Reversibility of the Curdlan Feeding Effects on the Morphological Structure of Intestinal Mucosa in Rats

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Summary We reported in the previous paper that rats fed a curdlan diet showed significant increases in the weight of the cecum and its contents, a decrease in fecal wet weight, a retardation in the transit time of the gastrointestinal tract, and morphological changes of the ileal and cecal mucosal surface when compared with the rats fed a cellulose diet. In the present study, we intended to learn if the curdlan effects on the morphological structure of intestinal mucosa were reversible. When rats were fed on the curdlan diet for 2 weeks followed by a cellulose diet for another 2 weeks, the cecum and cecal contents were not different from those of the cellulose group. The transit time of the gastrointestinal tract of the curdlan-followed-by-cellulose group was shorter than that of the curdlan group, whereas it was longer than that of the cellulose group. In scanning electron micrographs, the ileal villi of the curdlan-followed-by-cellulose group were normal, as in the cellulose group. However, their ileal and cecal microvilli were similar to those of the curdlan group, that is, the microvilli were crowded and more tightly packed, and some appeared to have been squeezed out. From these results, it was concluded that the effects of the curdlan feeding were only partially reversible, but the effects on the surface structure of intestinal mucosa were still sustained even after curdlan feeding of 2 weeks was discontinued. This might result from response to the high viscosity of the intestinal contents remaining after discontinuation of the curdlan.

Key Words curdlan diet, scanning electron microscopy, intestinal mucosa, transit time, rats

Some dietary fibers have been shown to cause alterations in the transit time of intestinal contents (1–3), to induce morphological changes of gastrointestinal mucosa (4, 5), and to exert physiological influence on epithelial cell proliferation (6). Cassidy et al have reported that the surface structure of small intestinal mucosa
was damaged in rats fed pectin, a soluble dietary fiber (7). We have observed that morphological changes of gastrointestinal mucosa in rats fed pectin at various levels depended on the amount of pectin in the diet (8). Morphological changes might be dependent on the type of dietary fiber and are likely to contribute to the effect of fiber on the digestive and absorptive functions. Thus it is of interest to examine the effects of dietary fiber intake on the morphological surface structure of the intestinal mucosa.

Recently, some undigestable polysaccharides have been used as food additives. In the digestive tracts, it is expected that they play roles as dietary fibers. Curdlan, a homopolymer of glucose with $\beta$-1,3-glucosidic linkages, is an undigestable polysaccharide. This is a bacterial polysaccharide and forms a gel by neutralization from its alkaline solution or by heating its aqueous suspension (9–12). It has been used by food manufactures as a gel-making agent and as a stabilizer in food processing.

In the previous paper (13), we reported that rats fed a curdlan diet showed significant increases in the weight of the cecum and its contents, a decrease in fecal wet weight, a retardation in the transit time of the gastrointestinal tract, and morphological changes of the ileal and cecal mucosal surfaces when compared with the rats fed a cellulose diet. It remained to be explained, however, whether these effects of curdlan were permanent or transient.

In this paper, we present results suggesting that the effect of curdlan feeding appeared not to be permanent but unexpectedly durable on the fine surface structure of cecal mucosa.

MATERIALS AND METHODS

Experimental animals. Male 3-week-old Sprague-Dawley rats (Japan SLC, Inc.) were fed laboratory chow (CE2; Clea Japan, Inc.) for 4 d before the experiment. Twenty-four rats were equally divided into three groups as follows: 1) 5% cellulose group ($n=9$); 2) curdlan-followed-by-cellulose group (that is 5% curdlan diet for the first two weeks and 5% cellulose diet for the next two weeks; $n=6$); and 3) 5% curdlan group ($n=9$). The experimental design and their diet compositions are shown in Fig. 1 and Table 1, respectively. The experimental procedure was approved by the ethical committee of Osaka City University. Cellulose was provided by Asahi Kasei Co., Ltd. (PH101), and curdlan was provided by Takeda Inc. (FD13A).

The rats were fed the diets for four weeks ad libitum. They were housed individually in a stainless cage placed in an air-conditioned room maintained at 22–24°C with a 12-h light-dark cycle. Throughout the experimental period, the body weight and food intake were monitored every 2 d. The transit time of food through the gastrointestinal tract was measured 5 d before the end of the feeding period.

