Effects of Curdlan and Gellan Gum on the Surface Structure of Intestinal Mucosa in Rats

Munehiro TETSUGUCHI, Shouko NOMURA, Masayuki KATAYAMA,1 and Yohko SUGAWA-KATAYAMA*

Department of Food and Nutrition, Faculty of Human Life Science, Osaka City University, Osaka 558, Japan
1 Department of Applied Biological Chemistry, Osaka Prefecture University, Sakai 593, Japan
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Summary The effects of curdlan and gellan gum on the gastrointestinal function were studied, and the morphological structure of the intestinal mucosal surface was observed by scanning electron microscopy of rats fed curdlan and gellan gum diets for four weeks. The rats fed the curdlan diet showed a significant increase in the weight of the cecum and its contents and a decrease in fecal weight as compared to the rats fed a cellulose diet. On the other hand, the rats fed the gellan gum diet showed a weight loss in cecal contents and weight gain in colonic contents. The transit time of the gastrointestinal tract was extended by curdlan supplementation whereas it was shortened by gellan gum supplementation. The surface structures of the ileal and cecal mucosa were markedly abnormal in the rats fed the curdlan diet: the microvilli were tightly packed and had fallen out at places. In the gellan gum-fed rats, the tops of the ileal and cecal microvilli adhered to one another and were covered with their contents. There was no difference in the surface structure of colonic mucosa among the cellulose, curdlan and gellan gum diet groups.

Key Words curdlan, gellan gum, intestinal mucosa, scanning electron microscopy, transit time

Curdlan, a homopolymer of glucose with β-1,3-glucosidic linkages, is a bacterial polysaccharide which forms a gel when its alkaline solution is neutralized or its aqueous suspension is heated (1–4). Gellan gum, a polysaccharide composed of tetrameric repeating units containing glucose, glucuronic acid and rhamnose in molar ratios of 2:1:1, is produced by a microorganism, and also forms a gel in the presence of cations (5,6). Both polysaccharides have been used as gel-making agents and stabilizers in food processing by food manufacturers. In the digestive...
tracts, they may play the role of dietary fiber.

Some dietary fibers have been shown to cause an alteration in the transit time of intestinal contents (7–9), induce morphological changes of gastrointestinal mucosa (10, 11) and exert physiological influence on epithelial cell proliferation (12). Cassidy et al have reported that the surface structure of small intestinal mucosa was damaged in rats fed pectin (13), a soluble dietary fiber. The morphological changes were different depending on the type of dietary fiber, and are likely to contribute to the effect of fiber on the digestive and absorptive functions. We have observed that morphological changes of gastrointestinal mucosa in rats fed pectin at various levels depended upon the amount of pectin in the diet. When pectin feeding was discontinued, the morphological changes were restored to normal (14). Thus, the examination of the effects of dietary fiber intake on the surface structure of the intestinal mucosa is considered to be of interest.

We intended to determine which portion of the digestive tract and what aspect of the intestinal function are affected more by some dietary fibers than by others. In this study, we observed that the morphological structure of intestinal mucosa was altered by curdlan and gellan gum diets in different aspects.

**MATERIALS AND METHODS**

*Experimental animals.* Male 3-week-old Sprague-Dawley rats (Japan SLC) were fed laboratory chow (CE2; Clea Japan) for four days before the experiment. Thirty-nine rats were equally divided into three groups as follows: (1) 5% cellulose diet group, (2) 5% curdlan diet group and (3) 5% gellan gum diet group. Their diet compositions are shown in Table 1. Curdlan and gellan gum were provided by Takeda (FD13A) and Saneigen FFI (16298A), respectively.

The rats were fed the respective diets for four weeks, ad libitum. They were housed individually in stainless cages 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 days.

*Sampling of the tissue specimens.* After four weeks on the experimental diets, the animals were anesthetized with Nembutal (pentobarbital sodium) by intraperitoneal injection (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 at the positions shown in Fig. 1. The weight and pH of the contents in the cecum and colon were measured.

*Transit time of intestinal contents.* The transit time of food through the gastrointestinal tract was measured five days before the end of the feeding period. The rats were fasted for 9 h before the measurement. Then, each rat was allowed to eat 2 g of the respective diet containing 3% carmine for 30 min, after which the respective diets were given ad libitum. The transit time was calculated as follows:

\[
\text{Transit time (h)} = \frac{F + L}{2},
\]
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Table 1. Compositions of the diets (%).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cellulose</th>
<th>Curdlan</th>
<th>Gellan gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>63</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Casein</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral mixture¹</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vitamin mixture²</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Curdlan</td>
<td>—</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Gellan gum</td>
<td>—</td>
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<td>5</td>
</tr>
</tbody>
</table>

¹ Mineral mixture (%): CaHPO₄·2H₂O, 14.56; KH₂PO₄, 25.72; NaH₂PO₄, 9.35; NaCl, 4.66; Ca-lactate, 35.09; Fe-citrate, 3.18; MgSO₄, 7.17; ZnCO₃, 0.11; MnSO₄, 0.12; CuSO₄·5H₂O, 0.03; and KI, 0.01.

