Curdlan and Gellan Gum, Bacterial Gel-Forming Polysaccharides, Exhibit Different Effects on Lipid Metabolism, Cecal Fermentation and Fecal Bile Acid Excretion in Rats

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Summary The effects of curdlan (CD) and gellan gum (GG), bacteria-producing polysaccharides, on lipid concentrations of serum and liver, fecal bile acid composition and intestinal fermentation products were studied in rats fed diets containing cellulose powder (CP), CD or GG at 5% for 4 wk. The cecal weight of the CD group increased significantly as compared to that of the other two groups and the pH of its contents was significantly low. The gastrointestinal transit time in the GG group was significantly shorter than that in the CP and CD groups. No significant inter-group differences were observed in the serum concentrations of total cholesterol and HDL-cholesterol, but a significant decrease was observed in the hepatic total cholesterol concentration of the CD group as compared to that of the CP and GG groups. No significant difference in the total bile acid excretion in feces was observed among the groups, but significantly low values were observed in the proportion of secondary bile acids in the CD group as compared to those of the CP and GG groups. Amounts of short-chain fatty acids (acetic, propionic and butyric acid) and lactic acid in the cecal contents were significantly higher in the CD group than in the other two groups. These results reveal that dietary CD is easily degraded and fermented by intestinal bacteria in the cecum and lowers cholesterol concentration in the liver, while dietary GG shortens the gastrointestinal transit time, suggesting the promotion of evacuation.

Key Words curdlan, gellan gum, short-chain fatty acids, fecal bile acid excretion, cholesterol metabolism

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Some bacteria-producing polysaccharides such as xanthan gum, pullulan, curdlan and gellan gum are used as food additives in the food industry. They have structures which cannot be degraded by human digestive enzymes and are considered as a type of dietary fiber. Curdlan (CD), a polysaccharide produced by *Alcaligenes faecalis* var. *myxogenes*, is β -1,3-glucan-linked to straight-chain D-glucose (1,2). Although CD is water-insoluble, it becomes gelled when its aqueous suspension is heated. Gellan gum (GG) produced by *Pseudomonas elodea*, on the other hand, is a water-soluble polysaccharide consisting of two D-glucose molecules and one each L-rhamnose and D-glucuronic acid molecules (3). CD is used as an improving agent of physical properties and GG as a gelling agent or thickening agent for various food processing. But their physiological functions have not yet been fully studied except for a few reports (4–7).

We previously studied the lipid metabolism in rats fed a hypercholesterolemic diet containing CD and GG at 5% for 2 wk (8). We observed that both substances did not affect the cholesterol concentrations of the serum and liver. But CD caused the cecal weight including contents to increase and the pH of cecal contents to decrease, while GG increased the number of fecal lumps and shortened the gastrointestinal transit time. We assumed that CD and GG would express different behaviors in the lower digestive tract of rats.

The present study was planned to further clarify the differences in the physiological functions of CD and GG. Therefore, their effects on the lipid concentrations of serum and liver, fecal bile acid composition and cecal fermentation products were investigated in rats fed cholesterol-free diets.

MATERIALS AND METHODS

Experimental animals, diet and rearing method. Four-week-old male Sprague-Dawley rats (Tokyo Experimental Animal, Co., Tokyo) were individually housed in stainless steel apartment cages and reared in a room ($23 \pm 1^\circ\text{C}$ temperature, $50 \pm 10\%$ humidity) maintained under a 12-h light/dark cycle (light cycle; 8:00–20:00). The three types of dietary fiber (DF) used for the experiments were cellulose powder (CP, Toyo Roshi Kaisya, Tokyo), curdlan (CD, MW = $7\text{--}9 \times 10^4$, Takeda Chemical Industries, Osaka) and gellan gum (GG, MW = $6\text{--}7 \times 10^5$, San-eigen FFI, Osaka). CP was used as the control.

