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Studies on Poisonous Metals. V.[1] Excretion of Cadmium through Bile and Gastrointestinal Mucosa and Effect of Chelating Agents on Its Excretion in Cadmium-Pretreated Rats

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The excretion of cadmium through the bile and gastrointestinal mucosa after the intraperitoneal administration of cadmium chloride and the effect of the chelating agents such as citric acid, D-cysteine, and DL-penicillamine on its excretion were studied in rats pretreated with cadmium. Cadmium pretreatment caused an appreciable decrease in the biliary excretion of subsequently administered cadmium and had no effect on the excretion of the metal through the gastrointestinal mucosa. The chelating agents used had no stimulatory effect on the biliary excretion of cadmium and scarcely affected the excretion of the metal through the gastrointestinal mucosa in the pretreated rats.

Moreover, a mechanism of the effect of cadmium pretreatment on the excretion of cadmium through the bile in rats was investigated. From findings relating to the contents of cadmium and zinc in the liver and kidney, the biliary and urinary excretion of both metals, and the binding characteristics of both metals to different proteins in the supernatant of liver, it was suggested that a part of subsequently administered cadmium replaced the zinc bound to metallothionein synthesized in the liver by the pretreatment, resulting in a decrease in the biliary excretion of cadmium and no stimulatory effect of the chelating agents on its excretion.

Keywords——metal; cadmium; biliary excretion; excretion through gastrointestinal mucosa; distribution; protein binding; chelating agent effect; cadmium-pretreated rat

Cadmium administered to experimental animals is largely accumulated in the liver, kidney, and intestine[3–5] and predominantly excreted in the feces.[5–8] Some investigators[8–10] have suggested that the excretory routes of cadmium through the bile and the mucosa of the gastrointestinal tract play an important role in the excretion of cadmium administered intravenously to rats. Recently, we reported that a large portion of cadmium administered intraperitoneally to rats was excreted in the gastrointestinal lumen through the bile and gastrointestinal mucosa, and that the chelating agents such as citric acid, D-cysteine, and DL-penicillamine increased the biliary excretion of cadmium.[11] It has been known that pretreatment of experimental animals with a low dose of cadmium induces the synthesis of metallothionein in the liver and kidney and, consequently, that a large part of cadmium is bound to a thionein, apoprotein, and is converted to the biologically inactive form.[12–16]

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According to such findings, it seems likely that the tissue distribution and excretion of cadmium administered to cadmium-pretreated animals are different from those of cadmium given to non-pretreated control animals.

The present study deals with the excretion of cadmium through the bile and gastrointestinal mucosa and the effect of some chelating agents on its excretion after the intraperitoneal administration of cadmium chloride to rats pretreated with cadmium.

Experimental

Materials and Equipment——Cadmium chloride, citric acid, D-cysteine, and DL-penicillamine were of reagent grade. Sephadex G-75 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden.

A Shimadzu AA-610S atomic absorption spectrophotometer, a Shimadzu QV-50 spectrophotometer, and Hitachi-Horiba F-7 pH meter were utilized.

Test Animals——Male Wistar rats weighing 180—220 g were used in the present study.

Experimental Procedures——A. Cadmium Pretreatment of Rats: Rats were injected intraperitoneally with 0.25 mg cadmium/kg/day for 7 days. The rats were used 3 days after the last injection of cadmium and starved for about 20 hr prior to use.

B. Surgical Procedure: The rat was operated by the method described previously.[11]

C. Administration of Cadmium Chloride and Chelating Agents: Cadmium chloride and chelating agents administered to rats were dissolved in physiological saline to give the concentrations of 0.2% (w/v) (as cadmium) and 0.178 mM, respectively. Cadmium (3 mg/kg) was administered intraperitoneally to rats and chelating agents (60—120 mg/kg) injected to the femoral vein of rats.

