

# Distribution of Metallothionein-Bound Cadmium and Cadmium Chloride in Mice: Preliminary Studies

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Metallothionein from livers of mice was isolated by gel chromatography and isoelectric focusing. One of two forms thus obtained contained 32% cysteine. This form, labeled *in vitro* with  $^{109}\text{Cd}$ , was injected intravenously in mice, and the distribution of  $^{109}\text{Cd}$  was studied. Animals killed after 4 hrs had over 80% of the injected dose in the kidneys. Protein obtained after gel chromatography, containing both forms of cadmium-binding protein, was also labeled *in vitro* with  $^{109}\text{Cd}$  and injected intravenously. Animals killed 4 hrs after injection had 50% of the injected dose in the kidneys. Whole-body measurements and whole-body autoradiography demonstrated that approximately 40-60% of the injected dose had been excreted in urine. The results show a selective accumulation of metallothionein-bound cadmium in the kidney and indicate possible differences in distribution and excretion of cadmium depending on binding to different forms of low molecular weight cadmium-binding proteins.

## Introduction

A low molecular weight protein with an unusually high content of cysteine was first reported by Margoshes and Vallee (1). Kägi and Vallee (2) published further data on this protein, at which time they gave it the name, metallothionein. A subsequent finding was that cadmium induced the synthesis of this protein (3).

The metabolic role of metallothionein is still under discussion (4), one suggestion being that it acts as a detoxifying agent for cadmium (5). In animal experiments, this role has been appeared to involve on the one hand a protective effect against testicular damage (6) and on the other hand a damaging action upon the kidney (7). A theory has

been advanced implying that metallothionein plays a part in the redistribution of cadmium from the liver to the kidney which occurs in long-term exposure to cadmium (5).

The studies presented here were designed to elucidate the fate of  $^{109}\text{Cd}$ -labeled metallothionein when given intravenously. The study was performed at two laboratories and therefore somewhat different techniques as well as two different mouse strains were used.

## Materials and Methods

### Labeling of Metallothionein

Mouse liver metallothionein was prepared as described by Nordberg, Nordberg, and Piscator (8). The protein, obtained after ultracentrifugation and chromatography on G-75 and G-50 Sephadex but not further purified, was designated cadmium-binding protein (Cd-BP).

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Characterization of this Cd-BP by isoelectric focusing revealed two main components. One contained 61% of the total cadmium and very little zinc, and had a pI of approximately 4 (identical with the cadmium-metallothionein form 1 to be described below). Another contained 24% of the total cadmium and the main part of the zinc that was present in the preparation. This component had a pI of approximately 6.

Isoelectric focusing was also employed for further purification of metallothionein. In order to optimize the output of purified protein, fractions corresponding to a pH-interval of 3.9-4.2 were pooled and deampholytized. The protein thus obtained was designated metallothionein form 1 (Cd-MT1). Characterization by amino acid analysis gave only minor differences from earlier findings by Nordberg, Nordberg, and Piscator (8). A high purity of the present metallothionein (Cd-MT1) was indicated by the high content of cysteine (32 residues - %) and the absence of aromatic amino acids. The metal content of lyophilized Cd-MT1 was 10% Cd (w/w) and approximately 0.4% Zn (w/w). The  $E_{250}/E_{280}$  ratio was 17. The labeling of Cd-BP and Cd-MT1 was performed in the same way. The respective proteins were exposed to  $^{109}\text{Cd}$  *in vitro* in an aqueous medium containing Tris-NaCl (Tris buffer 0.01M in 0.05M NaCl, pH 8.0, for the Cd-BP; Tris buffer 0.1M in 0.5M NaCl, pH 8.0, for Cd-MT1). That all  $^{109}\text{Cd}$  had been bound to the protein and that virtually no free cadmium remained were checked by chromatography on G-50 and G-75 Sephadex (Figs. 1 and 2). The radioactive cadmium peak appeared at the same elution volume as has the nonradioactive metallothionein cadmium peak earlier.

