

Metallothionein in the Extracellular Fluids as an Index of Cadmium Toxicity

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In rats injected with 5 μ mole CdCl₂/kg, 5 days/week, metallothionein was detected in plasma by gel filtration chromatography as early as four weeks. The mean renal concentration of cadmium was 80 μ g/g. The excretion of cadmium in urine at this time was rather low and amounted to 0.01% of the total dose. The amount of metallothionein in plasma, as determined by ¹⁰⁹Cd-binding to the 10,000 molecular weight fraction, increased markedly during week 14. Its excretion in urine, however, did not start until about 10 weeks, when the cadmium concentration in kidney approached a mean value of 212 μ g/g. Signs of renal toxicity were evident from glucosuria and proteinuria which became severe during the next four weeks. The excretion of cadmium in urine increased markedly and the majority of it was in the form of metallothionein. It is suggested that the appearance of metallothionein in plasma and urine can be used as specific indices of cadmium poisoning and that the assay of the protein in these fluids may be useful in screening for excessive cadmium exposure.

Introduction

The chronic exposure to cadmium is known to produce renal tubular dysfunction in animals as well as man when the concentration of cadmium in renal cortex exceeds 200 μ g/g wet weight (1). Measurement of cadmium concentration in urine or blood usually reflects the extent of recent exposure. Blood concentrations especially do not correlate with the body burden. There is, therefore, no threshold value for blood or urine cadmium concentration that can be used to predict the onset of renal dysfunction (1). Once the tubular damage has occurred in suspected populations of factory workers, the confirmation is usually made by measuring the urinary β_2 -microglobulin level (2, 3).

Most of the absorbed cadmium in the body is sequestered by metallothionein which under normal circumstances is an intracellular protein. However, after long-term exposure to low levels of cadmium, the presence of metallothionein in plasma and urine has been observed by others (4-6) and also in our laboratory (7). The physiological functions of the

protein are not yet fully understood. Of the various functions that have been ascribed to the protein, the two that have been advocated by Piscator and Nordberg are protection from the toxicity of cadmium and transport of the metal from liver to the kidneys (1). Contrary to this theory is the observation that intravenous administration of the protein into animals results in nephrotoxicity, characteristic of cadmium poisoning (8, 9). It could be postulated, therefore, that there is a connection between the presence of metallothionein in the plasma and subsequent renal toxicity in animals exposed to cadmium. The study described here provides for the first time some insight into the existence of such a relationship.

Materials and Methods

The study was carried out in Wistar rats injected subcutaneously with 5 μ mole ¹⁰⁹CdCl₂/kg, 5 days per week, for up to 14 weeks. The amount of cadmium in whole-body, tissues, and excreta was determined by counting the radioactivity and converting the values to mg cadmium using the specific activity of the injection solution. It was assumed that dietary intake of the metal resulted in negligible increase in body burden. Glucose in urine was tested with Multistix (Ames Company) and protein was assayed by Tsuchiya's reagent as described by Piscator (10). Analysis of metallothionein was car-

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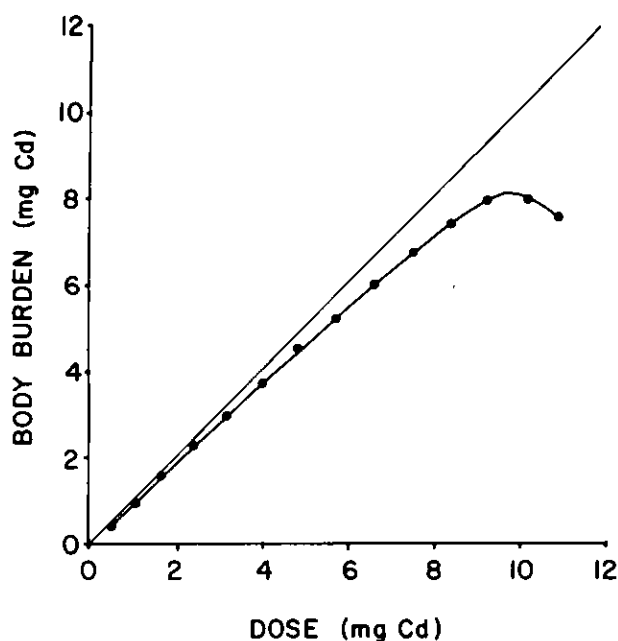


FIGURE 1. Relationship between the injected dose and the body burden of cadmium. Each point is a mean of 4 to 19 animals.

Table 1. Distribution of cadmium in liver and kidney.

