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General Aspects of Cadmium: Transport, Uptake and Metabolism by the Kidney

by Monica Nordberg*

Cadmium taken up from lung and gastrointestinal tract is transported via blood to liver and kidney. On long-term exposure to cadmium, renal tubular dysfunction develops in humans and experimental animals. Data from animal experiments demonstrate that initially after exposure cadmium in blood is bound to albumin and proteins with higher molecular weight. Such cadmium is mainly taken up in liver. For a few days after exposure cadmium exists as metallothionein in plasma and blood cells. After both single and long-term administration of cadmium bound to metallothionein, cadmium is taken up by the kidney. The concentration of metallothionein-bound cadmium in plasma is quite low due to continuous renal clearance. Cadmium from metallothionein is taken up in renal tubules by pinocytosis and subsequently degraded in lysosomes, thereby releasing cadmium which stimulates *de novo* synthesis of metallothionein but also binds to reabsorbed metallothionein. Catabolizing and rebinding are continuous and prevent excretion of cadmium. Because of differences in transport, renal metabolic handling forms of cadmium are also different for different forms of cadmium administered and rate of administration. A single dose of metallothionein-bound cadmium given intravenously is almost immediately and completely taken up in the renal tubule. Under such conditions, resynthesis and rebinding processes are insufficient to sequester cadmium from sensitive tissue receptors, and renal damage occurs at total tissue concentrations much lower than when renal cadmium concentrations rise slowly. This explains the wide range (10–200 μg Cd/g wet weight) of cadmium in the renal cortex that associated with renal tubular dysfunction in experimental animals.

Introduction

Cadmium is taken up from the lung and gastrointestinal tract and transported via the blood to liver and kidney. On long-term exposure to cadmium, renal tubular dysfunction may develop both in humans and experimental animals (*1*). Data from animal experiments demonstrate that initially after exposure cadmium in blood is bound to albumin and proteins with a molecular weight higher than albumin (*2*). Such cadmium is mainly taken up by the liver. Cadmium induces the synthesis of metallothionein in liver and other tissues (Table 1). Metallothionein is a low molecular weight protein involved in cadmium, zinc and copper metabolism (*3*) and has a molecular weight of around 6500 (Table 1). Metallothionein may serve in a protective function by binding cadmium in a stable biocomplex. In this way interference with other cellular components is decreased and the acute effects normally seen in acute exposure can be prevented. Data have been presented in support of this hypothesis, and such a course of events was demonstrated by Nordberg (*4*) in testicular and liver tissue.

Since proteins of a molecular weight similar to that of metallothionein are completely filtered through the glomerular membrane, metallothionein-bound cadmium when released into plasma will be filtered into renal tubular fluid. Such a mechanicsm and a subsequent tubular reabsorption was originally advanced by Piscator (*5*) as an explanation for the prominent accumulation of cadmium in kidneys of animals and human beings, chronically exposed to cadmium. These theories have been further discussed and developed by Nordberg (*6*) and reviewed by Friberg et al. (*1*) and Nordberg (*2*). The present studies aimed at confirming these theories and included investigations of the distribution of cadmium to different forms of metallothionein as well as assessment of adverse effects of such injections upon renal tubular cells. Autoradiographic techniques, metal

*Department of Environmental Medicine, Umeå University, S-901 87 Umeå, Sweden.

Table 1. Characteristics, occurrence and function of metallothionein.

Physical characteristics	
Molecular weight	6000–7000
Cd, Zn, Cu, Hg content	5–10%
Amino acids	30% cystine; no aromatics; no histidine
UV absorption	254 nm
Occurrence	Liver, kidney, plasma, erythrocytes
Functions	Storage of metals Detoxification of metals Protection from metal toxicity Transport of metals Metabolism of essential metals Immune response

analysis and various biochemical separation techniques have been used to gain information for identification of cadmium binding in the body with special interest focused on the transport, uptake and metabolism of cadmium by the kidney.

Material and Methods

In order to study the distribution of cadmium the following study was designed. Twenty-five male CBA mice obtained from Anticimex, Sweden (17–20 g), were divided into five groups and given a single subcutaneous injection of cadmium as radiolabeled cadmium chloride, 1 mg Cd/kg body weight, 100 μCi/mouse of ^{109}Cd. Immediately after exposure and before killing, the mice were taken for whole-body counting. Under slight ether anesthesia the animals were killed by cervical dislocation. They were killed after different time intervals: 20 min (n = 4) and 4 (n = 4), 24 (n = 4), 48 (n = 4), 96 (n = 4) and 192 (n = 5) hr. Liver, kidney, and testis were taken for metal analysis. For further study of the samples gel chromatography was performed on supernatants after ultracentrifugation (105,000g) of tissue homogenates.