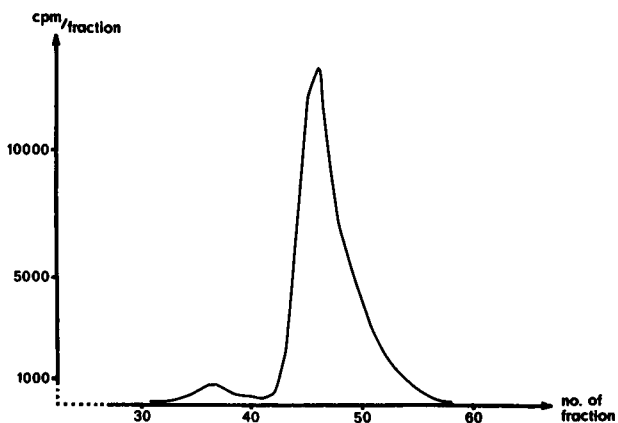


FIGURE 1. G-50 Sephadex chromatography of a sample of  $^{109}\text{CdBP}$ . Column dimensions 885 × 25 mm. Elution with 0.01M Tris buffer - 0.05M NaCl, pH 8.0, + 8°C. Fractions of 5 ml were collected at 28 ml/hr. Recovery of Cd, 98%.

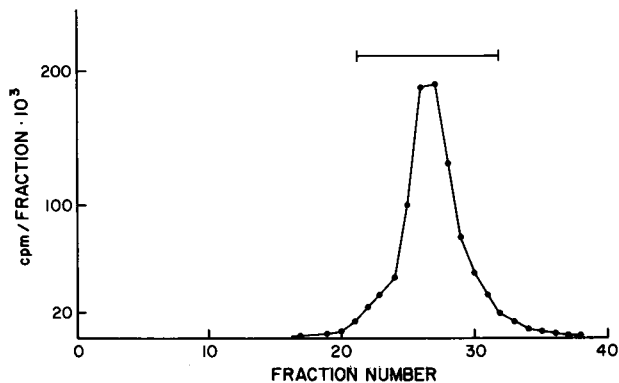


FIGURE 2. G-75 Sephadex chromatography of  $^{109}\text{CdMT-1}$ . Column dimensions, 365 × 25 mm. Elution with 0.1M Tris buffer - 0.5M NaCl, pH 8.0, + 4°C. Fractions of 5 ml were collected at 14 ml/hr. Fractions 21-31 indicated by the bold line were concentrated, buffer concentration and pH adjusted to physiological values (0.9% NaCl, pH 7.2), and injected intravenously in mice. Recovery of Cd, 98%.

## Animals

Twenty-five male C57BL16JH (J67) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine 04609, U.S.A. The mice, which had body weights of 25-33 g, were divided into two groups for studies on  $^{109}\text{Cd-MT1}$  and  $^{109}\text{CdCl}_2$  in comparable doses (Table 1).

Twenty male CBA-mice from the Institute of Genetics, University of Stockholm, Sweden, with body weights of 20.0-25.3 g, were divided into two groups and used for studies on  $^{109}\text{Cd-BP}$  and parallel injections of  $^{109}\text{CdCl}_2$  (Table 2).

## Treatment of Animals

All animals were given a single intravenous injection in a tail vein. Fourteen C57BL mice were given  $^{109}\text{Cd-MT1}$ , and eleven such mice were given the same doses of cadmium as radiolabeled  $\text{CdCl}_2$ . Subdivision of animals in two dose groups (0.03 and 0.08 mg Cd/kg) is seen in Table 1. In order to collect urine from mice with 4 hr survival time the animals were put in metabolism cages during the interval between injection and killing. Animals were killed after 5 min or 4 hr by heart puncture and cervical dislocation. Organs were removed and weights recorded. Cadmium content was calculated from values obtained by gamma counting of the organs (6).

**Table 1. Amount of  $^{109}\text{Cd}$  found in kidney and liver in mice injected intravenously with  $^{109}\text{Cd-MT1}$ ,  $^{109}\text{Cd-BP}$ , and  $^{109}\text{CdCl}_2$ .**