D. Collection of Bile and Other Specimens: Bile sample was collected continuously for 9 hr after the administration of cadmium. Other specimens, such as liver, both kidneys, gastrointestinal tract and its content, and urine, were collected according to the procedures reported previously.[11]

Gel Filtration——Liver or small intestine was homogenized in 3 volumes of chilled Tris-HCl buffer (0.01 M Tris-HCl–0.05 M NaCl, pH 8.0), using a glass homogenizer and a teflon pestle. The homogenates were centrifuged at 13000 *g* for 50 min at 4°. Aliquots (0.5 ml) of the supernatant were applied on a calibrated Sephadex G-75 column (1.5×40 cm). The column was eluted with the Tris-HCl buffer at a flow rate of 3.5—4.0 ml/hr at about 15° and the effluent was collected in 2 ml fractions. The absorbance at 280 nm and the concentrations of cadmium and zinc were determined on each fraction diluted with 1 ml of the Tris-HCl buffer. The molecular weight of metallothionein was estimated according to method of Andrews,[17] by using the following proteins of known molecular weight: cytochrome c (12500), chymotrypsin (25000), and ovalbumin (45000).

Analytical Procedures——The bile samples were diluted with distilled water to 3—5 ml. The tissues, intestinal washings, and urine were ashed by the $HClO_4$–HNO_3 method described previously.[18] The contents of cadmium and zinc in these samples were determined by the atomic absorption spectrophotometry. The analytical condition of cadmium was the same as that reported previously.[18] The content of zinc in aqueous samples was analyzed by the atomic absorption spectrophotometer at 2139 Å and compared to fresh dilution of 1000 ppm reference standard. The correlation between absorbance and concentration was linear to 0.5 μg zinc/ml.

Results and Discussion

Excretion of Cadmium through Bile and Gastrointestinal Mucosa in Cadmium-Pretreated Rats

The cumulative biliary excretion of cadmium in a 9 hr period in the pretreated rats after a single intraperitoneal injection of cadmium (3 mg/kg) was only 0.15% of the administered dose and extremly small as compared with that (0.85% of the dose) in the non-pretreated control animals.[11] Such a result as a decrease in the biliary excretion of cadmium in the pretreated rats is in agreement with findings of Stowe[19] and Cherian[20] who evaluated the effect of cadmium pretreatment on the biliary excretion of subsequently administered cadmium in rats.

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On the other hand, the content of cadmium in the gastrointestinal lumen was about 0.52% of the dose. A large portion of the administered cadmium was accumulated in the gastrointestinal mucosa. In addition, only a small amount of cadmium was excreted into the urine. These data were almost the same as those in the non-treated rats.[11] These results show that the pretreatment with cadmium causes an appreciable decrease in the biliary excretion of subsequently administered cadmium, and that a large portion of the administered cadmium is excreted in the gastrointestinal lumen through the gastrointestinal mucosa.

Effect of Some Chelating Agents on Excretion of Cadmium through Bile and Gastrointestinal Mucosa in Cadmium-Pretreated Rats

In the previous paper,[11] we reported that the chelating agents such as citric acid, D-cysteine, and DL-penicillamine increased the biliary excretion of cadmium administered intraperitoneally to control (non-pretreated) rats. However, as shown in Table I, these chelating agents had no stimulatory effect on the biliary excretion of cadmium in the pretreated rats. Also, as can be seen from Table II, all of the chelating agents used had little effect on the excretion of cadmium through the mucosa of the gastrointestinal tract. In addition, these chelating agents resulted in an increase in the content of cadmium in the kidney but had little effect on the distribution of cadmium in the liver and gastrointestine (Table III). These results suggest that the effects of the chelating agents on the excretion of cadmium *via* the gastrointestinal mucosa and on the distribution of cadmium in the various tissues in the pretreated rats are nearly similar to those in the non-pretreated animals.[11]

Table I. Effect of Some Chelating Agents on Biliary Excretion of Cadmium in Control and Cadmium-Pretreated Rats

