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Studies on Poisonous Metals. XIII.[1] Effect of Cadmium on Small Intestinal Absorption of L-Histidine in Rats

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The effect of cadmium on the small intestinal absorption of L-histidine was studied. The absorption of L-histidine (0.19—3.23 mM) from the small intestine of rats *in situ* was significantly depressed in the presence of cadmium (10—13 μg/ml) in the lumen. The absorption of L-histidine from the small intestine, after pretreatment with cadmium solutions (10 and 30 μg/ml) for 60 min, was significantly depressed as compared with the control. In addition, the absorption of L-histidine from the intestine of rats *in situ* at 1 and 5 h after a single oral administration of cadmium (3 mg/kg) was significantly decreased as compared with the control, although there was no significant decrease of absorption of L-histidine at 8 and 12 h after the administration of cadmium. In view of the accumulation of cadmium in the intestinal tissue and the result of Sephadex G-75 filtration of intestinal soluble fraction, it seems likely that the depressed intestinal absorption of L-histidine was due to the effect of free cadmium in the intestinal tissue. An *in vitro* intestinal circulation experiment confirmed that cadmium taken up in the intestinal tissue inhibits the active transport of L-histidine across the rat small intestine.

Keywords——L-histidine; intestinal absorption; cadmium; intestinal accumulation; metallothionein

Cadmium has recently been recognized as a dangerous environmental contaminant. Growth depression, anemia, hypertension, poor bone mineralization and kidney dysfunction have been documented as signs of cadminum toxicity in animals.[2] Cadmium is predominantly taken up into the human body through food and water which have been contaminated with cadmium. It has been reported that the *in vitro* intestinal transport of L-amino acids[3,4] and D-glucose,[5] which are actively transported,[6-9] is inhibited by sulfhydryl reagents such as mercuric ion and *p*-chloromercuribenzene sulfonate. In addition, cadmium is known to interact with sulfhydryl group(s)[10-12] and cause uncoupling of oxidative phosphorylation.[13] These findings suggest that cadmium may inhibit the intestinal absorption of nutrients, such as L-amino acids and D-glucose. Thus, the present study was designed to examine the effect of cadmium on the small intestinal absorption of L-histidine, which is an essential amino acid in human infants and young rats[14] and has a relatively high binding affinity for cadmium,[15,16] in rats.

Experimental

Materials and Equipment——Cadmium chloride and L-histidine were obtained from Wako Pure Chemicals Ind., Osaka. ^{109}Cd (specific activity, 1574 mCi/mg) was obtained from New England Nuclear, Boston, Mass. Sephadex G-75 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of reagent grade. A Shimadzu AA-610S atomic absorption spectrophotometer and an Aloka auto well gamma scintillation counter, model ARC 300, were utilized.

Test Animals——Male Wistar rats weighing 180—240 g were used for the experiments. They were fasted for about 20 h with drinking water *ad libitum* prior to the experiments.

***In Situ* Rat Experimental Procedures**——The procedure for studying the absorption of cadmium from the *in situ* rat small intestine followed the method reported in a previous paper.[17] The sample solution (40 ml) was continuously perfused at the rate of 5 ml/min through the small intestine for 2 h at 37 °C using a perfusion pump. A 1.0 ml aliquot was removed at periodic intervals for assay. The sampling at 0 h was performed 10 min after the beginning of perfusion. The concentration of L-histidine in the luminal solution was expressed as a relative value with respect to the concentration (100) at 0 h. For determination of cadmium accumulation in the intestinal tissue, the intestine was removed.

***In Vitro* Intestinal Transport Experiment**——The active transport of L-histidine from the mucosal to the serosal side was investigated by the circulation method.[18] The everted rat jejunum was circulated for 2 h at 37 °C using mucosal solution (60 ml) containing L-histidine (1.29 mM) together with cadmium and serosal solution (10 ml) containing L-histidine (1.29 mM) alone. At the termination of the experiment, the concentrations of L-histidine in the mucosal and serosal solutions were determined and the intestine was removed for the determination of cadmium accumulation.