Distribution of Cadmium in Blood

Whole blood was drawn from the eye vessels with heparinized capillaries, weighed and counted for radioactivity. Plasma and blood cells were separated by centrifugation. A few samples were totally excluded from the study due to hemolysis. Radioactivity was counted in plasma and blood cells. Blood cells were washed by adding 0.9% saline, and the radioactivity was counted. The cells were hemolyzed by adding deionized water and freezing at −60°C. The cadmium concentrations of blood cells and plasma are shown in Figure 1. Rapid clearance of cadmium was seen in plasma between 4 and 48 hr. In the same time interval an increase in cadmium concentration in the blood cells was observed.

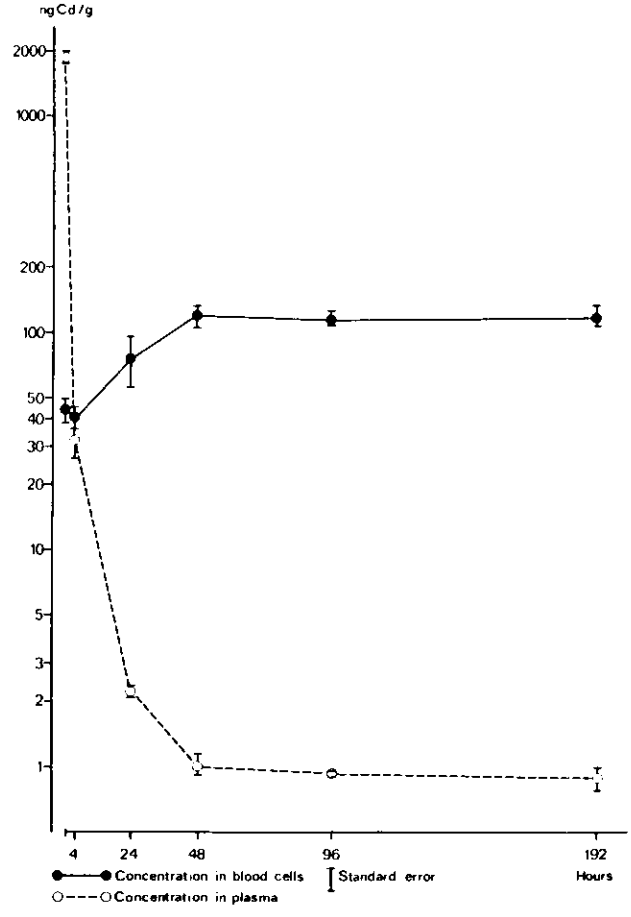

FIGURE 1. Concentrations of cadmium in (○) plasma and (●) blood cells, in mice given a single subcutaneous injection of $^{109}CdCl_2$ (1 mg of Cd/kg body weight) and killed at various times after injection. Vertical bars indicate the standard error and the circles indicate mean values. From Nordberg (2).

Plasma and blood cells were taken for gel chromatography. A 355 × 26 mm column was packed with Sephadex G-75 superfine gel. Elution was carried out with 0.01 M Tris buffer in 0.05 M NaCl (pH 8.0) at a flow rate of 14 mL/hr, and 5 mL fractions were collected. The optical density at 254 nm was continuously monitored.

The distribution pattern of cadmium after gel chromatography of plasma and hemolyzates from animals killed 20 min (plasma only) and 96 and 192 hr after injection is seen in Figures 2 and 3, respectively. Both high and low molecular weight fractions contained cadmium. Data from these animal experiments demonstrate that initially

