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Studies on Poisonous Metals. II.[1] Effect of Chelating Agents on Excretion of Cadmium through Bile and Gastrointestinal Mucosa in 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 ethylenediaminetetraacetic acid, citric acid, 2,3-dimercapto-1-propanol, L-cysteine, D-cysteine, and DL-penicillamine on its excretion were studied in rats. The cumulative biliary excretion of cadmium in a 9 hr period was about 0.85% of the injected dose. Citric acid, D-cysteine, and DL-penicillamine increased the biliary excretion of cadmium. All of the chelating agents used scarcely affected the excretion of cadmium through the gastrointestinal mucosa.

Moreover, chemical characteristics of cadmium complex in the bile were investigated by the chromatography on Sephadex G-75 of the bile from the rats receiving cadmium chloride. The results showed that cadmium was bound to several bile components with different molecular weights, and that cadmium in the bile of the rats administered cadmium chloride with the chelating agents such as citric acid, D-cysteine, and DL-penicillamine was largely bound to the substances with a low molecular weight.

It has been known that cadmium administered to experimental animals is predominantly accumulated in the liver, kidneys, and intestine,[3-6] and that a large portion of excreted cadmium is found in the feces.[6-9] From findings on radioautography of cadmium-109 given intravenously to rats, Berlin and Ullberg[3] suggested that cadmium was excreted in the bile and through the mucosa of the intestinal tract. Lucis, *et al.*[4] studied the distribution of subcutaneously injected cadmium-109 in rats and suggested that the appearance of cadmium in the lumen of the small intestine might be due to biliary, pancreatic, and intestinal secretions. Recently, some investigators[9-11] have suggested that the bile and the mucosa of the gastrointestinal tract may play an important role in the excretion of cadmium given intravenously to rats. However, little work has been done on the effect of chelating agents on the biliary and gastrointestinal excretion of cadmium.

The present study reports the excretion of cadmium through the bile and gastrointestinal mucosa, the effect of several chelating agents on its excretion, and chemical characteristics of cadmium complex in the bile after the intraperitoneal administration of cadmium chloride to rats.

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Experimental

Materials and Equipment——Cadmium chloride, ethylenediaminetetraacetic acid disodium salt (EDTA. 2Na), citric acid, 2,3-dimercapto-1-propanol, L-cysteine, 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 a Hitachi-Horiba F-5 pH meter were utilized.

Test Animals——Male Wistar rats weighing 180—220 g were used. Rats were fasted 24 hr prior to use, but drinking water was allowed *ad libitum*. Rats were kept in cages having a wide mesh floor to prevent coprophagy.

Experimental Procedures——A. Surgical Procedure: The rat was anesthetized with urethane (1 g/kg body weight, *i.p.*). The bile duct was exposed through a midline abdominal incision and cannulated with a 10-cm length of polyethylene tubing (PE-10). The rat was kept in a room maintained at about 28° during the experiment.

B. Administration of Cadmium Chloride and Chelating Agents: Cadmium chloride and chelating agents administered to rats were dissolved in 0.9% sodium chloride solution to give the concentrations of 0.2% (w/v) (as cadmium) and 0.178 M, respectively. Cadmium chloride (3 mg cadmium/kg) was administered intraperitoneally to rats. Chelating agents (60—120 mg/kg) were administered intravenously to rats from the femoral vein immediately after the administration of cadmium.

C. Collections of Bile and Other Specimens: Bile samples were collected every 20 min up to 1 hr after the administration of cadmium chloride and thereafter every 1 hr up to 9 hr. After 9 hr the rat was sacrificed by decapitation. The liver, both kidneys, and gastrointestinal tract were carefully removed from the abdominal cavity. The gastrointestinal tract was divided into three segments of stomach, small intestine, and cecum including colon. The contents of the individual segments of the gastrointestinal tract were washed out using 30—60 ml of distilled water and the washings were collected. The urine in the bladder was collected by an injection-syringe and combined with the urine excreted.

Analytical Procedures——The bile samples were diluted with distilled water to 3—5 ml. The tissues, intestinal washings, and urine were ashed by the method described previously.[1] The content of cadmium in those samples was determined by the atomic absorption spectrophotometry according to the method reported previously.[1]

Gel Filtration of Bile——The bile (0.5 ml) was fractionated using column chromatography. A glass column (1.5 × 40 cm) packed with Sephadex G-75 was eluted with a borate buffer solution (0.05 M sodium borate–0.1 M hydrochloric acid, pH 8.5) at a flow rate of 3.5—4.0 ml/hr at about 15°. The effluent was collected in 2-ml fractions. The absorbance at 280 nm and the concentration of cadmium were determined on each fraction diluted with 1 ml of the borate buffer.