Weeks	Dose, % ^a	
	Liver	Kidney
4	62.0 ± 3.4	6.3 ± 0.4
6	64.9 ± 3.3	6.6 ± 0.4
8	64.6 ± 3.3	6.8 ± 0.3
10	55.3 ± 3.5	6.9 ± 0.4
12	59.5 ± 1.4	6.7 ± 0.3
14	49.9 ± 4.3 ^b	5.0 ± 0.2 ^b

^a Mean ± S.D. of 3-4 values.

^b Significantly different from 4 week values ($p < 0.05$).

ried out in liver, kidney, plasma, and urine by the method described previously (11).

Results and Discussion

Continuous exposure to cadmium seemed to affect the growth of the rats. Although the mean weights of the control and cadmium-injected groups did not differ significantly except after 14 weeks, the mean weight of the cadmium-injected animals was consistently lower than that of the control group after the third week. Due to the small number of animals used for this study, this point cannot be further stressed. However, it does appear that the toxic effects of cadmium may have started after three weeks of exposure. Loss of body weight has been previously described in rabbits by Nomiyama (12).

The relationship between the injected dose of cadmium and the body burden of the rats is shown in Figure 1. With increasing dosage, smaller quantities of cadmium were retained. Between weeks 12 and 13, the rats accumulated and maintained the maximum quantity of cadmium (8 mg). Thereafter, the total retention declined although the exposure continued for another week. It thus appears that the maximum quantity of cadmium that can be accumulated by 300 to 350 g rats is 8 mg. The rats were probably suffering from severe renal toxicity during the last two weeks.

The rats were sacrificed at periodic intervals and their liver and kidneys were assayed for ¹⁰⁹Cd (Table 1). It was found that the liver contained about 60% of the injected dose during the first 12 weeks of exposure. However, during the next two weeks there was a significant (20%) reduction in cadmium content of the liver. A similar phenomenon was observed in the kidneys although the total cadmium content of the kidneys was almost ten times lower than the liver. The above observations indicate that the reduction in body burden during weeks 12-14 was caused mainly by the lower retention of cadmium by the liver and kidneys. Interestingly, at all times about 80% of the liver cadmium and 70% of the kidney cadmium were bound to metallothionein. This suggests that the decrease in tissue retention of cadmium is not caused by decreased binding by metallothionein.

When the cadmium content of the kidneys was expressed as $\mu\text{g/g}$ wet tissue weight, a rather interesting phenomenon was observed. As shown in Figure 2, the concentration of cadmium in kidney increased linearly up to ten weeks of exposure and reached a mean value of 212 $\mu\text{g/g}$. The cadmium concentration remained above 200 $\mu\text{g/g}$ during the next four weeks. In chronic cadmium toxicity kidney is regarded as the target organ and 200 $\mu\text{g/g}$ of renal cortex is considered as the critical concentration (1). In the present study, the kidneys were not dissected into the cortex and the medulla. Assuming that the cortex contains about three times as much cadmium as the medulla (13) it is possible that these rats contained more than 300 μg cadmium/g cortex at 10 weeks and thereafter. The symptoms of cadmium toxicity became evident after 10 weeks, as seen by the presence of glucosuria and proteinuria. During week 14, the urine contained as much as 100 mg protein/24 hr.

It was of interest to determine at what time period during the course of continuous exposure does the metallothionein begin to appear in the plasma. The samples of plasma collected from rats after only four weeks of exposure, when analyzed by gel filtration chromatography, showed a small but distinct

cadmium peak in the area where standard rat metallothionein is eluted (Fig. 3). The corresponding whole kidney cadmium concentration was 80 $\mu\text{g/g}$. With the duration of exposure, the amount of cadmium in plasma increased. After 10 weeks, although some cadmium was bound to metallothionein, most of it was associated with larger molecular weight proteins, probably albumin and globulins. After 14 weeks, however, about one-third of the plasma cadmium was bound to metallothionein. The