	Biliary excretion/9 hr (percent of dose)	
	Control[a]	Pretreated[b]
Cadmium alone	0.95±0.25	0.13±0.05
Citric acid	4.47±0.91	0.16±0.15
D-Cysteine	4.33±2.67	0.08±0.03
DL-Penicillamine	2.39±1.52	0.16±0.15

Doses of cadmium and chelating agents were: cadmium chloride 3 mg/kg; citric acid 60 mg/kg; D-cysteine 100 mg/kg; and DL-penicillamine 120 mg/kg.
a) The data were cited from ref. 11.
b) The values represent the mean±standard deviation for 3 to 5 animals.

Table II. Effect of Some Chelating Agents on Gastrointestinal Content and Urinary Excretion of Cadmium in Cadmium-Pretreated Rats

	Gastrointestinal content in 9 hr[a] (percent of dose)			Urinary excretion/9 hr[a] (percent of dose)
	Stomach	Small intestine	Cecum	
Cadmium alone	0.10±0.01	0.28±0.01	0.14±0.05	0.08±0.02
Citric acid	0.06±0.01	0.64±0.27	0.11±0.08	0.10±0.10
D-Cysteine	0.28±0.11	1.14±0.60[b]	0.18±0.10	0.25±0.15
DL-Penicillamine	0.11±0.07	0.65±0.40	0.09±0.06	6.53±1.11[c]

a) The values represent the mean±standard deviation for 3 to 5 animals.
b) Significantly different from cadmium alone, $p<0.05$.
c) Significantly different from cadmium alone, $p<0.01$.

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Table III. Effect of Chelating Agents on Distribution of Cadmium in Various Tissues of Cadmium-Pretreated Rats

	Percent of dose[a]				
	Liver	Kidney	Stomach	Small intestine	Cecum
Cadmium alone	64.02± 7.56	1.94±0.77	0.51±0.02	8.55±0.24	2.10±0.33
Citric acid	72.82± 7.94	4.08±0.80[b]	1.15±0.60	9.23±2.39	2.26±0.62
D-Cysteine	65.81±13.10	5.15±1.69[b]	1.46±0.29[c]	7.86±1.68	2.84±0.59
DL-Penicillamine	60.41± 1.31	13.21±1.76[d]	0.70±0.28	6.71±1.37	2.62±0.80

a) The values represent the mean±standard deviation for 3 to 5 animals.
b) Significantly different from cadmium alone, $p<0.01$.
c) Significantly different from cadmium alone, $p<0.05$.
d) Significantly different from cadmium alone, $p<0.001$.

Mechanism of Effect of Cadmium Pretreatment on Excretion of Cadmium through Bile and Gastrointestinal Mucosa

It has been reported that the administration of cadmium or zinc to rats results in a remarkable increase in the content of zinc in the liver.[21-23] Some workers have suggested that the increase of zinc in the liver mainly occurs in the soluble supernatant fraction,[21,24] and that the metal is bound to metallothionein in the supernatant.[15,23,25] These findings suggest a contribution of increase in the content of zinc in the liver to a decrease in the biliary excretion of cadmium and to no effect of the chelating agents on its excretion in cadmium-pretreated rats. Therefore, we examined the contents of cadmium and zinc in the liver and kidney and the biliary and urinary excretion of both metals in the pretreated rats given subsequently cadmium. The results obtained are given in Table IV and V. The cadmium pretreatment resulted in a remarkable increase in the content of zinc in the liver. When administered further cadmium (3 mg/kg, *i.p.*) to the pretreated rats, the content of zinc in the liver did not change, but the biliary and urinary excretion of zinc increased. In addition, there was a remarkably increased accumulation of cadmium in the liver.