After 7 d of preliminary rearing on the basal diet (AIN-76), rats were divided into three groups of seven (Experiment 1) or eight rat per group (Experiment 2) and given the following experimental diet (g/100 g): casein 20.0, DL-methionine 0.3, corn starch 15.0, sucrose 50.0, corn oil 5.0, mineral mixture (AIN-76) 3.5, vitamin mixture (AIN-76) 1.0, choline bitartrate 0.2 and DF 5.0. Water and food were given ad libitum and replaced everyday. After 4 wk on the experimental diets, blood samples were taken from the hearts of animals at 10:00, under sodium pentobarbital anesthesia (50 mg/kg body weight), without fasting. Care and use of the rats in the present study followed the guidelines of governmental legislation in Japan (1980).

Design of Experiment 1: After sampling blood from the heart, the liver was removed and weighed. The cecum was weighed with its contents. The cecal contents were then transferred to 50-mL plastic tubes, homogenized well and the pH was measured directly using a glass electrode. The cecal tissue was washed with physiological saline (4°C), blotted on filter paper and weighed. The small intestine and colon plus rectum were measured for their length and processed in the same way as the cecum. Feces were collected for the last 5 d preceding the end of the experimental period, weighed, freeze-dried and weighed again. After pulverizing in a mill, feces were placed in screw-capped tubes, sealed with nitrogen gas and stored (−30°C) until analysis. On days 8 and 15, the animals were starved from 8:00 until 20:00 and then given the diet containing 0.3% carmine (Wako Pure Chemical Industries, Osaka) as a marker at 20:00. Feces were checked once every 30 min for presence of the marker, and the time until the presence was first observed was set as the gastrointestinal transit time (mean of two observation times). On day 21, fresh feces were collected, placed in 2 mL of deionized water, homogenized and the pH was measured.

Design of Experiment 2: After anesthesia, blood samples were taken from the portal vein and abdominal aorta concurrently. The cecum was immediately removed and weighed. Cecal contents were obtained in the same manner as described in the design of Experiment 1. Portal venous blood and the homogenates of cecal contents were used for determination of ammonia. The remaining homogenates of cecal contents were placed in tubes, frozen with liquid nitrogen and stored at −80°C until analysis of short-chain fatty acids (SCFAs).

Lipid concentration. Blood samples were left standing for 30 min at room temperature in plastic tubes and then centrifuged (4°C, 1,500 × *g*, 15 min) to obtain serum. Serum concentrations of total cholesterol and HDL-cholesterol were determined with commercial kits (Wako Pure Chemical Industries). Lipids in the liver were extracted by the method of Folch et al (9). Concentrations of total cholesterol and triacylglycerol were determined by the Zak method (10) and Fletcher method (11), respectively. Phospholipid concentration was determined by commercial kits (Wako Pure Chemical Industries).

Fecal bile acids excretion. Bile acids in feces were extracted by the method of Locket and Gallaher (12). Conjugated bile acids were deconjugated by the enzyme method (13) using cholyglycine hydrolase (EC 3.5.1.24, Sigma Chemical, St. Louis, MO, USA). Bile acids were then extracted with diethyl ether under the presence of hydrochloric acid, methylated, trifluoroacetylated and determined by gas-liquid chromatography (Model GC-12A, Shimadzu, Kyoto). For analysis, glass columns (3.2 mm × 1.6 m) packed with 1.5% silicone AN-600 on 100/120 mesh Gaschrom Q (Chromatotec, Tokyo) were used (14). As the internal standard, 23-nor-deoxycholic acid was used. Standards of bile acids were purchased from Sigma Chemical or Steraloids (Wilton, NH, USA).

Ammonia concentration. Portal venous blood and cecal contents (~50 mg) were immediately added to polyethylene tubes containing deproteinized solution

(containing sodium tungstate dihydrate and sulfuric acid), mixed and centrifuged ($1,200 \times g$, 5 min). Ammonia concentrations in both supernatants were determined spectrophotometrically (15).

SCFAs and lactic acid in the cecal contents. SCFAs were determined by partially modifying the method of Deschner et al (16). After thawing, about 0.1 g of the cecal contents was transferred to 1.5 mL polyethylene tubes, weighed, added with 0.05 mL of 40 mM 2-ethyl-*n*-butyric acid (the internal standard) and 0.75 mL of 25% metaphosphoric acid, and homogenized by ultrasonication for 1 min at 4°C. Homogenate was held overnight at 4°C, and then centrifuged, $8,000 \times g$ for 30 min at 4°C. Supernatant was filtered through a 0.45- μ m filter (Dismic-13 Cellulose Nitrate, Toyo Roshi Kaisha, Tokyo). The sample was analyzed by gas-liquid chromatography (Model GC-12A, Shimadzu) equipped with a glass column (3.2 mm $\times$ 1.6 m) packed with 10% SP-1200/1% H₃PO₄ on 80/100 mesh Chromosorb W AW (Superlco, PA, USA). The concentration of lactic acid in the cecal contents was measured with a D-Lactic/L-Lactic acid kit (Boehringer-Mannheim, Mannheim, Germany).