Result and Discussion

Excretion of Cadmium through Bile and Gastrointestinal Mucosa

As shown in Fig. 1, cadmium began to be excreted in the bile about 1 hr after the administration of cadmium. Maximum biliary excretion rate of the metal was obtained about 4 hr after the intraperitoneal administration. As shown in Fig. 2, bile flow in control and cadmium-administered rats remained relatively constant over 9 hr. Furthermore, as can be seen in Table I, the cumulative biliary excretion of cadmium in a 9 hr period was about 0.85% of the dose. Content of cadmium in the gastrointestinal lumen was about 0.53% of the dose and a large portion of the metal excreted was found in the small intestinal lumen. An appreciable fraction of the administered cadmium was accumulated in the mucosa of the gastrointestinal tract, especially of the small intestine. A small amount of the metal was excreted into the urine.

These results support findings as described already[3,6,7] that a large portion of cadmium administered to rats is excreted in the gastrointestinal lumen through the bile and gastrointestinal mucosa, resulting in the excretion of the metal into the feces.

Effect of Various Chelating Agents on Excretion of Cadmium through Bile and Gastrointestinal Mucosa

Bile flow in rats administered cadmium with a chelating agent was approximately as much as that in control (see Fig. 2). As shown in Fig. 1 and Table II, the chelating agents

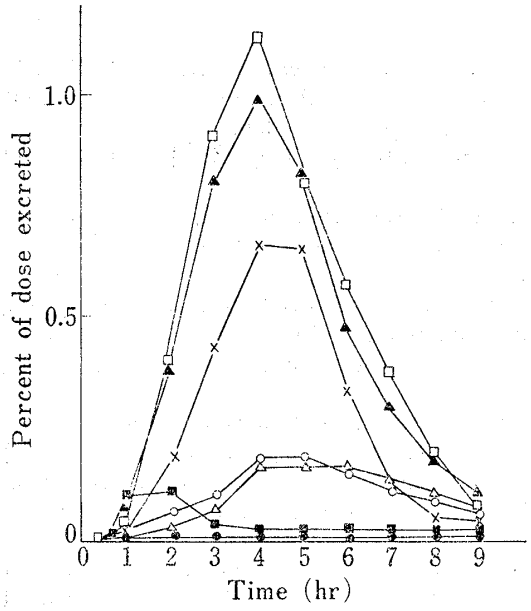

Fig. 1. Effect of Various Chelating Agents on Biliary Excretion of Cadmium in Rats

The values represent the mean of 3 to 6 animals.
—○— : control
—●— : EDTA
—□— : citric acid
—■— : 2,3-dimercapto-1-propanol
—△— : L-cysteine
—▲— : D-cysteine
—×— : DL-penicillamine

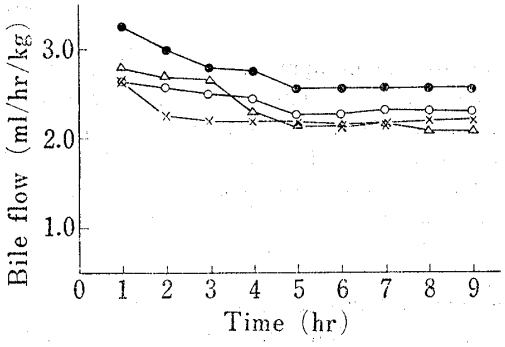

Fig. 2. Bile Flow following Administration of Cadmium or the Metal with Chelating Agents in Rats

Each value is expressed as the mean of 3 animals
—○— : control
—△— : cadmium chloride
—×— : cadmium chloride+citric acid
—●— : cadmium chloride+D-cysteine

such as citric acid, D-cysteine, and DL-penicillamine increased the biliary excretion rate of cadmium and the cumulative biliary excretion of the metal in a 9 hr period, although the increase by DL-penicillamine was not statistically significant. On the other hand, 2,3-dimercapto-1-propanol reduced the biliary excretion of cadmium. No biliary excretion of the metal was observed by the simultaneous administration of EDTA. L-Cysteine had no effect on the biliary excretion of the metal.

Table I. Biliary and Urinary Excretion of Cadmium and Content of the Metal in Mucosa and Lumen of Gastrointestinal Tract in Rats

	Percent of dose[a)]
Bile	0.85±0.46
Mucosa of gastrointestinal tract	
Stomach	0.69±0.17
Small intestine	9.70±1.30
Cecum	2.18±0.18
Lumen of gastrointestinal tract	
Stomach	0.06±0.04
Small intestine	0.34±0.08
Cecum	0.13±0.06
Urine	0.03±0.04

a) The values represent the mean ± standard deviation for 6 or 7 animals.