Time after injection	$^{109}\text{Cd}$ in kidneys, % of dose <sup>a</sup>						$^{109}\text{Cd}$ in liver, % of dose <sup>a</sup>					
	$^{109}\text{Cd-MT1}$		$^{109}\text{Cd-BP}$ , I	$^{109}\text{CdCl}_2$			$^{109}\text{Cd-MT1}$		$^{109}\text{Cd-BP}$ , I	$^{109}\text{CdCl}_2$		
	L	H		L	H	I	L	H		L	H	I
5 min	45.0	43.5	30.8	2.9			2.5	3.9	4.8	27.4		
	31.8	32.3	34.4	2.3	3.1	3.2	3.2	2.7	5.6	12.7	20.8	32.8
	39.0	38.9		2.3	4.0	3.2	5.8	2.8		19.3	23.8	40.8
Mean	38.6	38.2	32.6	2.5	3.6	3.2	3.8	3.1	5.2	19.8	22.3	36.8
4 hr	82.1	76.8		10.7	6.5	4.9	4.2	8.2		56.0	55.5	68.5
	93.4	89.5	43.7	5.8	6.6	4.6	10.6	8.9	4.3	48.6	56.0	60.8
	107.0	84.0	56.4	5.0	6.7		10.6	7.3	4.8	44.9	62.8	
	99.1	73.6					7.0	5.1				
Mean	95.4	81.0	50.1	7.2	6.6	4.8	8.1	7.4	4.6	49.8	58.1	64.7

<sup>a</sup>Dose levels: L = low dose = 0.03 mg Cd/kg; H = high dose = 0.08 mg Cd/kg; I = intermediate dose = 0.075 mg Cd/kg.

**Table 2. Body burden and concentration of cadmium in kidney and liver of mice injected intravenously with  $^{109}\text{Cd}$ -binding protein ( $^{109}\text{Cd-BP}$ ) and  $^{109}\text{CdCl}_2$ .**

$^{109}\text{Cd}$ compound	Time interval from injection to killing	Body burden, $\mu\text{g Cd/whole body}$		Cd, $\mu\text{g/g wet tissue}$	
		After injection	Just before killing	Kidney	Liver
$^{109}\text{Cd-BP}$	2 min	1.5	—	0.88	0.10
	5 min	1.5	—	1.54	0.06
	6 min	1.5	—	1.72	0.07
	20 min	1.6	—	1.08	0.05
	20 min	1.3	1.3	1.97	0.05
	4 hr	1.4	1.0 <sup>a</sup>	2.04	0.06
	4 hr	1.5	0.8 <sup>b</sup>	2.82	0.10
	24 hr	1.5	0.8	2.18	0.09
	96 hr	1.6	0.6	2.17	0.09
	96 hr	1.7	0.6	2.52	0.09
	$^{109}\text{CdCl}_2$	1 min	1.4	—	0.19
5 min		1.5	—	0.16	0.51
5 min		1.5	—	0.16	0.41
20 min		1.5	—	0.20	0.68
20 min		1.7	1.7	0.16	0.80
4 hr		1.7	1.9	0.28	0.97
4 hr		1.5	1.6	0.23	0.76
24 hr		1.4	1.4	0.24	0.79
96 hr		1.7	1.6	0.25	0.99
96 hr		1.6	1.3	0.25	0.84

<sup>a</sup>0.4  $\mu\text{g}$  measured in urine (and feces) collected immediately prior to whole body counting.

<sup>b</sup><0.0001  $\mu\text{g}$  measured in feces collected immediately prior to whole body counting.

Ten CBA-mice were given Cd-BP and ten mice were given radioactive cadmium as  $\text{CdCl}_2$ . Each animal was given approximately 1.6  $\mu\text{g Cd}$  and 0.4  $\mu\text{g Zn}$ , corresponding to a dose of 0.075 mg Cd/kg body weight and 0.02 mg Zn/kg body weight. The cadmium/zinc ratio is the same as in the Cd-BP. One or two animals were killed after 1, 5, and 20 min and 24 and 96 hr (Table 2). After injection and just before killing all animals were subjected to whole-body counting.

## Other Methods

Autoradiography was performed on the animals treated with  $^{109}\text{Cd-BP}$  and the corresponding ones injected with  $^{109}\text{CdCl}_2$ . The method was described by Ullberg (11). In the main, sections of the frozen mouse were prepared at 20  $\mu\text{m}$  on a tape and apposed to Structurix or Kodirex X-ray film.

Scintillation counting of organs removed from the thawed corpses of the mice (after sectioning)

was performed in a gamma counter as described by Nordberg (6).

## Results and Discussion

### Scintillation Counting

Table 1 gives the amounts of cadmium found in liver and kidney after injection of  $^{109}\text{Cd-MT1}$ . After 5 min, 38.6% (mean) of the total injected dose has already accumulated in the kidney in the lower dose group. The corresponding figure for the higher dose group was almost the same (38.2%). By 4 hr after the injection, the animals given the lower dose had an average of 95.4% of the total injected dose in the kidneys, and the corresponding figure for the higher dose was 81%.