Table IV. Contents of Zinc and Cadmium in Liver and Kidney of Cadmium-Pretreated Rats

Treatment	Tissue content (μg/g wet tissue)[a]			
	Liver		Kidney	
	Zn	Cd	Zn	Cd
Control (0.9% NaCl)	39.87±3.28	0.07± 0.03	23.30± 1.99	0.12±0.01
7×0.25 mg Cd/kg	61.63±5.98[b]	24.02± 3.32	28.47± 4.39	6.27±0.63
7×0.25 mg Cd/kg+3 mg Cd/kg	61.43±9.70[c]	68.95± 4.33	27.94± 4.10	8.90±1.25
7×0.25 mg Cd/kg+3 mg Cd/kg +120 mg DL-penicillamine/kg	50.04±7.97	66.28±17.50	25.35±10.59	36.72±7.54

a) The values represent the mean±standard deviation for 4 to 6 animals.
b) Significantly different from control, $p<0.01$.
c) Significantly different from control, $p<0.02$.

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Table V. Biliary and Urinary Excretion of Zinc and Cadmium in Cadmium-Pretreated Rats

Treatment	Excretion/9 hr (μg)[a]			
	Bile		Urine	
	Zn	Cd	Zn	Cd
Control (0.9% NaCl)	0.36±0.11	0.01±0.01	3.65± 0.54	0.27± 0.05
7×0.25 mg Cd/kg	0.69±0.15	0.15±0.21	4.40± 0.98	0.39± 0.08
7×0.25 mg Cd/kg+3 mg Cd/kg	2.13±0.67[b]	0.81±0.31	8.10± 2.17	0.88± 0.58
7×0.25 mg Cd/kg+3 mg Cd/kg +120 mg DL-penicillamine/kg	1.14±0.74	1.66±1.01	87.65±38.33[c]	54.83±24.76[b]

a) The values represent the mean±standard deviation for 4 to 6 animals.
b) Significantly different from control, $p<0.02$.
c) Significantly different from control, $p<0.01$.