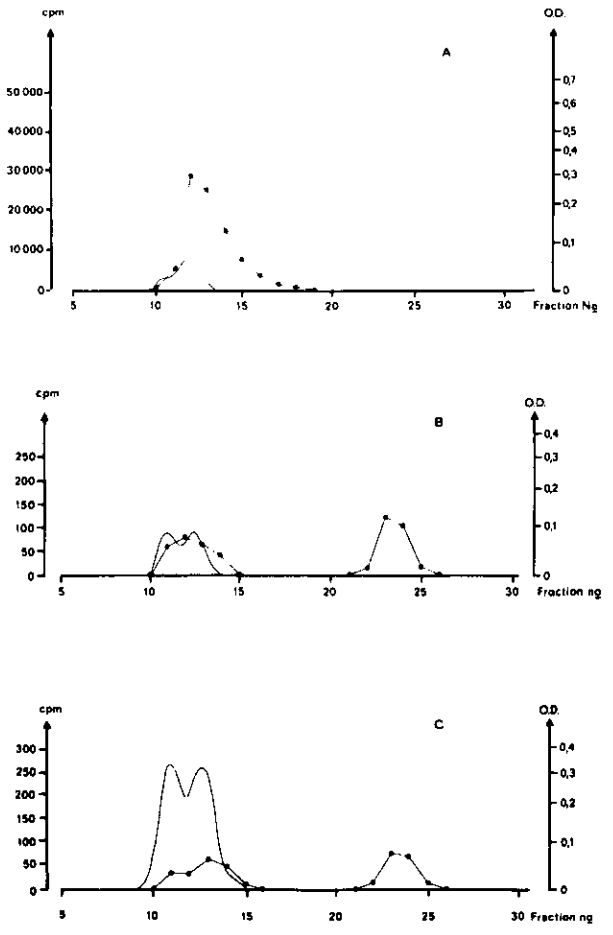

FIGURE 2. Gel chromatography on Sephadex G-75 of plasma from mice given a single subcutaneous injection of $^{109}CdCl_2$ (1 mg Cd/kg): (*A*) 20 min after injection, 20,000 cpm = 45.4 ng of Cd; (*B*) 96 hr after injection, 100 cpm = 0.028 ng of Cd; (*C*) 192 hr after injection, 100 cpm = 0.028 ng of Cd. Column dimensions were 355 × 26 mm. Elution with 0.01 *M* Tris buffer in 0.05 *M* NaCl (pH 8.0) at a flow rate of 14 mL/hr. Volume of fractions: 5 mL OD at 254 nm was continuously monitored. From Nordberg (*2*).

after exposure cadmium in blood is bound to albumin and proteins with molecular weight higher than that of albumin. However, cadmium was not found to be bound particularly to fractions containing hemoglobin. Low molecular weight fractions (fractions of 22–25) contained a major part of the cadmium, and the metal was thus likely to be bound to metallothionein. This evidence supports the idea that cadmium is bound to metallothionein in plasma and blood cells (*7*). Further evidence for the existence of increased metallothionein concentrations in blood plasma in animals and man after repeated exposure to cadmium has been provided by a radioimmunochemical method (*8,9*).

Low Molecular Weight Cadmium Binding Protein in Blood Cells

The indicated low molecular weight fractions (metallothionein) from blood cell hemolyzate were pooled and concentrated on an Amicon cell with a UM-2 filter. The concentrated protein solution was injected intravenously (IV) into mice in order to study the distribution of cadmium in mice.

The protein solution from blood cell hemolyzate was further characterized by isoelectric focusing according to a method described previously (*10*). Cadmium was discovered to be bound to the protein appearing at a pH slightly below 6. Zinc analyses, performed by atomic absorption spectrophotometry, showed that the fractions obtained by isoelectric focusing of cadmium binding protein from hemolyzates did inded contain zinc (Fig. 4). In accordance with a previous report (*11*), pI for metallothionein increases with increasing amount of zinc. This piece of evidence gives some support to the hypothesis that cadmium in blood exists mostly as metallothionein with zinc as the dominating metal. However, the possibility cannot be entirely ruled out that binding occurs to some other low molecular weight zinc-containing protein, since identification of the protein is still incomplete. Mice were intravenously injected with these solutions (approximately 2 ng Cd/mouse). The animals were divided into groups of two to five animals. They were killed 4 and 96 hr later. Animals intravenously injected with $^{109}CdCl_2$ served as controls. The animals were subjected to whole body counting which was performed immediately after injection and just before killing. Animals were put in metabolism cages, and urine was collected 4 hr after injection and 24 hr before killing. Animals injected with cadmium bound to protein isolated from mice killed 96 hr after subcutaneous (SC) Cd injection had kidney values of 66.3 and 58.9% of the IV dose, depending upon observation time, 4 or 96 hr. Corresponding figures for animals injected with protein isolated from hemolyzate of animals killed after 192 hr were 68.5 and 72.2% of the dose (Table 2). These values are about 10 times those seen in animals injected with $^{109}CdCl_2$. Autoradiography performed on mice killed 4 hr after IV injection of Cd-binding protein from blood cells showed accumulation of cadmium in the kidney cortex. High cadmium concentrations were seen in the liver of those given cadmium chloride (Fig. 5). The autoradiographic patterns were similar to those shown in mice injected with hepatic metallothionein (Fig. 6).