Statistical analyses. Values are expressed as mean $\pm$ SE. After processing with one-way ANOVA ($p < 0.05$), the significant differences among the diet groups were analyzed by Duncan's multiple range test (17) ($p < 0.05$, SPSS version 7.5.1J for Windows, SPSS Japan, Tokyo).

RESULTS

Body weight gain and food intake

Table 1 shows changes in body weight and food intake in Experiment 1. No significant differences in body weight were observed between the CD and GG groups and the CP group. During the periods of day 0 to day 21 and day 0 to day 28, food intake of the CD group showed significantly low values ($p < 0.05$) compared with that of the CP group (control). The food efficiency ratio (mean $\pm$ SE) was

Table 1. Effect of curdlan and gellan gum on body weight and total food intake in rats (Experiment 1).¹

Feeding period (d)	Body weight (g)			Total food intake (g)		
	Cellulose	Curdlan	Gellan gum	Cellulose	Curdlan	Gellan gum
0	138 $\pm$ 2	137 $\pm$ 2	137 $\pm$ 1	0	0	0
7	192 $\pm$ 2	194 $\pm$ 4	195 $\pm$ 2	135 $\pm$ 2	129 $\pm$ 3	132 $\pm$ 3
14	253 $\pm$ 3	253 $\pm$ 5	255 $\pm$ 3	298 $\pm$ 5	275 $\pm$ 6	286 $\pm$ 7
21	321 $\pm$ 5	311 $\pm$ 5	318 $\pm$ 5	475 $\pm$ 9 ^a	430 $\pm$ 10 ^b	452 $\pm$ 13 ^{ab}
28	377 $\pm$ 7	362 $\pm$ 8	363 $\pm$ 7	658 $\pm$ 14 ^a	595 $\pm$ 16 ^b	625 $\pm$ 19 ^{ab}

¹ Mean $\pm$ SE ($n = 7$): values in the same row not sharing a common superscript letter are significantly different at $p < 0.05$.

Table 2. Effect of curdlan and gellan gum on characteristics of digestive tracts, fecal weight and gastrointestinal transit time (GTT) in rats (Experiment 1).¹

Items	Cellulose	Curdlan	Gellan gum
Small intestine			
Weight (g)	7.52 ± 0.30	8.95 ± 0.81	7.92 ± 0.37
Length (cm)	124 ± 2	126 ± 2	123 ± 1
Cecum			
Weight (g)	0.50 ± 0.02 ^a	1.10 ± 0.11 ^b	0.45 ± 0.04 ^a
Contents (g)	2.11 ± 0.12 ^a	4.37 ± 0.43 ^b	1.86 ± 0.14 ^a
pH	7.35 ± 0.12 ^a	6.12 ± 0.21 ^b	7.47 ± 0.14 ^a
Colon plus rectum			
Weight (g)	1.08 ± 0.05 ^a	1.39 ± 0.09 ^b	1.36 ± 0.08 ^b
Length (cm)	19.7 ± 0.7	20.9 ± 0.4	20.1 ± 0.5
Feces			
Lumps (number/d)	16.3 ± 0.7 ^a	12.4 ± 1.6 ^a	32.7 ± 1.6 ^b
Wet weight (g/d)	2.20 ± 0.08 ^a	1.39 ± 0.17 ^b	2.42 ± 0.19 ^a
Dry weight (g/d)	1.87 ± 0.06 ^a	0.88 ± 0.09 ^b	1.60 ± 0.09 ^c
Moisture (%)	15.2 ± 0.7 ^a	35.5 ± 1.9 ^b	33.1 ± 1.9 ^b
pH	7.62 ± 0.12 ^a	5.69 ± 0.13 ^b	7.23 ± 0.10 ^c
GTT (min)	684 ± 12 ^a	673 ± 31 ^a	514 ± 23 ^b

¹ Mean ± SE ($n=7$): values in the same row not sharing a common superscript letter are significantly different at $p<0.05$.