Analytical Procedures——Cadmium was determined by the method reported previously.[18] The intestine was wet-ashed by using perchloric acid and nitric acid. The content of cadmium in the tissue specimen was determined with an atomic absorption spectrophotometer at 2288 Å by comparison with standards (0.1—0.5 μg Cd/ml). L-Histidine was analysed by the method of Macpherson.[19] The ^{109}Cd radioactivity in samples was determined using a gamma counter.

Gel Filtration of Small Intestinal Soluble Fraction——Rats starved for 24 h were orally given radioisotopic cadmium (3 mg Cd and 20 μCi ^{109}Cd/kg). Rats were killed by decapitation at 1 and 8 h after administration. The small intestine was homogenized in 3 volumes of chilled 0.25 M sucrose solution (pH 6.0), using a glass-Teflon homogenizer. The homogenate was centrifuged at 105000 × *g* for 1 h at 4 °C. An aliquot (1 ml) of the supernatant was applied to a Sephadex G-75 column (1.5 × 41 cm). The column was eluted with Tris–HCl buffer (0.01 M Tris–HCl, 0.05 M NaCl, pH 8.0) at a flow rate of 3.5—4.0 ml/h at 4 °C and the effluent was collected in 2 ml fractions. The absorbance at 280 nm and the ^{109}Cd radioactivity of each fraction were determined. The molecular weight of metallothionein was estimated according to the method of Andrews,[20] by using the following proteins of known molecular weight: cytochrome c (12500), chymotrypsin (25000), and ovalbumin (45000).

Statistics——The results were statistically evaluated by using Student's *t*-test.

Results and Discussion

We investigated the *in situ* small intestinal absorption of L-histidine in the presence of cadmium (5—30 μg/ml). The results are shown in Fig. 1. Cadmium significantly depressed the intestinal absorption of L-histidine at cadmium concentrations above 10 μg/ml. The inhibitory effect of cadmium on the absorption of L-histidine was observed in the luminal solution at 0.19—3.23 mM L-histidine and tended to increase with increasing luminal concentration of cadmium. In addition, cadmium caused a significant decrease in the absorption of L-histidine at 60 min after the beginning of perfusion. As shown in Table I, the accumulation of cadmium in the intestinal tissue after a 2-h perfusion increased with increasing cadmium concentration in the luminal solution but was little affected by the L-histidine concentration in the luminal solution. These results suggest that the *in situ* intestinal absorption of L-histidine is probably inhibited by cadmium taken up in the intestinal tissue and that some lag time (30—60 min) is

Table I. Accumulation of Cadmium in Intestinal Tissue after a 2-h Perfusion

Concentration of Cd (μg/ml)	Cadmium (μg/g wet tissue)[a] Concentration of L-histidine (mM)			
	3.23	1.29	0.65	0.19
5	—	12.90 ± 1.56	—	13.73 ± 1.17
10	17.93 ± 0.53	19.76 ± 3.70	19.27 ± 3.08	17.76 ± 1.44
20	—	32.14 ± 2.01	—	—
30	51.50 ± 4.45	58.60 ± 3.70	—	53.60 ± 7.50

a) Each value is the mean ± standard deviation for 3 or 4 animals.