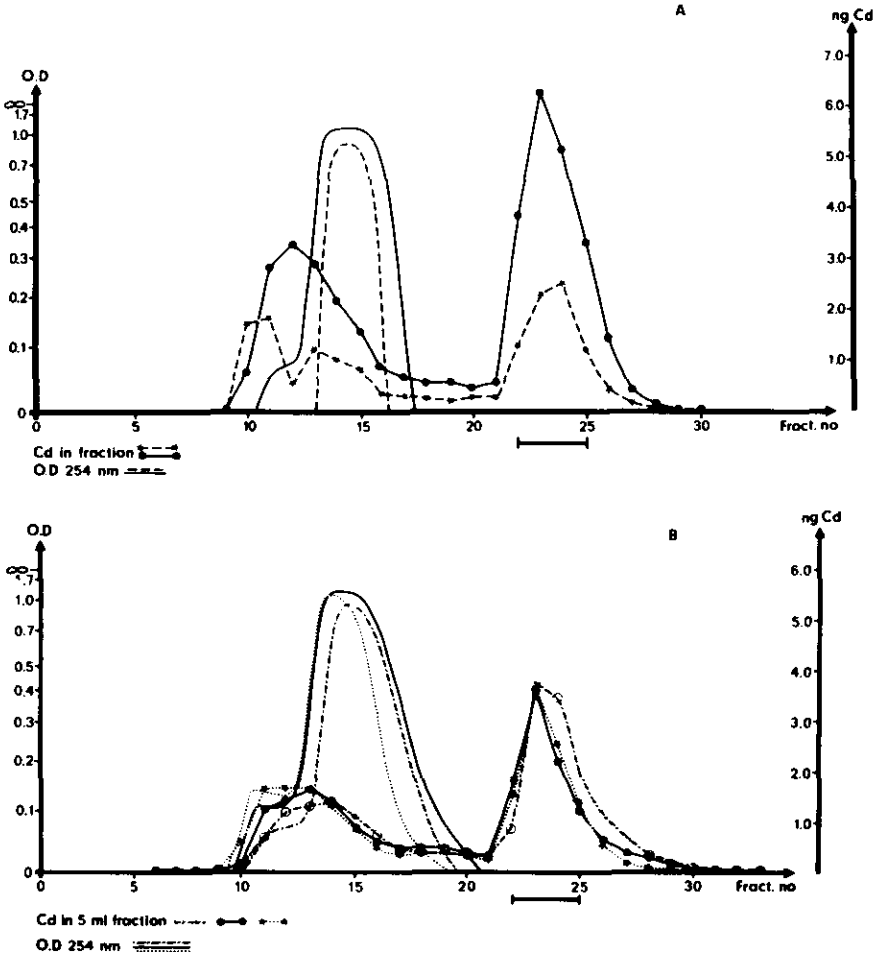

FIGURE 3. Gel chromatography on Sephadex G-75 of hemolyzate of blood cells from mice given a single subcutaneous injection of $^{109}CdCl_2$ (1 mg Cd/kg): (*A*) 96 hr after injection; (*B*) 192 hr after injection. Column dimensions were 355 × 26 mm. Elution with 0.01 *M* Tris buffer in 0.05 *M* NaCl (pH 8.0) at a flow rate of 14 mL/hr. Volume of fractions = 5 mL. OD at 254 nm was continuously monitored. The indicated fractions were pooled, concentrated and taken for further studies. From Nordberg (2).

These findings showed that a few days after exposure, cadmium exists in a biocomplex similar to metallothionein both in plasma and in blood cells. Preliminary results from further separation of blood cells (Nuottaniemi, to be published) into lymphocytes and erythrocytes by Ficoll separation of blood from cadmium single subcutaneously dosed rats and CBA mice showed that cadmium was present mainly in the erythrocytes, with almost no cadmium in the lymphocytes. Cadmium uptake in blood cells may occur either directly from plasma or via the bone marrow (*12*).

Relationship to Toxicity of Cd Binding to Metallothionein

Single as well as long-term parenteral administration (*13*) of metallothionein-bound cadmium gives rise to an uptake of cadmium in the kidney. Metallothionein, the protein that stores cadmium in the body, is found to act as a detoxifying agent for cadmium and is involved in the transport of cadmium from liver via blood to kidney. These mechanisms may explain both the protective effect (*4*) against acute cadmium toxicity, e.g., the testicular damage, and the elicitation of long-term toxicity, i.e., effect upon the kidney (*14*). The renal damage was actually observed as a side effect when using metallothionein as a protective agent against testicular necrosis (*4*).