Sampling of the tissue specimens. After four weeks on experimental diets, the
Fig. 1. Experimental design. Sprague-Dawley strain, male rats 3 weeks old were prefed for 4 d and divided into three groups, as indicated.

Table 1. Diet composition (g/100 g).

<table>
<thead>
<tr>
<th>Diet groups of</th>
<th>Cellulose</th>
<th>Curdlan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture(^1)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mixture(^2)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Curdlan</td>
<td>—</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\) Mineral mixture (%): CaHPO\(_4\)·2H\(_2\)O 14.56, KH\(_2\)PO\(_4\) 25.72, NaH\(_2\)PO\(_4\) 9.35, NaCl 4.66, Ca-lactate 35.09, Fe-citrate 3.18, MgSO\(_4\) 7.17, ZnCO\(_3\) 0.11, MnSO\(_4\) 0.12, CuSO\(_4\)·5H\(_2\)O 0.03, KI 0.01.

\(^2\) Vitamin mixture: vitamin A acetate 50,000 IU, D\(_3\) 10,000 IU, B\(_1\) hydrochloride 120 mg, B\(_2\) 400 mg, B\(_6\) hydrochloride 80 mg, B\(_12\) 0.05 mg, C 3,000 mg, E 500 mg, K\(_3\) 520 mg, biotin 2 mg, pantothenic acid calcium salt 20 mg, p-aminobenzoic acid 500 mg, nicotinic acid 600 mg, inositol 600 mg, choline chloride 20,000 mg.

animals were anesthetized by intraperitoneal injection of Nembutal (pentobarbital sodium, 5 mg/100 g body weight) and dissected from the abdomen. Tissue samples for scanning electron microscopy were excised from the ileum, cecum, and colon.

Transit time of intestinal contents. Five days before the end of the feeding period, the rats were fasted for 9 h for measurement of the transit time. Each rat was then allowed to eat 2 g of its respective diet containing 3% carmine for 30 min, after which the diets were given ad libitum. The transit time was calculated as follows;
Transit time (h) = (F + L) / 2
where F and L were the first and last appearance times (h), respectively, of carmine in the feces as detected visually after the ingestion of carmine (t = 0, when the rats were allowed access to the carmine diet).

Scanning electron microscopy. The samples of the intestinal tracts were carefully cut into approximately 1 cm² pieces and rinsed in cold saline. The samples for scanning electron microscopy were taken at 2–3 cm proximal to the cecum (ileum) and to the center of the cecum (cecum), and 2–3 cm distal to the cecum (colon). They were pre-fixed by infiltration with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and post-fixed with 1% osmic acid in the same buffer overnight. After fixation, they were dehydrated with ethanol of graded increasing concentrations, infiltrated with 3-methylbutyl acetate, and dried by a critical point dryer (Hitachi HCP-2) with liquid carbon dioxide. The samples were mounted on aluminum stubs with electroconductive silver paste (Dotite) and coated with gold or platinum-palladium with an ion-sputter (Hitachi E-101).

The morphological structure of the surface of intestinal mucosa was observed under a scanning electron microscope (Hitachi S-800) at the accelerating voltage of 20 kV.

Protein and DNA in the cecal mucosa. After a sampling of the scanning electron microscopy, the cecal mucosa was peeled off with a microscopic slide glass. The isolated mucosa was homogenized in 12 mL of the medium (12 mM histidine monohydrochloride monohydrate, 12 mM imidazole, 2 mM NaEDTA, pH 7.10). The protein was determined by the method of Lowry et al (14), using bovine serum albumin as a standard. DNA was measured by the Burton method using diphenylamine (15). Salmon sperm DNA was used as a standard. Reagents were analytical Reagent grade or Guaranteed Reagent (JIS) grade.

Statistics. All results were performed by one-way analysis of variance (ANOVA) and were subjected as means ± SE. Significant differences between groups were tested by using Duncan’s multiple-range test and were assessed at p < 0.05.

RESULTS

Weight gain and food intake
No significant differences were observed in final body weight and weight gain among the three groups. The amounts of food intake were also similar in all three groups.

