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Studies on Poisonous Metals. IX.[1,2] Effects of Dietary Fibers on Absorption of Cadmium in Rats

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The effects of dietary fibers on the gastrointestinal absorption of cadmium were studied. The fibers, such as lignin, cellulose, and sodium carboxymethylcellulose (Na CMC), produced a slight decrease in the contents of cadmium in the tissues of rats following a single oral administration of cadmium. In addition, in rats fed continuously with the experimental diets containing cadmium together with fibers, lignin and Na CMC significantly decreased the contents of cadmium in the tissues. These results show that the fibers depressed the intestinal absorption of cadmium. It is suggested that the inhibitory effects of these fibers on the gastrointestinal absorption of cadmium were due to their intrinsic properties, such as ability to bind cadmium and effect on viscosity, and that the differences of inhibitory effects of the fibers on the intestinal absorption of cadmium were related to differences in such physical properties.

Keywords——poisonous metal; cadmium; absorption; distribution; excretion; dietary fiber; rat

Dietary fibers have been shown to influence the gastrointestinal absorption and toxicity of various organic chemicals and metals in animals. For example, the toxic effects of amaranth are counteracted by dietary fibers.[3-5] A number of plant fibers and fiber-containing materials have marked ability to counteract the toxic effects induced in rats fed massive doses of sodium cyclamate and polyoxyethylene sorbitan monostearate.[6] In addition, it was recently reported that high intakes of fiber impair the utilization of zinc, calcium, and magnesium in man,[7] and that various indigestible fibers reduce the intestinal absorption of calcium, iron, zinc, copper, chromium and cobalt in rats.[8]

In evaluating the hazard from exposure to cadmium, for which a major route of entry into the body is through the gastrointestial system, it is important to understand the extent to which dietary factors influence the absorption of the metal. Recently, we reported that the absorption of cadmium from the small intestine of rats is inhibited by proteins such as glycinin, β-lactoglobulin, and ovalbumin, and in contrast, is enhanced by amino acids such as L-cysteine and L-histidine.[9,10] In addition, we reported that some dietary fibers depress the absorption of cadmium from the small intestine of rats *in vitro* and *in situ*.[1] The purpose of this study was to further examine the effects of various dietary fibers on the *in vivo* absorption of cadmium in rats.

Experimental

Materials——Cellulose (200—300 mesh, Toyo Roshi Co., Tokyo), glucomannan (Tsuruta Shoji Co., Gunma), pectin (from citrus, Katayama Chemical Industries Co., Osaka), lignin (Tokyo Kasei Kogyo Co., Tokyo), and sodium alginate (Tokyo Kasei Kogyo Co., Tokyo), which are all found in nature, and sodium carboxymethylcellulose (Na CMC, Tokyo Kasei Kogyo Co., Tokyo) were chosen as test fibers in this study. Cadmium chloride and all other chemicals were of reagent grade.

Diets——Rats used in the single oral dose experiments were allowed food (Nosan Lab Chow) *ad libitum*. The compositions of diets in the continuous oral dose experiments are shown in Table I.

TABLE I. Compositions of Basal and Experimental Diets

Ingredient	Basal diet	Experimental diet	
		Control	Fiber
		(g/kg diet)	
Cadmium	—	0.1	0.1
Fiber	—	—	50
Potato starch	780	780	730
Milk casein	100	100	100
Soybean oil	50	50	50
Salt mixture[a]	55	55	55
Vitamin mixture[b]	15	15	15

a) The following salts were made up to 55 g by adding lactose: $Ca(H_2PO_4)_2 \cdot H_2O$, 35.69 g; NaCl, 0.844 g; Na_2CO_3, 1.76 g; $CaCO_3$, 8.81 g; K_2CO_3. 3.88 g; $MgCO_3$, 1.144 g; $ZnCO_3$, 0.042 g; $FeSO_4 \cdot 7H_2O$, 0.136 g; $CuSO_4 \cdot 5H_2O$, 0.0215 g; $MnSO_4 \cdot 7H_2O$, 0.169 g; KI, 0.4 mg.

b) The preparation was the same as described in a previous paper.[12]

Single Oral Administration Experiment——Male Wistar rats, weighing about 150 g, were fasted for about 20 h with drinking water *ad libitum* prior to use. Cadmium (10 mg/kg) was given to the rats as an aqueous solution (1.5 mg/ml) *via* a stomach tube. Fibers were dissolved or suspended in the above cadmium solution and given to the rats. The rats were housed in individual metabolic cages with water *ad libitum* and the urine was collected 24 h after administration. The rats were killed with urethane 24 h after administration, and various tissues, such as blood, liver, kidney, spleen, testis and carcass, were collected and stored in a freezer until required for analysis. The residue after removal of the tissues described above and the gastrointestinal tract (including contents) from the whole body was designated as the carcass.