Metabolism by the Kidney and Renal Toxicity

Subcutaneous injection of metallothionein in mice at doses of 6, 2.5, 1.5, 1.3 and 1.1 mg of Cd/kg

Table 2. Amount of cadmium in the kidney of mice injected intravenously with hemolyzate of mice.

Cadmium compound	Time after injection		% of amount injected
$^{109}Cd\ Cl_2$	4 hr		6.1
			6.9
			10.1
			6.5
			4.5
		Mean	6.8
	96 hr		6.2
			6.6
		Mean	6.4
^{109}Cd-binding protein from 96-hr hemolyzate	4 hr		55.9
			74.9
			68.2
		Mean	67.4
	96 hr		67.4
			50.3
		Mean	58.9
^{109}Cd-binding protein from 192-hr hemolyzate	4 hr		75.9
			66.6
			63.0
		Mean	68.5
	96 hr		71.3
			73.1
		Mean	72.2

body weight and control animals showed a marked difference of cadmium toxicity. Animals in the metallothionein group did not survive these doses of cadmium (*14*). The histological examination showed dose-dependent renal damage in animals exposed to cadmium (0.05–0.6 mg Cd/kg body weight) as metallothionein (*14*) and a decreased urinary excretion of creatinine was recorded. This provides evidence that the death of the animals in the high Cd-metallothionein dose group was due to renal damage. An experiment (Table 3) with doses of 0.03, 0.08 and 0.075 mg Cd/kg as metallothionein or $CdCl_2$ showed that 4 hr after injection 95% of injected dose was accumulated in the kidney when pure metallothionein was administered (*15*).

The concentration of metallothionein-bound cadmium in plasma is quite low due to continuous renal clearance. Cadmium-metallothionein is taken up in the renal tubule by pinocytosis (*16, 17*) and may subsequently be degraded in the lysosomes, thereby releasing cadmium. This theory has been confirmed in several papers by Fowler et al. (*18*). Cherian et al. (*19*) have presented evidence that, according to our interpretation, is not contradictory to the findings of Fowler

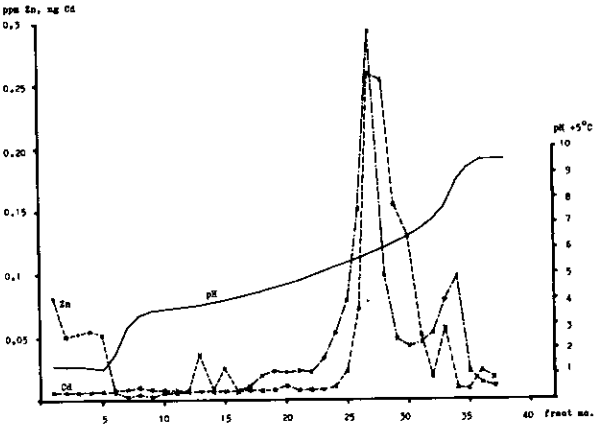

FIGURE 4. Isoelectric focusing of pooled fractions indicated in Fig. 3*B*. Carried out by use of a sucrose gradient 0–50% (w/v). The following carrier ampholytes were used (Ampholine 40 % w/v): in the less dense fraction, 0.4 mL pH 4–6 and 1.6 mL pH 3.5–5; in the more dense fraction, 0.1 mL pH 3.5–5, 0.4 mL pH 4–6 and 0.4 mL pH 6–8.

et al. (*18*). Intracellularly released cadmium may subsequently stimulate *de novo* synthesis of metallothionein but may also bind to reabsorbed metallothionein that has been transported from the liver. The mechanism for release of liver metallothionein to blood has not been studied.

Uptake of cadmium in the kidney has been demonstrated by autoradiographic study of mice intravenously injected with cadmium as metallothionein isolated from livers of rabbit and mouse and from hemolyzate of mouse. Neither specifies differences nor differences in source for isolation of metallothionein were observed. A prominent uptake by the cells in kidney cortex

Table 3. Amount of ^{109}Cd found in kidneys of mice injected intravenously with ^{109}Cd-MT1, ^{109}Cd-BP and $^{109}CdCl_2$.