Appearances of feces (Fig. 2)
The feces of the curdlan group were soft and long, and their mean wet weight was significantly lower than that of the cellulose group. However, when the curdlan feeding was discontinued and replaced with cellulose feeding, the appearances of the feces were restored to normal in a week, and their final wet weight was
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Fig. 2. Wet weights of the feces. The feeding conditions of rats were described in Fig. 1. Values are expressed as means ± SE. Mean values with different superscript letters express that they are significantly different (Duncan's multiple-range test; p < 0.05).

Fig. 3. Transit time of the intestinal contents. The feeding conditions of rats were described in Fig. 1. Values are expressed as means ± SE. Mean values with different superscript letters express that they are significantly different (Duncan's multiple-range test; p < 0.05).

significantly higher than that of the curdlan group.

Transit time (Fig. 3)

The transit time of intestinal contents through the gastrointestinal tract was significantly longer in rats fed the curdlan diet than in the cellulose group. The transit time of the rats fed the curdlan-followed-by-cellulose diet was shorter than in the curdlan group, whereas it was longer than in the cellulose group.

Cecum and colon and their contents (Table 2)

The cecum of rats fed the curdlan diet was markedly enlarged. The weight of the cecum was significantly higher in rats fed the curdlan diet than in rats in the
Table 2. Wet weights of the cecum and colon and their contents.

<table>
<thead>
<tr>
<th>Diet groups of</th>
<th>Cellulose</th>
<th>Curdlan-Cellulose</th>
<th>Curdlan</th>
</tr>
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<tbody>
<tr>
<td>Cecum</td>
<td>0.62 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cecal contents</td>
<td>3.03 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.13 ± 0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.27 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon</td>
<td>0.89 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon contents</td>
<td>0.86 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE. The feeding conditions of rats were as described in Fig. 1. Mean values with different superscript letters express that they are significantly different (Duncan’s multiple-range test; p < 0.05).

*cellulose* group. The weight of cecal contents was slightly greater than in the *cellulose* group. However, the weight of cecal contents of rats fed a curdlan-followed-by-cellulose diet was not different from that of the *cellulose* group. Furthermore, the water percentage of cecal contents did not differ significantly among the three groups, that is, the *cellulose* (69.9 ± 1.12%), *curdlan*-followed-by-*cellulose* (73.4 ± 0.54%), and *curdlan* groups (77.5 ± 2.25%).

The weights of the colon and colonic contents were significantly lower in the rats fed the curdlan diet than those in the *cellulose* group. However, those of the rats fed the *curdlan*-followed-by-*cellulose* diet showed no differences from the *cellulose* group.

*Morphological changes*

Ileum (Fig. 4): Scanning electron micrographs of the ileum of rats fed the cellulose diet revealed typical leaf-shaped villi of the small intestine, and an individual villus appeared smooth and round in conformation. Their microvilli showed regular arrangements. The villi of rats fed the curdlan diet were thinner compared with those of the *cellulose* group, and the microvilli were more tightly packed, and some were lying down. When curdlan feeding was discontinued, the villi became normal, as in the *cellulose* group. However, the microvilli were more tightly packed, and some were lying down as in the *curdlan* group.

Cecum (Fig. 5): Scanning electron micrographs of the cecum in rats fed the cellulose diet revealed a normal histological arrangement. In rats of the *curdlan* group, the mucosal surface of the cecum was markedly abnormal. The cecal mucosal surface was crowded with microvilli and showed a wavy mucosal surface. The microvilli of the *curdlan* group were tightly packed, and some appeared to have been squeezed out. When curdlan feeding was discontinued, the surface of the cecum was covered with the contents. The microvilli of the *curdlan* group were crowded and tightly packed, and some appeared to have been squeezed out.

Colon: No significant differences were found in the surface structure of...
Fig. 4. Scanning electron micrographs of the ileal mucosal surface. The feeding conditions of rats were described in Fig. 1. From the top, the photographs are the cellulose group (A, B), the curdlan-followed-by-cellulose group (C, D), and the curdlan group (E, F). The left and right bars represent 100 μm and 1.0 μm, respectively.
Fig. 5. Scanning electron micrographs of the cecal mucosal surface. The feeding conditions of rats were described in Fig. 1. From the top, the photographs are the cellulose group (A, B), curdlan-followed-by-cellulose group (C, D), and curdlan group (E, F). The left and right bars represent 100 μm and 1.0 μm, respectively.
colonic mucosa among the three groups.