0.36 ± 0.00 in the CP group, 0.38 ± 0.01 in the CD group and 0.36 ± 0.01 in the GG group, indicating no inter-group differences.

Experiment 2 showed a tendency similar to Experiment 1 (data not shown), but none of the items showed significant differences among groups.

Characteristics of the digestive tract, fecal weight and gastrointestinal transit time

Table 2 shows the results of Experiment 1. The weight of small intestine in the CD group was not significantly different ($p<0.05$) from those in the CP and GG groups. The length was substantially the same in all the groups. The cecal weight of the CD group increased to approximately twice that of the CP group. On the other hand, no difference was observed between the GG group and the CP group. The weight of cecal contents in the CD group also significantly increased ($p<0.05$) as compared to that in the CP and GG groups. The cecal contents of the CD group looked highly viscous, but that of the GG group appeared to be mostly gelled particles. There was no difference in the pH of the cecal contents between the CP group and GG group, but that of the CD group was significantly lower ($p<0.05$) than that of the other two groups. The weight of colon plus rectum was significantly higher ($p<0.05$) in the CD and GG groups than in the CP group, but the length was not different among the groups.

The number of fecal lumps in the GG group increased significantly ($p<0.05$)

Table 3. Effect of curdlan and gellan gum on weights of cecum and colon plus rectum, and ammonia concentrations of portal vein blood, and cecal contents in rats (Experiment 2).¹

Items	Cellulose	Curdlan	Gellan gum
Cecum			
Weight (g)	0.46 ± 0.01 ^a	1.11 ± 0.06 ^b	0.57 ± 0.02 ^a
Contents (g)	2.11 ± 0.12 ^a	4.37 ± 0.43 ^b	1.86 ± 0.14 ^a
pH	7.22 ± 0.14 ^a	6.12 ± 0.09 ^b	7.45 ± 0.10 ^a
Colon plus rectum			
Weight (g)	1.09 ± 0.03 ^a	1.32 ± 0.11 ^b	1.37 ± 0.04 ^b
Ammonia concentration			
Portal vein blood (µg/dL)	183 ± 13	210 ± 7	208 ± 7
Cecum (µg/g contents)	24.8 ± 2.5 ^a	15.6 ± 1.5 ^b	12.2 ± 1.8 ^b
(µg/contents)	51.1 ± 6.0 ^a	63.0 ± 9.8 ^a	24.1 ± 4.0 ^b

¹ Mean ± SE (n = 8); values in the same row not sharing a common superscript letter are significantly different at $p < 0.05$.

as compared to that in the CP and CD groups. The shape was varied and more or less elongated in the CD group, while it was normally elliptical in the CD and GG groups, and the lump size, although it was not measured, looked to be smaller in the GG group than in the CP group. Wet and dry fecal weights per day of the CD group decreased significantly ($p < 0.05$) as compared to those of the CP and GG groups. The fecal moisture in the CD and GG groups was significantly increased ($p < 0.05$) as compared to that in the CP group. No diarrhea was observed.

The gastrointestinal transit time in the GG group was significantly shortened ($p < 0.05$) as compared to that in the CP and CD groups, but there was no relation observed between the gastrointestinal transit time and fecal weight.

Table 3 shows the results of Experiment 2. Substantially similar results as Experiment 1 were observed in respect of the cecal weight and the weight and pH of the cecal contents. Ammonia concentration of the portal vein blood in the CD and GG groups was not different from that in the CP group. Ammonia concentration per gram of the cecal contents in the CD and GG groups decreased significantly ($p < 0.05$) as compared to that in the CP group. As for the amount of ammonia in entire cecal contents, no significant difference was observed between the CP group and CD group, but the GG group showed a significant decrease ($p < 0.05$) as compared to the other two groups.