Time after injection	^{109}Cd in kidneys, % of dose[a]					
	^{109}Cd-MT1		^{109}Cd-BP	$^{109}CdCl_2$		
	L	H	I	L	H	I
5 min	45.0	43.5	30.8	2.9		
	31.8	32.3	34.4	2.3	3.1	3.2
	39.0	38.9		2.3	4.0	3.2
Mean	38.6	38.2	32.6	2.5	3.6	3.2
4 hr	82.1	76.8		10.7	6.5	4.9
	93.4	89.5	43.7	5.8	6.6	4.6
	107.0	84.0	56.4	5.0	6.7	
	99.1	73.6				
Mean	95.4	81.0	50.1	7.2	6.6	4.8

[a]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).

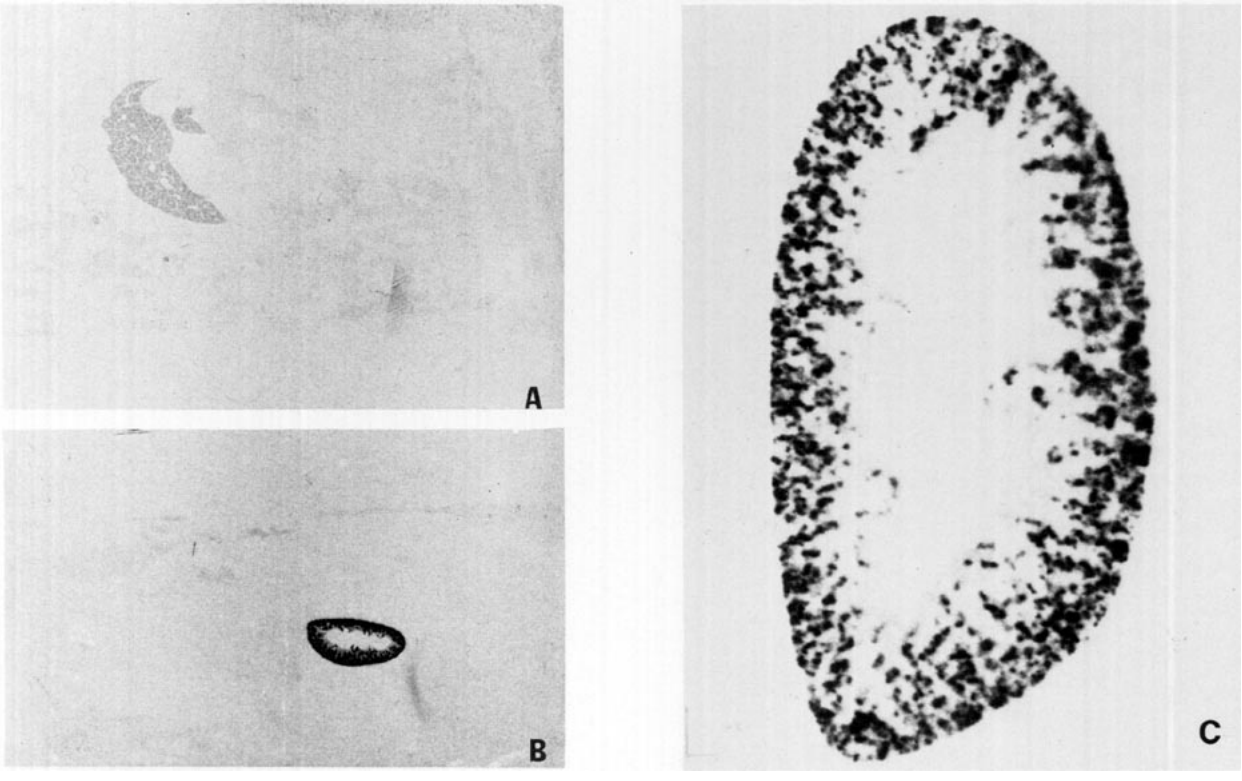

FIGURE 5. Whole body autoradiograms of mice: (*A*) 4 hr after a single intravenous injection with $^{109}CdCl_2$; darkened parts correspond to high accumulation of cadmium, and the highest Cd concentration is seen in the liver; (*B*) 4 hr after a single intravenous injection with fractions indicated in Fig. 3*B*; accumulation is seen in the kidney; (*C*) detail (kidney) of whole-body autoradiograms from a mouse (Fig. 5*B*).