Protein and DNA in the cecal mucosa (Fig. 6)

The protein and DNA contents in the cecal mucosa were significantly higher in rats fed the curdlan diet than in rats in the cellulose group. However, the protein and DNA contents of rats fed the curdlan-followed-by-cellulose diet were not different from those of rats in the cellulose group.

DISCUSSION

Curdlan, a homopolymer of glucose with β-1,3-glucosidic linkages, is an undigestable polysaccharide and has been used by food manufacturers as a gel-making agent and a stabilizer in food processing. When this polysaccharide was fed to the rats, the cecum was markedly enlarged and weights of the cecum and of the cecal contents were significantly increased. Some dietary fibers were reported (16, 17) to enlarge the cecum and colon of rats, depending on the amount of undigested residues in the cecum and colon. Our results showed that the protein and DNA contents of the cecal mucosa of rats fed the curdlan diet were greater than those of the cellulose diet (Fig. 6), but the protein/DNA ratios were unchanged.
Loeschke and Resch (18) have also suggested that the tissue enlargement by dietary fiber intake may be caused by an increase in cell number. On the other hand, the cecum of the curdlan-followed-by-cellulose group did not show the enlargement, and the weights of the cecum and of the cecal contents were not significantly different from the cellulose group. The protein and DNA contents of the cecal mucosa were also similar to those of the cellulose group. These results indicated that the tissue enlargement by the curdlan feeding was a temporary effect (reversible reaction) and did not persist. When the curdlan feeding was discontinued, the transit time of the contents through the intestinal tract was shorter than that of the curdlan group and longer than that of the cellulose group.

It was concluded that one cause for prolongation of the transit time might be an alteration of the properties of cecal contents, where the curdlan might have been adapted to the longer retention time of intestinal contents in the cecum and rather adhered to the intestinal mucosa. On dissection, the cecal contents of the cellulose group appeared solid, whereas those of the curdlan group were fluid and flowed out of the cecum. In the group in which curdlan feeding was discontinued, the cecal contents were similar to those of the cellulose group, whereas they were soft ingredients in them. However, the water percentages of the cecal contents were middle value between the cellulose and the curdlan group. According to the observation under the scanning electron microscope, the ileal villi of the curdlan-followed-by-cellulose group were normal, as in the cellulose group. However, the ileal and cecal microvilli were similar to those of the curdlan group; their microvilli were crowded and more tightly packed, and some appeared to have been squeezed out.

Undigested soluble polysaccharides form a highly viscous gel in the gastrointestinal tract. Their gel-forming properties are thought to cause damage to the adaptive response (19). Curdlan is an undigestable insoluble polysaccharide and has been used by food manufacturers as a gel-making agent and as a stabilizer in food processing. Curdlan is thought to have some effects on properties of the contents in the intestinal tract.

Ikegami et al (20) reported that the viscous property of a dietary fiber is a major factor affecting the gastrointestinal function. They suggested that an increased bulk and viscosity in the intestinal contents considerably decreases the diffusion processes of substrates and enzymes and hinders their effective interaction at the mucosal surface. From our present study of observed the fine structure of the intestinal mucosa, it is suggested that the properties (viscosity) of the cecal contents caused an effect on the morphological changes of ileal and cecal surface mucosa.

Furthermore, it is considered that one of the factors that affects the environment in the cecum may be fermentation by the bacterial flora and production of the short chain fatty acids. On discontinuation of the curdlan feeding, the properties of the small intestinal contents might return to those of the cellulose group, but
the cecal mucosal surface and transit time might remain altered for the same reason that the fermentable substrates were still remaining in the cecum. We conjecture that the morphological changes and the transit time may return to those of the cellulose group if the cellulose feeding period is further extended.

In conclusion, it is indicated that the effects of the curdlan feeding were temporary, although the effects of the surface structure of intestinal mucosa continued to be present even after curdlan feeding under this condition was discontinued.

REFERENCES