Continuous Oral Administration Experiment——Male Wistar rats, weighing about 150 g, were housed in individual metabolic cages. The rats were fed on the basal diet for the first 6 d and then on the experimental diets containing cadmium with and without fibers for 7 d. The body weights of the rats and the quantity of the diet consumed were measured every day. Thereafter, the rats were fasted for 20 h, killed with urethane, and the various tissues were removed.

Equilibrium Dialysis Experiment——Seamless cellulose tubing (Visking Co., 24/32 in size) was cut to 100 mm in length, and tied at one end to make a cellulose bag. The bag was heated in distilled water for 4 h and placed between filter papers to eliminate water completely. It was then attached to the lower end of a glass cylinder (15 × 100 mm) with the aid of a cotton thread. Ten milliliters of physiological saline (pH 6.0) was pipetted into the bag. The bag was inserted into a test tube (45 × 100 mm) containing 100 ml of saline solution (pH 6.0) of cadmium and fibers and fixed with the aid of a cork stopper. The inner and outer solutions were stirred at 37°C for 5 h, which was a sufficient period of time for equilibrium to be attained between the solutions inside and outside the dialysis bag. The extent of binding of cadmium with each fiber was calculated from the difference between the concentrations of cadmium in the inner and outer solutions.

Determination of Viscosity——The viscosity of fiber solutions was determined by using an Ostwald viscometer at 37°C.

Determination of Diffusion Coefficient——The diffusion coefficient of cadmium ion in various fiber solutions was determined according to the porous disc method of McBain and Liu.[11]

Analytical Procedures——The tissues and urine were wet-ashed in concentrated nitric acid followed by 60% perchloric acid on a temperature-controlled hot plate. Then, the content of cadmium in the specimens from blood and urine was determined by Zeeman atomic absorption spectrometry using a Hitachi 170-70 graphite furnace atomizer; the correlation between absorbance and concentration was linear up to 4 ppb cadmium. Cadmium in the specimens from other tissues was chelated with sodium diethyldithiocarbamate, extracted with methylisobutylketone, and determined with a Shimadzu AA-610S flame-type atomic absorption spectrometer. The correlation between absorbance and concentration was linear up to 0.5 ppm cadmium.

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

Results and Discussion

We investigated the tissue distribution and urinary excretion of cadmium 24 h after a single oral administration of cadmium with and without fibers in rats. The results are shown in Table II. The urinary excretion of cadmium was little influenced by administration of these fibers. However, lignin significantly decreased the total cadmium in the tissues. In addition, cellulose, glucomannan, Na alginate, and Na CMC fibers caused a slight decrease in the total cadmium in the tissues, although the effects were not statistically significant. These fibers did not induce diarrhea in the rats. Since the cadmium amounts in the feces and gastroinstinal contents after administration were not determined, the effect of these fibers on the gastrointestinal transit time is unknown. However, the inhibitory effect of the fibers on the intestinal absorption of cadmium is supported by the *in vitro* and *in situ* results which were reported in our previous paper.[1]

TABLE II. Tissue Distribution and Urinary Excretion of Cadmium 24 h after a Single Oral Administration of Cadmium and Fibers in Rats

Fiber	Cadmium (% of dose)[a]							
	Tissue							Urine
	Blood[b]	Liver	Kidney	Spleen	Testis	Carcass[c]	Total	
Control	0.014 ±0.006	2.981 ±0.850	0.100 ±0.028	0.009 ±0.008	0.019 ±0.011	2.589 ±0.542	5.819 ±0.881	0.008 ±0.004
Cellulose	0.020 ±0.007	2.631 ±0.543	0.081 ±0.009	0.005 ±0.002	0.010 ±0.003	2.040 ±0.111	4.749 ±0.553	0.013 ±0.003
Pectin	0.021 ±0.003	3.272 ±0.352	0.091 ±0.011	0.006 ±0.001	0.013 ±0.004	2.264 ±0.193	5.676 ±0.511	0.011 ±0.003
Glucomannan	0.030 ±0.005	2.910 ±0.618	0.102 ±0.003	0.010 ±0.003	0.013 ±0.003	2.083 ±0.245	5.163 ±0.820	0.015 ±0.013
Lignin	0.000 ±0.000	2.310 ±0.320	0.091 ±0.030	0.011 ±0.001	0.017 ±0.004	1.889 ±0.085	4.313 ±0.398[d]	0.007 ±0.006
Na alginate	0.021 ±0.003	2.903 ±0.388	0.101 ±0.001	0.007 ±0.003	0.008 ±0.003	2.333 ±0.180	5.454 ±0.579	0.011 ±0.004
Na CMC	0.011 ±0.003	2.670 ±0.466	0.087 ±0.012	0.005 ±0.001	0.011 ±0.002	1.750 ±0.213	4.573 ±0.705	0.016 ±0.009

Doses of cadmium and fibers were 10 mg/kg and 133 mg/kg, respectively.
a) The values represent the mean ± standard deviation for 3 to 7 animals.
b) Blood value was estimated as 9% of body weight.[13]
c) Residue after removal of the tissues described above and the gastrointestinal tract (including contents) from the whole body.
d) Significantly different from control values, $p<0.02$.