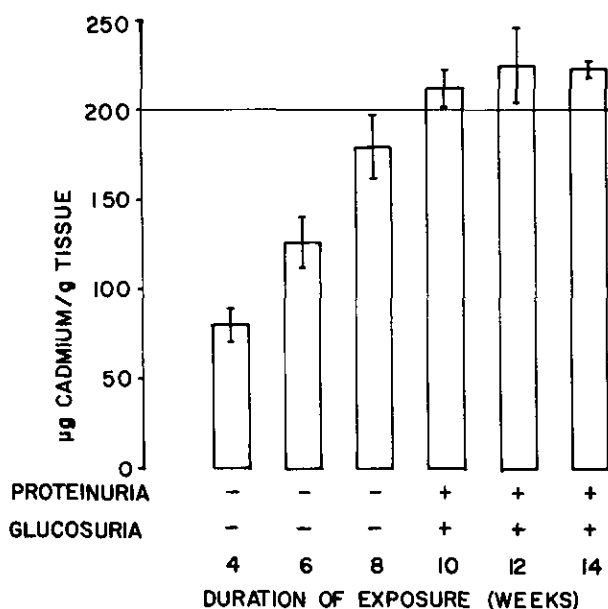


FIGURE 2. Concentration of cadmium in the kidney and the occurrence of renal damage. Each point is the mean \pm S.D. of data obtained from the three animals, except after 14 weeks, when four rats were sacrificed.

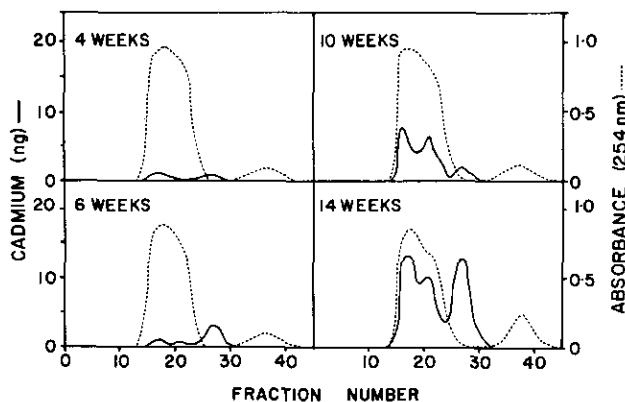


FIGURE 3. Gel filtration chromatography of rat plasma. 1 ml of plasma was applied to 0.9×60 cm column packed with Sephadex G-75. The column was eluted with 1M Tris-HCl, pH 8.6 buffer, at 10 ml/hr. The eluant was monitored at 254 nm and 1 ml fractions were collected.

origin of the protein in plasma is not known. It is possible, though, that it may have been released from the liver and to some extent from other tissues as well.

The elimination of cadmium took place mainly via the fecal route (Fig 4). At the end of the second week 6.4% of the total dose was excreted in the feces. There was a linear increase in fecal excretion of cadmium with the increase in exposure up to week 10, and 9.7% of the dose was excreted in feces. The fecal excretion was relatively high during the next two weeks and was much more pronounced during the final weeks. The liver, kidney and other tissues which accumulate cadmium had apparently reached their saturation level and the excess cadmium was simply not retained. Total excretion during the 14 weeks accounted for almost 22% of the injected dose. Biliary and pancreatic secretions, desquamation of intestinal epithelium cells, and direct excretion of cadmium through the intestinal wall may have all contributed to the loss of cadmium through the fecal route (14, 15).

Excretion of cadmium in urine was relatively small, but it increased linearly during the eight weeks of exposure (Fig. 5). The amount excreted in urine was 0.01% of the total dose after four weeks and it doubled in eight weeks. The appearance of glucosuria and proteinuria after ten weeks was also accompanied by relatively large excretion of cadmium in urine. By the end of 14 weeks, the rats had excreted 7.25% of the injected dose in urine.

In order to examine the form of cadmium that was being excreted in the urine, an aliquot of the fresh

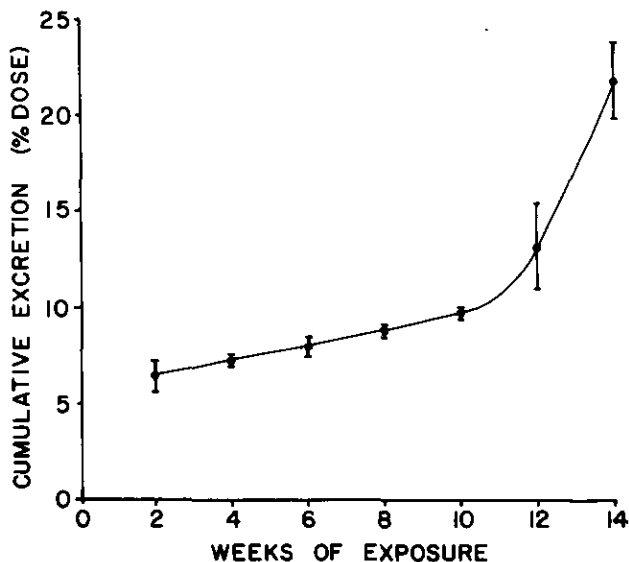


FIGURE 4. Excretion of cadmium in feces. Each point is the mean \pm S. D. of data from four rats.