² Vitamin mixture: vitamin A acetate, 50,000 IU; D₃, 10,000 IU; B₃, hydrochloride, 120 mg; B₂, 400 mg; B₆, hydrochloride, 80 mg; B₁₂, 0.05 mg; C, 3,000 mg; E, 500 mg; K₃, 520 mg; biotin, 2 mg; pantothenic acid calcium salt, 20 mg; p-aminobenzoic acid, 500 mg; nicotinic acid, 600 mg; inositol, 600 mg; and choline chloride, 20,000 mg.

Fig. 1. Positions of tissue sampling for scanning electron microscopy. Samples were taken at 2–3 cm proximal to the cecum (ileum), center of the cecum (cecum) and 2–3 cm distal to the cecum (colon), respectively.

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 given 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), center of the cecum (cecum) and 2–3 cm distal to the cecum (colon), respectively. 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 using a critical point dryer (Hitachi HCP-2) with liquid carbon dioxide. The samples were mounted on aluminium stubs with electro-conductive silver paste (Dotite) and coated with gold or platinum-paradium using an ion sputter (Hitachi E-101).

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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.

Statistics. For statistical analysis, Student's t-test was used to assess the significance of the differences between the control (cellulose) group and curdlan or gellan gum groups. Results are expressed as means ± SD.

RESULTS

Weight gain and food intake

Body weight gains of the rats are shown in Fig. 2. The rats fed the gellan gum diet showed a slightly greater body weight gain than those of the other groups. However, no significant differences were observed in final body weight among the three groups. The amounts of food intake were also similar in all three groups; the means ± SD of food intake in the cellulose, curdlan and gellan gum groups were 15.10 ± 2.38, 15.09 ± 2.74 and 16.33 ± 2.09 g per day, respectively.

Appearances of feces

The feces of the curdlan diet group were soft and long, and their mean wet weight (1.26 ± 0.32 g per day) was significantly lower than that of the cellulose diet group (1.86 ± 0.36 g per day). On the other hand, the fecal lumps of the gellan gum diet group were smaller in size and round in shape, and showed an extraordinary blackish color. The number of fecal lumps was more than that of the cellulose diet group, but their total wet weight (1.83 ± 0.40 g per day) was not significantly different from the control group.

Cecum and its contents (Fig. 3)

The ceca of rats fed the curdlan diet were markedly enlarged. The cecal wet weight and the weight of cecal contents were significantly greater for the rats fed the curdlan diet than those of the cellulose diet group. Furthermore, the mean pH of the cecal contents was lower in the curdlan group (pH 6.37 ± 0.40) than that of

![Fig. 2. Changes in body weight.](J Nutr Sci Vitaminol)
the control group (pH 7.38 ± 0.25). For the rats of the gellan gum group, the wet weight of cecal contents was lower than that of the cellulose group, but the pH values (pH 7.13 ± 0.34) were similar between the two groups.

Water percentage of cecal contents (Fig. 4)
The water percentage of cecal contents was significantly higher for the rats fed the curdlan diet as compared to the cellulose diet group. However, there was no significant difference in the water percentage of cecal contents between the cellulose and gellan gum groups.
Colon and its contents (Fig. 5)

There was no difference in colon weight among the three groups. The wet weight of colonic contents of the rats fed the gellan gum diet was significantly greater than that of the cellulose group.

Transit time (Fig. 6)

The transit time of intestinal contents through the gastrointestinal tract was significantly longer for the rats fed the curdlan diet than that for the cellulose group, whereas it was significantly shorter for the rats fed the gellan gum diet than for the cellulose group.
Fig. 7. Scanning electron micrographs of the ileal mucosal surface. From the top, the photographs are the cellulose group (A, B), curdlan group (C, D) and gellan gum group (E, F). The left and right bars represent 100 μm and 1.0 μm, respectively.
Fig. 8. Scanning electron micrographs of the cecal mucosal surface. From the top, the photographs are the cellulose group (A, B), curdlan group (C, D) and gellan gum group (E, F). The left and right bars represent 100 μm and 1.0 μm, respectively.
Fig. 9. Scanning electron micrographs of the colonic mucosal surface. From the top, the photographs are the cellulose group (A, B), curdlan group (C, D) and gellan gum group (E, F). The left and right bars represent 100 μm and 1.0 μm, respectively.
Morphological changes

Ileum (Fig. 7). Scanning electron micrographs of the ileum of the 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 arrangement. The villi of the rats fed the curdlan diet were thinner compared to those of the control group, and the microvilli were more tightly packed and some were lying down. The villi in the rats fed the gellan gum diet were also thinner than those of the cellulose group, and an exfoliating feature was often observed at the top of the villi. The microvilli of the gellan gum group showed irregularities such as adherence to each other at the top.