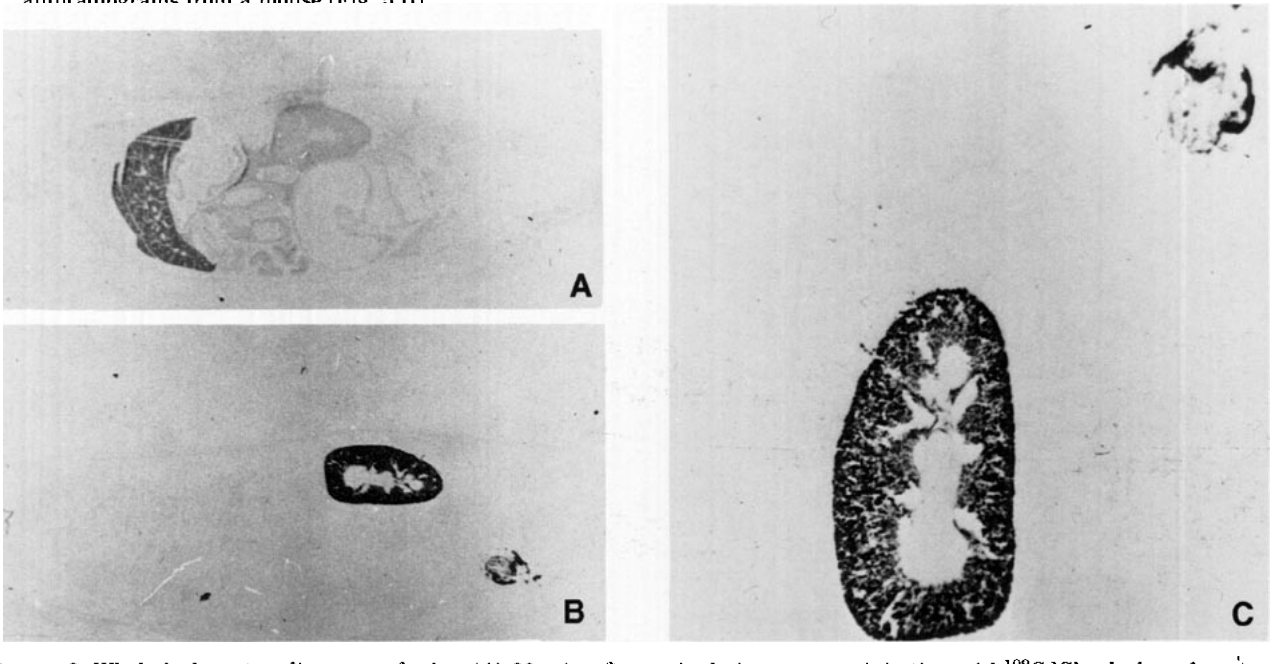

FIGURE 6. Whole body autoradiograms of mice: (*A*) 20 min after a single intravenous injection with $^{109}CdCl_2$; darkened parts correspond to high accumulation of cadmium with the highest Cd concentration seen in the liver; (*B*) 20 min after a single intravenous injection with $^{109}CdBP$; accumulation is seen in the kidney and in the urinary bladder; (*C*) Detail (kidney) of whole-body autoradiogram from a mouse intravenously injected with $^{109}CdBP$ and killed after 4 hr. From Nordberg and Nordberg (*10*).