Moreover, rats were continuously fed on the experimental diets containing cadmium alone and cadmium together with fibers, such as lignin, Na CMC or cellulose. Table III shows the food intake, body weight gain, and feces weight. The daily food intake was not effected by the presence of cadmium or cadmium together with each type of fiber. These fibers did not induce diarrhea. The daily feces weight was not affected by the presence of cellulose or lignin, but was decreased in the case of the diet containing Na CMC. Thus, it was suggested that these fibers did not shorten the gastrointestinal transit time when given continuously in the diet. In addition, the body weight gain was little effected by the addition of cadmium alone or cadmium together with cellulose to the basal diet. On the other hand, the body weight gain in the rats fed on the diet containing cadmium together with lignin or Na CMC was significantly reduced in comparison with that in the rats fed on the basal diet. At present, however, the mechanism of the decreases in body weight gain is unknown.

Table IV shows the tissue contents of cadmium in the rats after continuous administration. Lignin and Na CMC significantly decreased the contents of cadmium in the blood, liver, kidney, spleen and testis, and considerably reduced it in the carcass. On the other hand, cellulose decreased the tissue contents of cadmium to only a small extent. These results suggested that the fibers such as lignin and Na CMC had a much more inhibitory effect on the absorption of cadmium than did cellulose. The inhibitory effect of fibers on the absorption of

Table III. Average Daily Food Intake, Body Weight Gain and Feces Weight

Diet	Food intake (g/d)	Body weight gain (g/d)	Feces weight[a] (g/d)
Basal	24.0±1.7	6.6±0.4	10.8±1.2
Basal+cadmium (100 ppm)	24.7±1.9	5.9±0.6	11.3±1.7
Basal+cadmium (100 ppm) +cellulose (5%)	22.2±1.0	6.8±0.4	10.1±1.1
Basal+cadmium (100 ppm) +lignin (5%)	26.4±2.0	2.3±2.0[b]	9.5±1.8
Basal+cadmium (100 ppm) +Na CMC (5%)	20.2±4.0	1.8±0.3[c]	8.3±0.8[b]

a) Feces were dried for 3 h at 100°C.
b) Significantly different from the basal diet, $p<0.05$.
c) Significantly different from the basal diet, $p<0.001$.

Table IV. Tissue Distribution of Cadmium after Continuous Oral Administration of Cadmium and Fibers for 7 Days

Fiber	Total dose of Cd (mg)	Cadmium (% of dose)[a]						
		Blood	Liver	Kidney	Spleen	Testis	Carcass	Total
Control	17.30	0.0015 ±0.0002	0.161 ±0.011	0.071 ±0.018	0.0009 ±0.0003	0.0013 ±0.0004	0.185 ±0.035	0.419 ±0.055
Cellulose (5%)	15.54	0.0020 ±0.0006	0.153 ±0.039	0.060 ±0.011	0.0006 ±0.0002	0.0015 ±0.0006	0.162 ±0.005	0.379 ±0.047
Lignin (5%)	18.49	0.0007 ±0.0001[b]	0.083 ±0.008[b]	0.023 ±0.004[c]	0.0004 ±0.0002[c]	0.0005 ±0.0001[c]	0.139 ±0.018	0.246 ±0.011[c]
Na CMC (5%)	13.96	0.0003 ±0.0003[b]	0.096 ±0.016[b]	0.024 ±0.006[c]	0.0005 ±0.0002	0.0008 ±0.0002[d]	0.125 ±0.021	0.247 ±0.038[c]

Concentration of cadmium in the experimental diets was 100 μg/g.
a) The values represent the mean± standard deviation for 3 to 6 animals.
b) Significantly different from control values, $p<0.001$.
c) Significantly different from control values, $p<0.01$.
d) Significantly different from control values, $p<0.05$.

Table V. Binding of Cadmium to Various Fibers

Fiber	Bound cadmium[a] (μg cadmium/mg fiber)
Cellulose	0.101±0.005
Glucomannan	0.121±0.013
Pectin	0.333±0.007
Na alginate	0.343±0.008
Na CMC	0.624±0.0005
Lignin	0.713±0.001

Equilibrium dialysis was carried out for 5 h at 37°C.
The concentrations of fibers were: glucomannan 0.25%; and cellulose, pectin, lignin, Na alginate and Na CMC 1%.
The initial concentration of cadmium in the outer solution was 10 μg/ml.
a) Each value represents the mean±standard deviation of duplicate experiments.

cadmium was much more marked in the case of continuous administration than single administration. Such a phenomenon might be attributable to the difference in the fiber/cadmium ratios, which were 500 in the diets used in the continuous administration and 13.3 in the single dose.