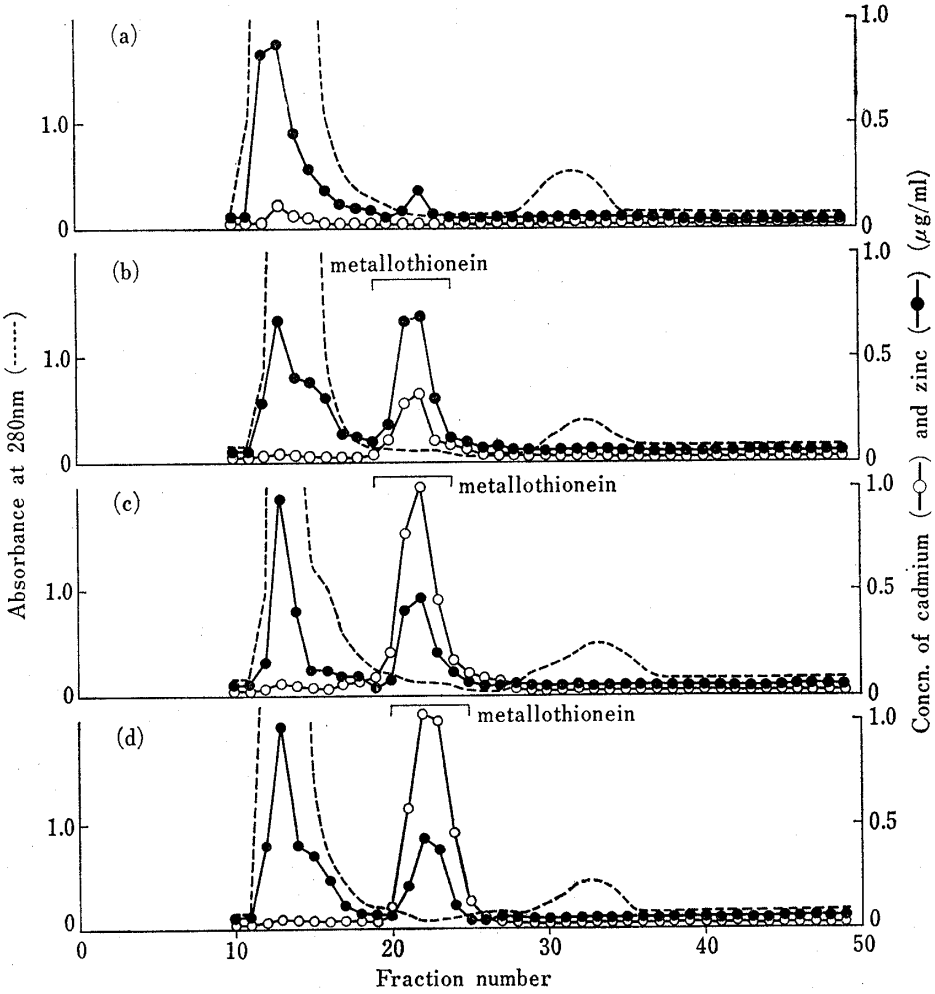

Fig. 1. Sephadex G-75 Chromatographic Profile of Liver Supernatant

(a) control rat, (b) cadmium-pretreated rat, (c) rat administered cadmium (3 mg/kg) after cadmium pretreatment, (d) rat administered cadmium (3 mg/kg) and DL-penicillamine (120 mg/kg) after cadmium pretreatment.

Furthermore, a difference in the binding of cadmium and zinc to different proteins in the liver of control and cadmium-pretreated rats was studied by fractionating liver supernatant (13000 ***g***) on a Sephadex G-75 (Fig. 1). In control rats, most of the zinc was associated with a high-molecular-weight protein (Fig. 1a). However, as shown in Fig. 1b, gel filtration

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of the supernatant from liver of the pretreated rats showed that cadmium pretreatment increased remarkably the zinc content in a low molecular weight protein (10600), which corresponded to metallothionein occurring in the liver of cadmium-treated rats.[15,23,25] The zinc in the hepatic metallothionein is replaced by a part of cadmium after subsequent administration with a larger amount of cadmium, resulting in the binding of cadmium to the metallothionein fraction (Fig. 1c). Such a replacement may cause an increase in the binding of zinc to high-molecular-weight proteins and in the excretion of the metal into the bile and urine (Fig. 1c and Table V). In addition, it is presumed from a remarkable increase of cadmium in the metallothionein fraction that the further exposure of cadmium to the pretreated rats brings about not only the replacement of zinc from zinc-thionein but also more rapid synthesis of the metallothionein which is then available to bind a larger part of the administered metal, as reported by Webb and Verschoyle.[26] Cherian[20] has reported that cadmium-thionein is exclusively excreted in the urine and little secreated into the bile.

From these findings, it is suggested that a part a of the cadmium administered after the cadmium pretreatment replaced the zinc bond to metallothionein synthesized in the liver by the pretreatment and, further, that because of the increased capacity for the hepatic metallothionein synthesis, a larger part of the administered cadmium is retained in the metallothionein fraction of liver, resulting in a decrease in the biliary excretion of cadmium.

In addition, in cadmium-pretreated rats, the contents of cadmium and zinc in the liver and the biliary excretion of both metals did not show a conspicuous difference between the rats administered cadmium with and without DL-penicillamine. However, DL-penicillamine greatly increased the content of cadmium in the kidney and the urinary excretion of the metal. As shown in Fig. 1d, also, gel filtration of the supernatant from liver of cadmium-pretreated rat administered cadmium with DL-penicillamine showed that the administered cadmium was mainly bound to the metallothionein fraction in a similar manner as that in rat administered cadmium alone (Fig. 1c). These results suggest that in cadmium-pretreated rats receiving the administration of cadmium with DL-penicillamine, a portion of cadmium is strongly bound to the hepatic metallothionein synthesized by cadmium pretreatment, and that the other portion is rapidly excreted into the urine in the form of cadmium-DL-penicillamine complex, resulting in no stimulatory effect of DL-penicillamine on the biliary excretion of cadmium.

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