Lipid concentration in serum and liver

Table 4 shows the results of Experiments 1 and 2. In both experiments, there were no significant inter-group differences observed in the total cholesterol and HDL-cholesterol concentrations in serum. The liver weight did not differ among groups. Total cholesterol concentration of the liver in the CD group decreased

Table 4. Effect of curdlan and gellan gum on serum and liver lipid concentrations and liver weight in rats.¹

Items	Cellulose	Curdlan	Gellan gum
Experiment 1 (<i>n</i> = 7)			
Serum			
Total cholesterol (mg/dL)	129 ± 7	123 ± 6	130 ± 8
HDL-cholesterol (mg/dL)	71.0 ± 6.7	72.4 ± 5.4	62.0 ± 8.5
Liver			
Weight (g)	17.9 ± 0.6	16.3 ± 0.5	16.9 ± 0.7
Total cholesterol (mg/g)	3.62 ± 0.33 ^a	2.65 ± 0.10 ^b	3.56 ± 0.33 ^a
Triacylglycerol (mg/g)	54.7 ± 7.3 ^a	31.5 ± 4.2 ^b	45.8 ± 5.7 ^{ab}
Phospholipids (mg/g)	26.4 ± 1.6	24.5 ± 1.0	27.5 ± 1.6
Experiment 2 (<i>n</i> = 8)			
Serum			
Total cholesterol (mg/dL)	130 ± 10	114 ± 5	123 ± 8
HDL-cholesterol (mg/dL)	73.5 ± 3.8	66.6 ± 5.5	75.1 ± 4.9
Liver			
Weight (g)	18.5 ± 0.8	18.0 ± 0.4	17.4 ± 0.8
Total cholesterol (mg/g)	3.68 ± 0.31 ^a	2.86 ± 0.16 ^b	3.59 ± 0.23 ^a
Triacylglycerol (mg/g)	62.3 ± 6.3 ^a	41.9 ± 4.9 ^b	53.0 ± 6.6 ^{ab}
Phospholipids (mg/g)	18.8 ± 0.9	18.5 ± 0.7	19.7 ± 0.8

¹ Mean ± SE; values in the same row not sharing a common superscript letter are significantly different at $p < 0.05$.

significantly ($p < 0.05$) as compared to that in the CP and GG groups. Triacylglycerol concentration in the liver in the CD group decreased significantly ($p < 0.05$) as compared to that in the CP group, but the decrease seen in the GG group was not significant. Phospholipid concentration remained unchanged.

Fecal bile acid excretions

Table 5 shows the results of Experiment 1. Lithocholic acid was significantly higher ($p < 0.05$) in the CD group than in the other two groups. No significant difference was observed in deoxycholic acid. Significantly higher ($p < 0.05$) values of α -muricholic acid and β -muricholic acid were observed in the CD group than in the CP and GG groups, but hyodeoxycholic acid in the CD group was significantly lower ($p < 0.05$) than that in the CP and GG groups. In the CP and GG groups, the major bile acid was hyodeoxycholic acid, while in the CD group, it was β -muricholic acid; both acids accounting for about half of the total amount. No inter-group difference was significant in the total bile acid excretion. The proportion of secondary bile acids to total bile acids was significantly lower ($p < 0.05$) in the CD group than in the CP and GG groups.

Table 5. Effect of curdlan and gellan gum on fecal bile acids and ratio of secondary bile acids to total bile acids in rats (Experiment 1).¹

Bile acid	Cellulose	Curdlan	Gellan gum
		(mg/d)	
Lithocholic acid	0.22 ± 0.01 ^a	0.57 ± 0.10 ^b	0.24 ± 0.07 ^a
Deoxycholic acid	0.43 ± 0.04	0.30 ± 0.04	0.43 ± 0.07
α-Muricholic acid	0.04 ± 0.01 ^a	0.26 ± 0.05 ^b	0.12 ± 0.05 ^a
Hyodeoxycholic acid	2.54 ± 0.16 ^a	0.19 ± 0.08 ^b	2.09 ± 0.31 ^a
Cholic acid	0.05 ± 0.01 ^a	0.18 ± 0.03 ^b	0.09 ± 0.03 ^a
β-Muricholic acid	0.11 ± 0.02 ^a	2.02 ± 0.34 ^b	0.45 ± 0.14 ^a
ω-Muricholic acid	0.04 ± 0.01 ^a	0.05 ± 0.01 ^a	0.10 ± 0.02 ^b
Total	4.04 ± 0.24	3.91 ± 0.54	3.83 ± 0.56
Secondary/total bile acids	0.80 ± 0.02 ^a	0.28 ± 0.04 ^b	0.76 ± 0.04 ^a

¹ Mean ± SE (*n* = 7): values in the same row not sharing a common superscript letter are significantly different at *p* < 0.05.