In some cases more than 100% of the injected dose were recovered in liver and kidney, particularly in the lower dose group. Indeed, an error of the order 20% may easily arise and be explained by the small volume of solution injected (0.05 ml), since the syringe was graduated in 0.01 ml units. In the higher dose group, larger volumes (0.15 ml) were used, the relative error upon injection therefore being smaller.

It is further evident from Table 1 that more than 10 times as much cadmium was accumulated in the kidneys when the cadmium was injected as Cd-MT1 than as CdCl<sub>2</sub>; liver values, on the other hand, were lower.

Values for animals injected with Cd-BP and with survival times corresponding to those in the Cd-MT1 study are also given in Table 1. In these animals approximately 10-fold larger amounts of cadmium were found in kidneys than with the corresponding CdCl<sub>2</sub> animals.

A direct comparison of Cd-BP animals with CdMT1 ones is obviously somewhat limited because different strains of mice were used for the two studies. It might be appropriate to point out, however, that the renal accumulation expressed as per cent of injected dose in Cd-MT1 animals (81%) seems higher than when approximately the same dose of cadmium was injected as Cd-BP (50%).

This observation was further supported by the whole body measurements in Table 2. Whereas a very minor tendency, i.e., approximately 10%, towards a decrease arose in CdCl<sub>2</sub> animals during the 96 hr, the whole body values of the Cd-BP mice decreased by approximately 40–60% by 4–96 hr after injection.

Urine values for the CdMT1 injected mice (4-hr survival time) showed a broad range for the lower

dose group. They varied from 0.03–1.23% of the injected dose. In the higher dose group, corresponding values were 0.03–2.18% of the injected dose. For the Cd-BP injected mice some occasional excretion values were also obtained. One 4-hr mouse excreted 0.4  $\mu\text{g}$  (24%) of cadmium in urine and feces. The second 4-hr mouse excreted less than 0.0001  $\mu\text{g}$  Cd in feces during the same time interval. Urine was not collected. From this it seems most likely that the cadmium has been excreted via urine. This also indicates a difference in Cd-excretion of the two protein forms Cd-MT1 and Cd-BP. The mice injected with  $^{109}\text{CdCl}_2$  had a low excretion of cadmium in the urine and the values (4 hr) ranged from 0.02 to 0.08% of the injected dose.

### Autoradiography

**Distribution among Organs:** The general distribution pattern of injected  $^{109}\text{CdCl}_2$  was the same as that reported by Berlin and Ullberg (9) and Nordberg and Nishiyama (10) with the highest concentration in the liver and somewhat lower concentrations in pancreas and kidney at the survival times employed (2 min–1 day).

A markedly different pattern made itself evident in autoradiograms from animals injected with  $^{109}\text{Cd-BP}$ . By 2 min after injection, substantial uptake of cadmium in the renal tissue had already occurred, this organ standing out with considerable blackening against a very pale background representing other tissues. Blackening of an intensity similar to the one corresponding to the kidney was also seen to represent the contents of the urinary bladder in 20-min animals, but not in animals having shorter or longer survival times. The difference in the distribution patterns between CdCl<sub>2</sub> mice and Cd-BP mice is illustrated in Figure 3.

**Distribution within the Kidneys:** Autoradiographic studies employing the whole body sectioning technique also enable determination of distribution among various structures within organs (9, 11). In the present study the distribution within the kidney is of particular interest. In autoradiograms from animals injected with  $^{109}\text{CdCl}_2$ , the distribution pattern in the kidney was in accord with that reported previously (9, 10, 12), i.e., a maximum spotwise darkening in the outer zone of the kidney cortex, probably corresponding to the most proximal tubule. At variance with what was reported by Berlin and Ullberg (9), the present