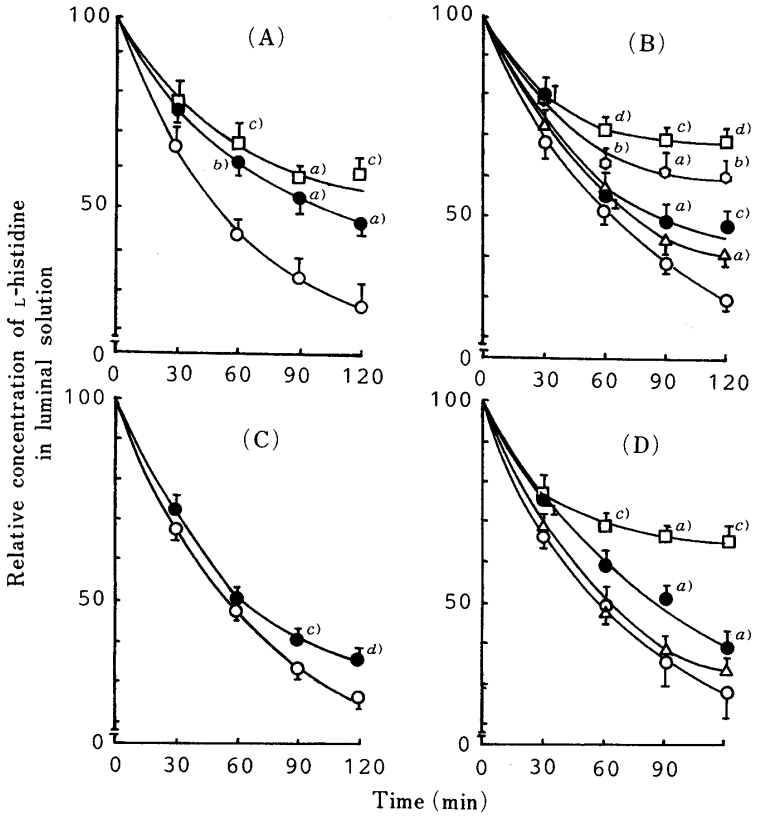

Fig. 1. Effect of Cadmium on the Absorption of L-Histidine by *in Situ* Rat Small Intestine in a 2-h Perfusion Experiment

Each value is the mean ± standard deviation for 3 or 4 animals. The initial concentrations of L-histidine were: (A) 3.23 mM, (B) 1.29 mM, (C) 0.65 mM, (D) 0.19 mM. The initial luminal concentration of cadmium were: 0 μg/ml (control, ○), 5 μg/ml (△), 10 μg/ml (●), 20 μg/ml (◇), 30 μg/ml (□). Significantly different from the control values, *a*) $p<0.05$; *b*) $p<0.02$; *c*) $p<0.01$; *d*) $p<0.001$.

required for the intestinal accumulation of sufficient cadmium to inhibit the absorption of L-histidine. In order to test this possibility, the *in situ* absorption of L-histidine was examined in the small intestine, after pretreatment with cadmium solutions (5—30 μg/ml) for 60 min. As shown in Fig. 2, the intestinal absorption of L-histidine was significantly depressed at 30 min after the beginning of perfusion following pretreatment with cadmium solution of 10 or 30 μg/ml, although the absorption was not affected at a cadmium concentration of 5 μg/ml. These results may reflect the differences of cadmium accumulation in the intestinal tissue.

Next, we investigated the absorption of L-histidine from the small intestine of rats *in situ* at 1, 5, 8, and 12 h after a single oral administration of cadmium (3 mg/kg) (Fig. 3). The intestinal absorption of L-histidine at 1 and 5 h after the administration of cadmium was significantly decreased as compared with the control. However, there was no significant decrease of absorption of L-histidine at 8 and 12 h after the administration of cadmium. In addition, as shown in Table II, the intestinal accumulation of cadmium at 8 h was little changed as compared with that at 1 and 5 h. In order to examine further this phenomenon, the chemical forms of cadmium in the intestinal tissue were investigated by fractionating soluble supernatant of the small intestine of rats orally given cadmium on a Sephadex G-75 column. Representative elution profiles of intestinal supernatant of rats at 1 and 8 h after the administration of cadmium are shown in Fig. 4. The amount of cadmium bound to a high molecular weight protein (fraction I), which probably has a low binding affinity for cadmium, at 8 h after the administration of cadmium was remarkably decreased as compared