Cecum (Fig. 8). Scanning electron micrographs of the cecum from the rats fed the cellulose diet revealed normal histological arrangement. For the 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. The surface of the cecum of the rats fed the gellan gum diet had a doughnut-like appearance. The microvilli of this group showed irregular arrangement, and their tops adhered to each other. The contents were difficult to remove by usual treatment for scanning electron microscopy, and their thin layers remained on the surface.

Colon (Fig. 9). There was no significant difference in the surface structure of colonic mucosa among the three groups.

DISCUSSION

Figure 3 shows that the cecum of the rats fed the curdlan diet was markedly enlarged, and the wet weights of the cecum and its contents were greater than those of the cellulose diet group. Some dietary fibers have been reported (15, 16) to enlarge the cecum and colon of rats, depending upon the amount of undigested residues in the cecum and colon. Tissue enlargement might be caused by an increase in the number and/or size of cells. Our results showed that the protein and DNA of the cecal mucosa of rats fed the curdlan diet were greater than those of the cellulose diet (Fig. 10), but protein/DNA ratios were unchanged (cellulose 113.65 ± 9.36, curdlan 117.17 ± 7.73). Loeschke and Resch (17) also reported that the protein/DNA, protein/RNA and DNA/RNA ratios of rats fed polyethylene glycol remained unchanged. These results suggest that the tissue enlargement by dietary fiber intake may be caused by an increase in cell number.

The lower digestive tract, especially the cecum, was markedly influenced by curdlan addition to the rat diet. Figure 8 shows that the cecal mucosa in the rats fed the curdlan diet was irregular in shape, and their microvilli were packed tightly and some appeared to squeeze out in comparison with those of the rats fed the cellulose diet. One of the causes for these morphological changes may be alteration of the properties of cecal contents. The water percentage of cecal contents was
significantly higher for the rats fed the curdlan diet as compared to the cellulose diet group. When observed at the time of dissection, the cecal contents of the control group appeared solid, whereas those of the curdlan diet group were fluid and flowed out of the cecum on dissection. The pH of cecal contents for the rats fed the curdlan diet was significantly lower than that of the cellulose group. These results suggest that curdlan may have been fermented by the bacterial flora to produce short chain fatty acids. The increase in the number of epithelial cells may supposedly be caused by short chain fatty acids produced in the cecum, resulting in the morphological change of the mucosal surface structure.

The transit time of intestinal contents through the gastrointestinal tract of the rats fed the curdlan diet was significantly longer than that of the control rats. The cecum of the rats fed the curdlan diet might have been adapted to a longer retention time of intestinal contents in the cecum. Moreover, carmine (marker), which was used for the measurement of transit time, may mix with the contents, and adhere to the intestinal surface mucosa. It is possible that this adhesion was stronger in the curdlan-fed rats than in the control rats and transit to the colon was slower. This may explain the finding that the fecal weight of the curdlan diet group was significantly lower than that of the cellulose diet group.

On the other hand, for the rats fed the gellan gum diet, the wet weight of cecal contents was smaller and the wet weight of colonic contents was greater than those of the cellulose diet group. These results suggest that the intestinal contents of the gellan gum-fed rats did not remain in the cecum for long hours, but were rapidly forced out to the colon in the digestive tract. Thus, the transit time was significantly shorter for the rats fed the gellan gum diet as compared to the cellulose group.

The microvilli of the ileum and cecum of the rats fed the gellan gum diet showed irregular features such as adherence to each other at the top. Moreover, in the gellan gum diet group, thin layers of residues covered the surface of the microvilli (Figs. 7, 8).

In contrast to the rats fed the curdlan diet, the cecal contents of the rats fed...
the gellan gum diet were as solid as those of the cellulose group. The rats fed the gellan gum diet showed a slightly greater water percentage of cecal contents than that of the cellulose group; however no significant difference was observed between the two groups. For the rats fed the gellan gum diet, the pH of cecal contents was similar to that of the cellulose diet group. It is probable that gellan gum was not fermented unlike curdlan, and the intestinal contents of the rats fed gellan gum contained rather higher amounts of undigested polysaccharides. 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 (18) as well as the intestinal mucosal fine structure (Figs. 7–9). Perhaps, when rats were fed the gellan gum diet, their intestinal contents may also have formed a highly viscous gel and given damage to the intestinal mucosal surface structure, although we did not measure the viscosity of intestinal contents. It is believed that this viscosity may be one of the factors which affects the properties of cecal contents. But the transit time for the rats fed the gellan gum diet was significantly shorter than that of the cellulose group. This is because the ceca of the rats fed the gellan gum diet might have been adapted to a shorter retention time of intestinal contents in the cecum, and the force of propulsion to the colon may have been stronger than that of the cellulose diet group. This may explain the finding that the weight of cecal contents was smaller and the weight of colonic contents was greater in the gellan gum group as compared to the cellulose diet group.

In conclusion, curdlan and gellan gum diets affected different aspects of the morphological fine structure of the digestive tract of rats fed those dietary fibers for 4 weeks. These different morphological changes brought about by the dietary fibers may reflect the different manners of effects caused by those fibers on the mucosa of the digestive tract.

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