Table 6. Effect of curdlan and gellan gum on amounts of short-chain fatty acids (SCFAs) and lactic acid in cecal contents of rats (Experiment 2).¹

Items	Cellulose	Curdlan	Gellan gum
		(μmol/g cecal contents)	
Acetic acid	38.9 ± 4.1 ^a	50.7 ± 2.9 ^b	32.0 ± 1.5 ^a
Propionic acid	16.7 ± 1.1 ^a	20.8 ± 1.7 ^b	9.6 ± 0.7 ^c
Butyric acid	8.6 ± 1.1 ^a	12.0 ± 1.2 ^b	6.6 ± 0.5 ^a
Total SCFAs	72.8 ± 6.5 ^a	95.6 ± 3.9 ^b	54.9 ± 2.8 ^c
Lactic acid	1.53 ± 0.27 ^a	6.86 ± 1.39 ^b	0.61 ± 0.11 ^a
		(μmol/cecal contents)	
Acetic acid	80 ± 9 ^a	199 ± 14 ^b	62 ± 5 ^a
Propionic acid	35 ± 3 ^a	83 ± 10 ^b	19 ± 2 ^a
Butyric acid	18 ± 3 ^a	49 ± 9 ^b	13 ± 1 ^a
Total SCFAs	139 ± 16 ^a	337 ± 30 ^b	97 ± 9 ^a
Lactic acid	3.0 ± 0.4 ^a	25.8 ± 4.2 ^b	1.2 ± 0.2 ^a

¹ Mean ± SE (*n* = 8): values in the same row not sharing a common superscript letter are significantly different at *p* < 0.05.

SCFAs and lactic acid in the cecal contents

Table 6 shows the amounts of SCFAs and lactic acid in the cecal contents in Experiment 2. The CD group revealed a significantly high value (*p* < 0.05) in both the concentration and total amount of SCFAs as compared to the CP and GG groups. The GG group values tended to be lower than the CP group. The concentration and amount of lactic acid in the CD group was significantly higher

($p < 0.05$) than those in the CP and GG groups. No difference was observed between the CP group and GG group.

DISCUSSION

We observed enlargement of the cecum and lowering of pH in the cecal contents in rats fed the CD diet. Similar results have been observed under feeding conditions of cholesterol-free (7) or -added diets (8). These observations may indicate that CD is easily degraded and fermented by intestinal bacteria in the cecum as in the case with many water-soluble dietary fibers. Lowered pH of the cecal contents is recognized to be attributable mainly to increased concentrations of organic acids such as lactate and succinate (18). In the CD group, the lactic acid concentration in the cecal contents was actually elevated significantly as compared to the CP and GG groups. On the other hand, in the GG group, the pH of the cecal contents was significantly higher and the total SCFA amount was significantly lower than that of the CD group. Edwards and Eastwood (6) reported that GG had little effect on the cecal SCFA. Thus, GG is considered to be hardly degraded and fermented by intestinal bacteria.

Many water-soluble dietary fibers are characterized by their tendency to become easily fermented by intestinal bacteria (19) and extend the gastrointestinal transit time. However, we observed that the gastrointestinal transit time in the GG group was significantly shorter than that in the other two groups. This phenomenon is supported by the paper of Tetsuguchi et al (7). GG is known to become highly viscous when dissolved in water and to easily form gels under the condition of low concentration of cations. As the cecal contents in the GG group consisted of gel-like particles, it is considered that GG administered in powder form became gelled in the rat stomach and went down to the cecum in the same state. It is assumed that one reason for the resistance against degradation of GG by bacteria is attributable to such physical properties of the cecal contents. However, non-degradation of GG in the large intestine may perhaps be caused essentially by the absence of bacteria by which the sugar chain of GG could be cleaved. Thus, we presume that the shortening of gastrointestinal transit time by GG feeding may contribute to relieving constipation and to creating smooth evacuation.

