (Figure 4 and Table 1). In addition, MT-I synthesis rate in the liver after a single injection of Cd was lower in the Zn-deficient rats than in the Zn-adequate rats (unpublished result). This may cause Cd to bind with non-MT fractions, thus possibly increasing tissue toxicity of Cd. In fact, the percentage of Cd in the HMWP of the hepatic and renal cytosols was greater in the Cd-treated group of Zn-deficient rats than in that of Zn-adequate rats (Figure 3A). We have earlier reported that an increase in the amount of Cd in the fractions other than MT in both the supernatant and particulate fractions is related to the development of Cd toxicity (Sato and Nagai 1982). Cain and Holt (1983) also demonstrated that even small amounts of non-MT bound Cd^{2+} was toxic to the kidney after injection of Cd-MT. Furthermore, since repeated exposure to Cd caused further decreased amounts of available Zn bound to non-MT (Figure 3B), the development of Cd toxicity may be accelerated by the induced secondary Zn deficiency in the kidney. Repeated injections of Cd into rats caused a decreased activity of Zn-enzymes such as alkaline phosphatase and leucine aminopeptidase in the kidney (Sato and Nagai 1982) and this reduction was thought to be caused by exchange of Zn with Cd in the enzyme molecule (Friberg *et al.* 1974). Another possibility for this reduction in enzyme activity is a decrease in Zn available for these enzymes as mentioned above. Further studies on relationships between Cd-induced alteration in Zn metabolism in the tissue and development of Cd toxicity are required.

Templeton and Cherian (1984) demonstrated a lability of the pancreatic Zn pool, as compared with that of the liver and kidney, in rats which were pretreated with Cd prior to being made Zn-deficient. The present study showed that the amount of Zn bound to MT was reduced to 30% in hepatic MT and to 60% in renal MT in the Cd-treated group of Zn-deficient rats (−Zn + Cd), as compared with that in the Zn-adequate rats (+Zn + Cd) (Figure 5). Furthermore, the net increase of Zn in renal MT induced by Cd exposure in the Zn-deficient rats (−Zn + Cd) was almost the same as that in the Zn-adequate rats (+Zn + Cd), while the liver amount of Zn bound to MT was sensitive to the Zn status of the whole body. Thus, these data suggest that Zn deficiency causes different influences on the accumulation of Zn in the MT of different tissues.

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