Table II. Effect of Various Chelating Agents on Biliary Excretion of Cadmium in Rats

	Biliary excretion/9 hr[a)] (percent of dose)
Control	0.95±0.25
EDTA	0
Citric acid	4.47±0.91[b)]
2,3-Dimercapto-1-propanol	0.40±0.13[c)]
L-Cysteine	0.89±0.61
D-Cysteine	4.33±2.67[c)]
DL-Penicillamine	2.39±1.52

Doses of cadmium and chelating agents were: cadmium chloride 3 mg Cd/kg; EDTA. 2Na 90 mg/kg; citric acid 60 mg/kg; 2,3-dimercapto-1-propanol, L-cysteine and D-cysteine 100 mg/kg; and DL-penicillamine 120 mg/kg.
a) The values represent the mean ± standard deviation for 3 to 7 animals.
b) significantly different from control, $p<0.01$
c) significantly different from control, $p<0.05$

The effect of the chelating agents on the content of cadmium in the lumen of the gastrointestinal tract and the tissue distribution of the metal in rats are summarized in Tables III and IV, respectively. All of the chelating agents used had little effect on the excretion of

Table III. Effect of Various Chelating Agents on Gastrointestinal Content and Urinary Excretion of Cadmium in Rats

	Gastrointestinal content in 9 hr[a] (percent of dose)			Urinary excretion/9 hr[a] (percent of dose)
	Stomach	Small intestine	Cecum	
Control	0.07±0.04	0.32±0.07	0.14±0.05	0.03±0.04
EDTA	0.06±0.04	0.15±0.02	0.14±0.07	52.59±9.10[b]
Citric acid	0.09±0.04	0.53±0.27	0.32±0.16	0.01±0.01
2,3-Dimercapto-1-propanol	0.05±0.03	0.09±0.02[c]	0.09±0.01	0.11±0.12
L-Cysteine	0.05±0.02	0.43±0.21	0.15±0.04	0.04±0.02
D-Cysteine	0.03±0.01	0.78±0.83	0.28±0.18	0.01±0.01
DL-Penicillamine	0.06±0.03	0.48±0.06[b]	0.10±0.02	5.45±6.46

a) The values represent the mean ± standard deviation for 3 to 6 animals.
b) significantly different from control, $p<0.05$
c) significantly different from control, $p<0.01$

Table IV. Effect of Chelating Agents on Distribution of Cadmium in Various Tissues of Rats

	Percent of dose[a]				
	Liver	Kidneys	Stomach	Small intestine	Cecum
Control	57.79±5.91	1.84±0.43	0.72±0.14	10.03±1.11	2.16±0.18
EDTA	20.50±4.15[b]	4.26±1.96	0.27±0.05[b]	3.17±0.68[b]	0.88±0.14[b]
Citric acid	55.33±3.25	1.82±0.16	0.74±0.11	10.59±3.29	2.83±0.45
2 3-Dimercapto-1-propanol	23.51±4.45[b]	3.49±1.21	0.49±0.25	13.71±4.70	1.83±0.53
L-Cysteine	59.77±5.36	2.01±0.66	0.67±0.13	9.65±2.08	2.56±0.25
D-Cysteine	55.08±7.16	3.14±0.80[b]	0.57±0.06	8.31±1.21	2.10±0.43
DL-Penicillamine	47.12±2.97[c]	14.51±1.64[b]	0.44±0.05[c]	8.97±1.62	2.05±0.52

Doses of cadmium and chelating agents were the same as described in Table II.
a) The values represent the mean ± standard deviation for 3 to 6 animals.
b) significantly different from control, $p<0.01$
c) significantly different from control, $p<0.05$

cadmium *via* the mucosa of the gastrointestinal tract. Citric acid, L-cysteine, and D-cysteine scarcely affected the distribution of cadmium in the various tissues and the urinary excretion of the metal. In contrast, EDTA, DL-penicillamine, and 2,3-dimercapto-1-propanol,[12-14] which were known to enhance the urinary excretion of cadmium, resulted in a decrease in the content of the metal in the liver. In addition, EDTA decreased the content of the metal in the gastrointestinal tissue.

Binding of Cadmium with Bile Components

The bile of rats between 2 and 6 hr after the administration of cadmium with or without the chelating agents such as citric acid, D-cysteine, and DL-penicillamine was collected and chromatographed on the column of Sephadex G-75. The elution profile of the bile obtained from the rat receiving cadmium alone is shown in Fig. 3a. Three peaks containing cadmium were obtained. The ratios (Ve/Vo) of the elution volume (Ve) of three peaks to the void volume (Vo) were 1.0, approximately 2.6, and approximately 3.1, respectively. Havrdova,