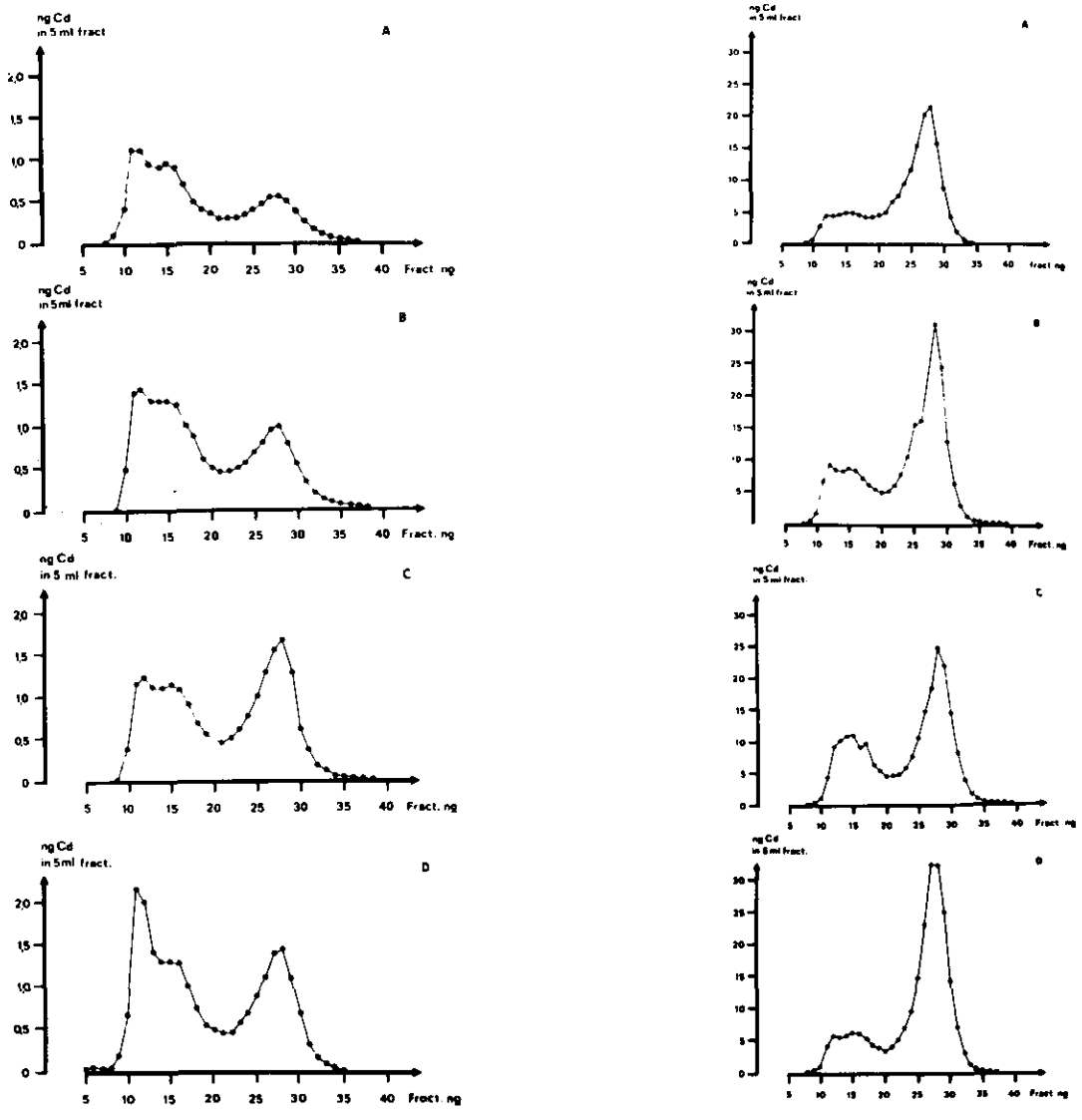

FIGURE 7. Gel chromatography on G-75 Sephadex of kidneys from mice exposed to: (left) $^{109}CdCl_2$ and (right) $^{109}CdBP$: (*A*) 5 min after injection; (*B*) 4 hr after injection; (*C*) 24 hr after injection; (*D*) 96 hr after injection. Column dimensions were 371 × 26mm. Elution was carried out with 0.01 *M* Tris buffer in 0.05 *M* NaCl, pH 8.0, at a flow rate of 14 mL/hr. volume of fractions 5 mL. From Nordberg (*2*).

was observed (Figs. 5 and 6). In control animals injected intravenously with radiolabeled cadmium chloride, cadmium was mainly distributed to the liver. Kidneys from animals exposed to crude metallothionein (*15*) subjected to gel chromatography showed that for $^{109}CdBP$ most of the radioactivity was in the low molecular weight fractions 5 min after injection (Fig. 7). At 24 hr, less cadmium was detected in the low molecular weight fractions. At 96 hr the distribution pattern was more similar to that of 5 min. Further studies were made by Squibb and Fowler (*20*). Kidneys of cadmium-exposed rabbits preserved for transplantation did not lose cadmium as metallothionein during storage (*21, 22*) which showed that metallothionein is a stable biocompound of cadmium.

Concluding Remarks

From the data presented it is obvious that, because of differences in transport, the ability of the kidney to handle metabolism of cadmium is entirely different for different forms of cadmium

originally administered and depends also on the rate of administration. Cadmium in blood plasma as well as in blood cells is to a large extent bound to a low molecular weight protein. These observations have been confirmed by other investigators (*13,19,23*). When a single dose of metallothionein-bound cadmium is given intravenously, an almost immediate and complete uptake occurs in the renal tubule. It is conceivable that under such conditions the resynthesis and rebinding processes are insufficient to sequester cadmium from sensitive tissue receptors, and renal damage occurs at total tissue concentrations much lower than when renal cadmium concentrations rise slowly. This explains the wide range of cadmium concentration (10–200 μg Cd/g wet weight) in the renal cortex associated with renal tubular dysfunction in experimental animals.

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