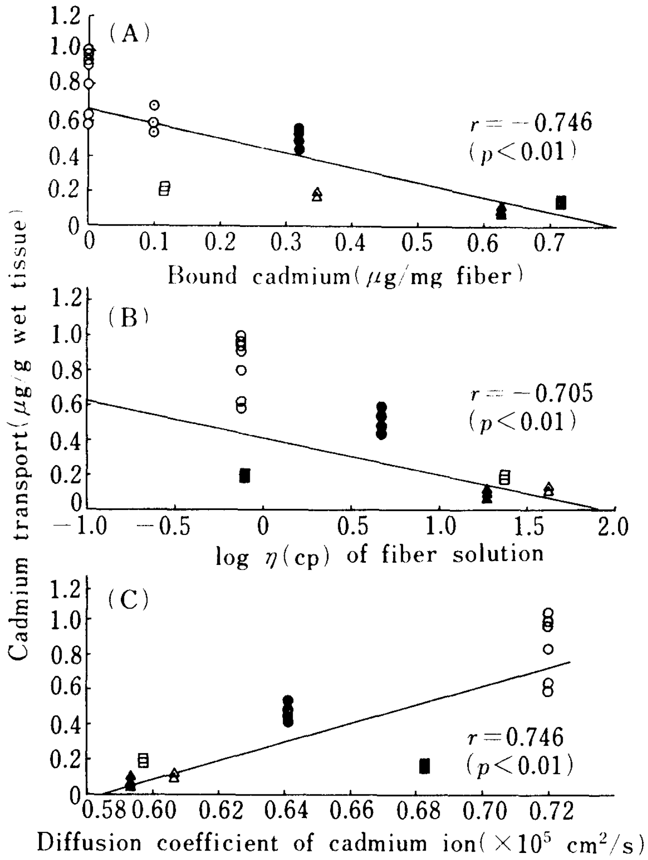

Fig. 1. Correlation between Binding of Cadmium to Fibers (A), Viscosity (η) of Fiber Solution (B), or Diffusion Coefficient of Cadmium Ion (C) and *in Vitro* Transport of Cadmium across Everted Rat Small Intestine[a]

a) The values are cited from our previous paper.[1]
The concentrations of fibers: (A) glucomannan 0.25%; other fibers 1%, (B) glucomannan 0.25%; other fibers 1%, (C) glucomannan 0.09%, other fibers 0.1%.
Key: ○, control; □, glucomannan; ⊙, cellulose; ●, pectin; △, Na alginate; ▲, Na CMC; ■, lignin.

Next, the mechanism of the inhibitory effect of fibers on the intestinal absorption of cadmium was examined from various standpoints, such as the binding of cadmium with fibers and the effect of the viscosity of fiber solution on the diffusion of cadmium. As shown in Table V, the extent of cadmium binding to the fibers was in the order lignin>Na CMC>Na alginate>pectin>glucomannan>cellulose. Lignin and Na CMC, which had a much greater inhibitory effects on the absorption of cadmium, showed stronger binding with cadmium than did other fibers. The correlation between the binding of cadmium to fibers and the *in vitro* intestinal transport of cadmium, which was reported in our previous paper,[1] is shown in Fig. 1(A). The correlation coefficient was −0.746 ($p<0.001$), indicating that the binding of cadmium to fibers was related to the depression of intestinal absorption of cadmium. In addition, the viscosity of fiber solutions was measured. As shown in Fig. 1 (B), a correlation coefficient of −0.705 ($p<0.01$) was obtained, indicating that the viscosity of the fiber solutions influences the intestinal absorption of cadmium. Furthermore, the correlation between the diffusion coefficient of cadmium ion measured in the fiber solutions and the transport of cadmium was examined and a correlation coefficient of 0.746 ($p<0.01$) was obtained as shown in Fig. 1 (C). These results suggest that the fibers forming solutions of higher viscosity decrease the concentration of cadmium at the intestinal mucosa surface by reducing the diffusion rate of cadmium, resulting in depression of the intestinal absorption of cadmium.

In summary, the fibers used in this study were considered to inhibit the gastrointestinal absorption of cadmium due to intrinsic properties, such as ability to bind cadmium and effect on viscosity, and the differences of inhibitory effects of these fibers on the intestinal absorption of cadmium are considered to be related to differences in such physical properties.

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