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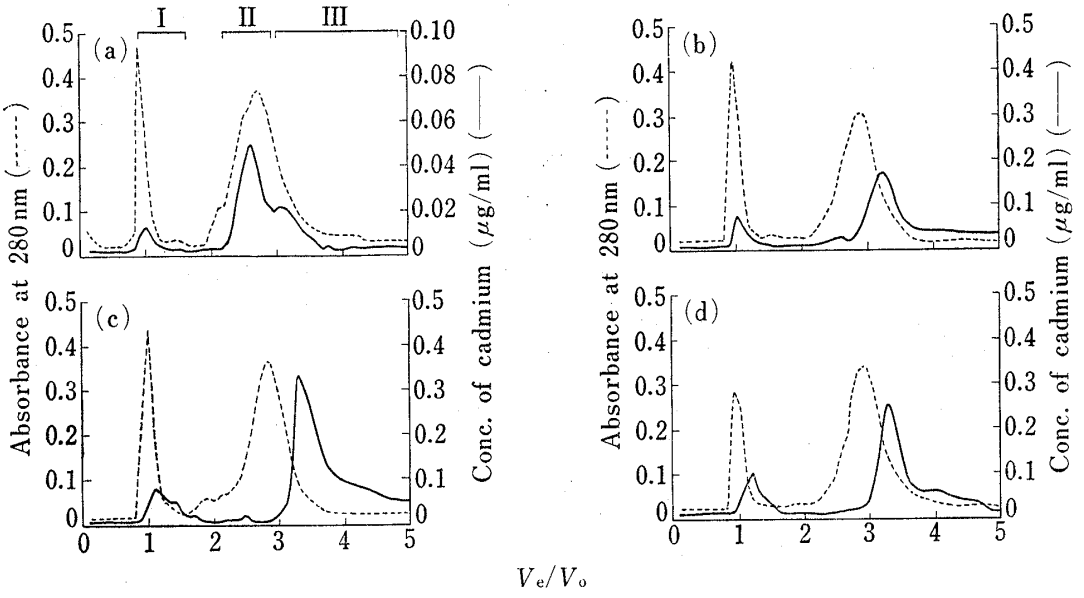

Fig. 3. Sephadex G-75 Chromatographic Profile of Bile following Administration of Cadmium Chloride with and without Chelating Agents

The bile applied to the gel filtration was collected between 2 and 6 hr after the administration of cadmium chloride.
(a) cadmium chloride alone, (b) with citric acid, (c) with D-cysteine, (d) with DL-penicillamine

et al.[15] investigated the binding of cadmium with the components of the bile from the rat exposed to cadmium chloride using the chromatographic fractionation on Sephadex G-100 and observed two main peaks eluted at the void volume and in the fraction with Ve/Vo about 3. These results suggest that cadmium in the bile is bound to several bile components with different molecular weights.

It has been reported[16–20] that cadmium-binding protein with a low molecular weight, metallothionein, appears in the liver and kidneys after the administration of cadmium chloride to rats and mice. It is probable that a part of cadmium is excreted as metallothionein into the bile of the rat receiving cadmium. However, the detailed character of the low molecular weight substances bound by cadmium in the fractions with Ve/Vo 2.6 and 3.1 is obscure at the moment.

The bile from the rat administered cadmium chloride with citric acid, D-cysteine, or DL-penicillamine was chromatographed on the column of Sephadex G-75 as described above. The elution curves obtained were summarized in Fig. 3b, 3c, and 3d. The amount of cadmium in the fractions I (Ve/Vo 0.9—1.6), II (Ve/Vo 2.2—2.9), and III (Ve/Vo 3.0—5.0) in Fig. 3 was shown in Table V as the percent of the total cadmium in the bile applied to the gel filtration. The amount of cadmium bound to the bile components of high molecular weight in the fraction I was little influenced by the administration of the chelating agent. However, those chelating agents markedly decreased the amount of cadmium in the fraction II. On the contrary, the chelating agents increased the amount of cadmium in the fraction III. This increase, however, was statistically significant only in the case of citric acid. These results strongly suggest that cadmium in the bile of the rat administered cadmium with the chelating agents is largely bound to the substances with a low molecular weight.

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Table V. Content of Cadmium present in Fractions on Sephadex G-75 Chromatography of Bile obtained from Rats given Cadmium Chloride with or without Various Chelating Agents

	Percent of total cadmium applied to gel filtration[a] Fraction[b]		
	I	II	III
Control	8.23±3.21	25.79±7.10	30.35±12.40
Citric acid	13.27±4.26	9.30±0.68[c]	49.90± 6.88[c]
D-Cysteine	8.61±2.03	4.29±6.06[c]	40.52± 9.86[d]
DL-Penicillamine	6.40±1.43	1.40±1.03[e]	58.85±21.88[d]

a) The values represent the mean ± standard deviation for 3 or 4 animals.
b) Each fraction is shown in Fig. 3.
c) significantly different from control, $p < 0.05$
d) not significantly different from control, $p > 0.05$
e) significantly different from control, $p < 0.01$

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