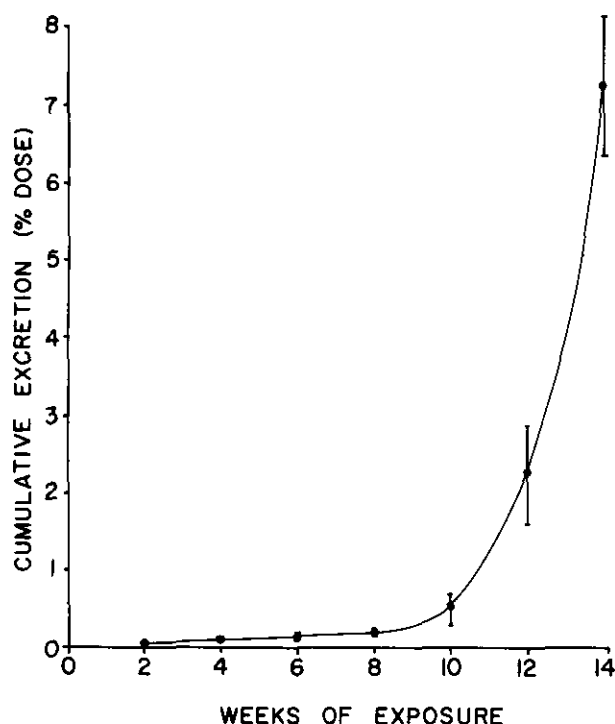


FIGURE 5. Excretion of cadmium in urine. Each point is the mean \pm S. D. of data from four rats.

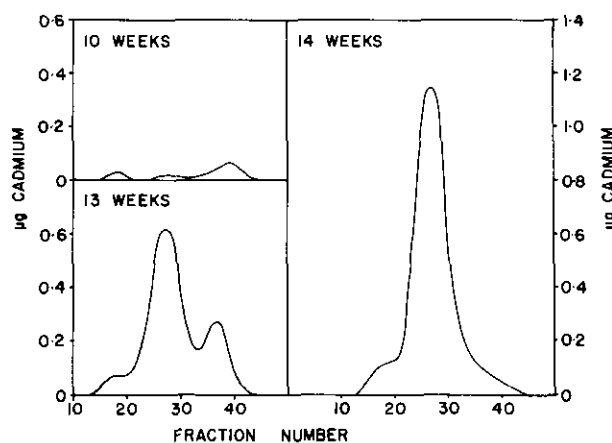


FIGURE 6. Gel filtration chromatography of urine; 1 ml of clear urine was applied to the column. Other details were the same as described for Figure 3.

urine sample was separated by gel filtration chromatography. As seen in Figure 6, the urine from an animal injected with cadmium for ten weeks separated into three cadmium-binding peaks. Of these, the middle peak corresponded with the elution volume of standard rat metallothionein. No such peak of cadmium was detected in urine samples analyzed prior to 10 weeks. The excretion of cadmium associated with metallothionein increased as the

plasma metallothionein level increased and the renal damage progressed. After 14 weeks about 90% of the cadmium in urine was excreted bound to metallothionein. Previous work reported from our laboratory has shown that intravenously injected ^{109}Cd or ^{35}S -labeled metallothionein is excreted in rat urine as intact protein, and its excretion is dependent on the injected dose (16, 17).

Conclusions

Under conditions of continuous exposure, it appears that more cadmium is excreted as the duration of exposure is increased. Before the onset of renal dysfunction, both fecal and urinary excretion are proportional to the exposure. After the toxicity symptoms appear, the excretion is considerably higher through both routes. There is less retention of the dose by liver, kidney and other tissues.

Renal toxicity occurs when the concentration of cadmium in whole kidney exceeds $200 \mu\text{g/g}$. Metallothionein appears in the urine along with other proteins and its concentration increases with the severity of the damage. In plasma, however, the protein is present long before the onset of nephrotoxicity. If metallothionein is monitored in plasma of exposed individuals, it would be an excellent screening tool for excessive exposure. Its presence may serve as a warning against potential renal toxicity of cadmium. By measuring metallothionein in urine from industrial workers and severely exposed populations it may be possible to determine the extent of damage. Such a test would be more meaningful and more specific than the measurement of β_2 -microglobulin in urine. More work is needed to test the applicability of the observations in animals to the human situations. There is also a need to develop a sensitive and selective method to measure metallothionein in biological fluids.

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