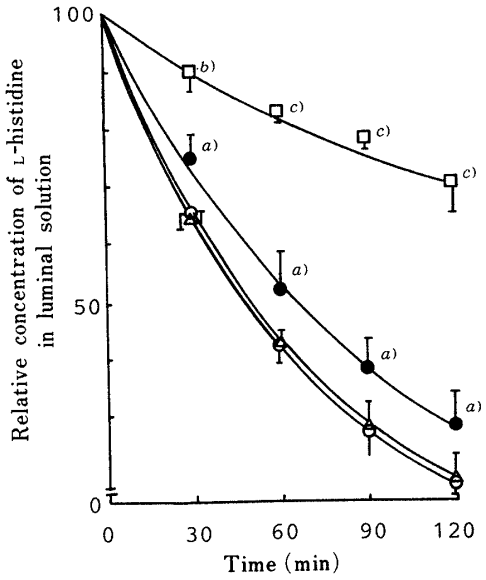

Fig. 2. Absorption of L-Histidine by *in Situ* Rat Small Intestine Pretreated with Cadmium in a 2-h Perfusion Experiment

Each value is the mean ± standard deviation for 3 or 4 animals. The small intestine was pretreated with various cadmium solutions (5—30 μg/ml) for 60 min. 0 μg/ml (control, ○), 5 μg/ml (△), 10 μg/ml (●), 30 μg/ml (□). The initial luminal concentration of L-histidine was 1.29 mM. Significantly different from the control values, *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$.

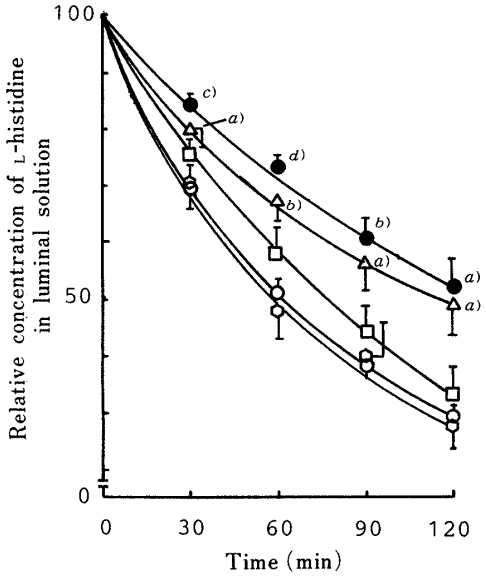

Fig. 3. Absorption of L-Histidine by *in Situ* Small Intestine of Rat Following a Single Oral Administration of Cadmium in a 2-h Perfusion Experiment

Each value is the mean ± standard deviation for 3 animals. The dose of cadmium was 3 mg/kg. The times after the administration of cadmium were: 0 h (control, ○), 1 h (●), 5 h (△), 8 h (□), 12 h (⬡). The initial luminal concentration of L-histidine was 1.29 mM. Significantly different from the control values, *a*) $p<0.05$; *b*) $p<0.02$; *c*) $p<0.01$; *d*) $p<0.001$.