The ammonia concentration in the cecal contents of the CD group markedly decreased as compared to that of the CP group. This might be due to the dilution effect accompanied by the increased weight of the cecal contents, as the total amount of ammonia in the contents was not different between the CD group and CP group. In the CD group, ammonia concentration in the portal venous blood was assumed to rise as the ammonia-absorbing mucosal surface increased accompanying enlargement of the cecum. However, the concentration in the CD group was not different from that in the CP group. Castell and Moore (20) reported that ammonia becomes ionized by the increase of SCFAs in the large intestine and is less likely to become absorbed. Accordingly, the reason for no rise in the ammonia con-

centration in portal venous blood in the CD group might be attributable to the increased amount of SCFAs in the cecal contents. This does not necessarily reflect directly the elevation of ammonia in the cecum of the CD group, because ammonia is known to be utilized by intestinal bacteria as a nitrogen source for proliferation. The decrease of total amount of ammonia in the cecal contents of the GG group might be attributed to the decreased bacterial growth in the cecum on the account of less utilization of GG as a carbon source.

In this experiment, we observed a significantly lower total cholesterol concentration in the liver, but no inter-group changes in serum cholesterol concentration. The theory that the effects of dietary fibers on cholesterol metabolism are mediated by increased steroid excretion into feces (21) has been quite influential so far, but there are also reports that no relevance is observed between the two (22, 23). In the present study, the excretion of bile acids into feces was not different between the CP group and CD group. Therefore, hepatic cholesterol 7 α -hydroxylase activity is not likely to be increased in the CD group. On the other hand, there is a report that SCFAs produced by intestinal bacteria in the large intestine participate in affecting the cholesterol metabolism (24). This is because propionic acid among SCFAs was observed to lower cholesterol synthesis in an *in vitro* experiment using cultured hepatocytes. The amount of propionic acid in the cecal contents of the CD group was significantly higher than that of the CP and GG groups. Topping and Pant (25) pointed out that a comparison is difficult because the concentration of propionic acid differs in *in vitro* and *in vivo* experiments. However, there exists undeniably a relation between cholesterol metabolism and cecal fermentation products, because SCFAs are constantly being produced by intestinal bacteria in the rat cecum. SCFAs are also reported to increase the blood flow in the mucosa of the intestinal tract (26). Since enlargement of the cecum was observed in the CD group, we assume that a considerable amount of SCFAs might be absorbed. Accordingly, it is considered that the effect of SCFAs on hepatic cholesterol concentration is certainly not negligible.

Bile acids are the final metabolites of cholesterol, and secondary bile acids converted by intestinal bacteria are considered to promote mutagenicity in the colon (27). Many intestinal bacteria have 7 α -dehydroxylase which catalyzes the conversion of primary bile acids to secondary bile acids, and the activity of this enzyme is clearly inhibited below pH 6.5 (28). Thornton (29) assumed that the intestinal environment is improved by the acidification of intestinal contents and feces by the mechanism which inhibits formation and activation of carcinogens derived from bile acids and cholesterol. An increase in secondary acids, particularly of lithocholic acid and deoxycholic acid, is considered to increase cancer risk as the results of studies on humans (30) and *in vitro* studies (31). The main bile acid in the feces of the CD group was β -muricholic acid, which is the primary bile acid of rat. In this group, the ratio of secondary bile acids/total bile acids was significantly lowered, suggesting that CD inhibited the formation of secondary bile acids owing to a lower pH in the cecum. In the CD group, the fecal excretion of lithocholic

acid was observed to be significantly high. However, lithocholic acid is a highly hydrophobic monohydroxy bile acid and may precipitated under acidic conditions. Therefore, it develops cancer-promoting activity only when it is dissolved in the large intestine. It is not clear whether the increased excretion of lithocholic acid in the CD group was due to accelerated conversion from chenodeoxycholic acid to lithocholic acid by intestinal bacteria or to accelerated excretion into feces by lowered re-absorption.

Based on the foregoing, dietary CD is degraded and fermented by intestinal bacteria in the cecum, and thereby may act to improve the intestinal environment and lower cholesterol concentration in the liver. Dietary GG shortens the gastrointestinal transit time, suggesting that it promotes evacuation. A study of the relationship between the degradation mechanism of CD and intestinal flora is also warranted.

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