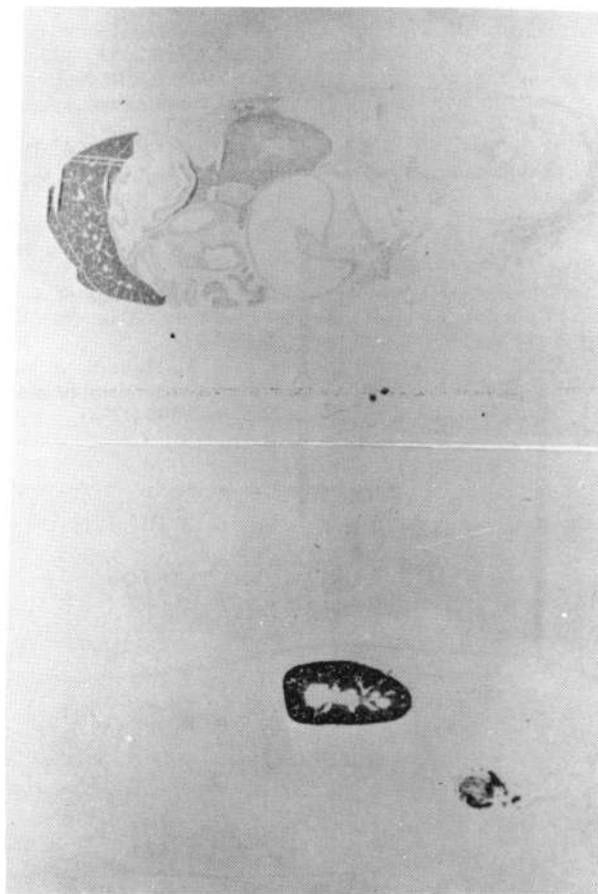


FIGURE 3. Whole-body autoradiograms of mice. (Top) 20 min after a single intravenous injection with  $^{109}\text{CdCl}_2$ . Darkened parts correspond to high accumulation of cadmium. The highest Cd-concentration is seen in the liver. (Bottom) 20 min after a single intravenous injection with  $^{109}\text{Cd-BP}$ . Accumulation is seen in the kidney and urine in the urinary bladder.

autoradiograms demonstrated that the darkening in the renal cortex, even at the darkest spots, never exceeded the darkening of the liver during the shorter times after injection. This may be explained by the highest doses of cadmium used in this study, since it is known (5) that a smaller proportion of cadmium will be found in the kidney of experimental animals given larger doses.

The whole body countings (Table 2) show that the amount of cadmium retained in the group injected with  $^{109}\text{CdCl}_2$  was approximately constant, while appreciable excretion of cadmium occurred already between 20 min and 4 hr in mice injected with  $^{109}\text{Cd-BP}$ . This suggests that the blackening in the central parts of the kidney observed in

autoradiograms obtained at the shortest survival times represents an excretion of cadmium into the urine. Autoradiograms from longer times after injection showed most of the cadmium retained in these  $^{109}\text{Cd-BP}$ -injected mice to be confined to the kidney cortex (Fig. 4). The spotwise appearance of the blackening in the outer cortex of these animals is similar to the pattern unfolded (12) for cadmium chloride injected mice and shown to correspond to an uptake in the first part of the proximal tubule. More detailed studies are warranted in order to define more precisely the localization of metallothionein-bound cadmium.

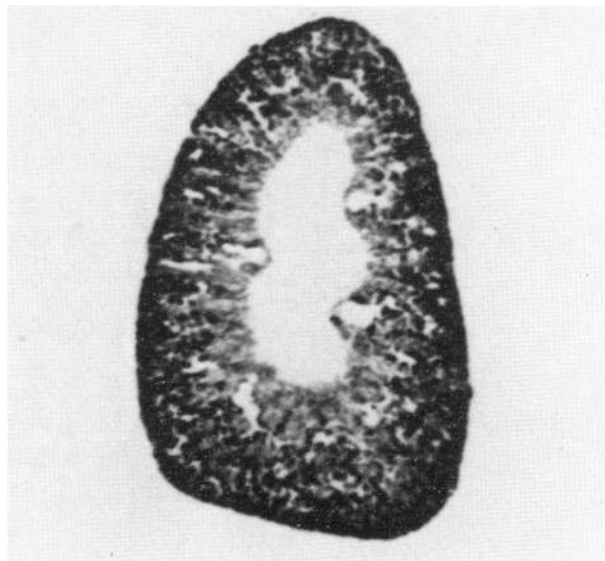


FIGURE 4. Detail (kidney) of whole-body autoradiogram from a mouse intravenously injected with  $^{109}\text{CdBP}$  and killed after 4 hr.

In summary, the present results show that, once it has reached the blood, metallothionein-bound cadmium is transported to the kidney where it accumulates selectively. This supports our previously advanced theory pointing to an involvement of metallothionein in the redistribution of cadmium from liver to kidney in long-term exposure and a role of this protein in the elicitation of renal tubular effects of cadmium.

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