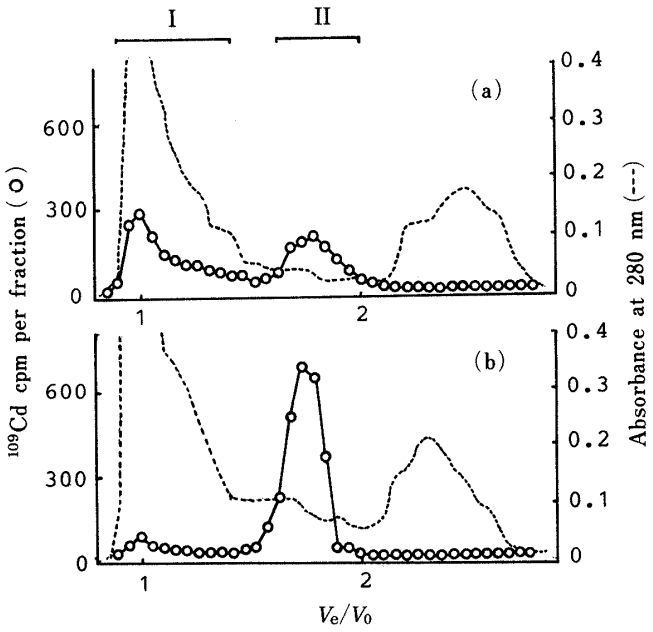

Fig. 4. Sephadex G-75 Chromatographic Profile of Rat Intestinal Soluble Fraction

The intestine was removed at 1 (a) or 8 h (b) after a single oral administration of cadmium (3 mg/kg).

with that at 1 h. Cadmium is known to induce the synthesis of metallothionein in the intestinal tissue.[21] The amount of cadmium bound to a low molecular weight (10000) protein (fraction II) corresponding to metallothionein, which has a high binding affinity for cadmium, was remarkably increased at 8 h after the administration of cadmium as compared with that at 1 h. These results suggest that the synthesis of metallothionein induced in the intestinal tissue after the administration of cadmium causes a marked decrease of

TABLE II. Intestinal Accumulation of Cadmium after a 2-h Perfusion Experiment using *in Situ* Small Intestine of the Rat Following Oral Administration of Cadmium

Time after administration of Cd (h)	Cadmium (μg/g wet tissue)[a]
1	7.02±1.15
5	5.98±0.30
8	5.67±0.88
12	2.17±0.48

The dose of cadmium was 3 mg/kg. The initial luminal concentration of L-histidine was 1.29 mM. *a*) Each value is the mean±standard deviation for 3 or 4 animals.

TABLE III. Effect of Cadmium on Transport of L-Histidine across Rat Small Intestine *in Vitro*

Concentration of cadmium (μg/ml)	Transport of L-histidine (serosal to mucosal concentration ratio)	Tissue accumulation of cadmium (μg/g wet tissue)
Control	1.94±0.19	—
5	1.81±0.30	20.81± 3.76
7	1.45±0.20	31.79± 4.28
10	1.28±0.09[a]	81.50±15.15

Each value is the mean±standard deviation for 3 animals. Mucosal and serosal solutions contained L-histidine (1.29 mM) together with cadmium, and L-histidine (1.29 mM) alone, respectively. *a*) Significantly different from the control, $p<0.05$.

free cadmium available to inhibit the absorption of L-histidine, resulting in no depression of the absorption of L-histidine at 8 h after the administration of cadmium.

Furthermore, in order to clarify the mechanism of inhibitory effect of cadmium on the intestinal absorption of L-histidine, the transport of L-histidine from the mucosal to the serosal side was examined by means of an *in vitro* intestinal circulation experiment (Table III). L-Histidine was shown to be actively transported across the small intestine against a concentration gradient. The serosal-to-mucosal concentration ratio of L-histidine was significantly decreased at the cadmium concentration of 10 μg/ml, although the active transport of L-histidine was not significantly decreased at cadmium concentrations of 5—7 μg/ml. Moriuchi *et al.*[22] have reported that when the everted rat intestine was perfused *in vitro* for 1 h using mucosal solution containing 0.25 mM $CdCl_2$ (28.1 μg Cd/ml), damage to the mucosal membrane was not observed on scanning electron micrographs. This finding suggests that in our perfusion experiment using mucosal solution containing cadmium (10 μg/ml), cadmium does not produce any morphological lesion in the intestinal mucosal membrane. In addition, the intestinal accumulation of cadmium was large at the cadmium concentration of 10 μg/ml. These results indicate that cadmium taken up in the intestinal tissue inhibits the active transport of L-histidine across the rat small intestine.

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