GASTROINTESTINAL RESPONSES TO GRADED LEVELS OF CELLULOSE FEEDING IN CONVENTIONAL AND GERM-FREE MICE

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Summary Conventional (CV) and germ-free (GF) mice were fed on a semi-synthetic diet containing graded levels of cellulose (0, 5, 15 and 30%), and thereafter the length and wet weight of intestine, the morphology of mid-jejunum epithelium and the turnover of mid-jejunum epithelial cells were determined. The following results were obtained. Enlarged stomachs were observed in CV mice fed on a non-cellulose or 30% cellulose diet, but there were no differences found among the four dietary groups in GF mice. On the other hand, no effect of intestinal bacteria was observed, at least with regard to caecum weight, since the responses of caecum wet weight to the graded cellulose intake in CV mice were similar to those in GF mice.

The responses of intestine length to graded cellulose intake differed between CV and GF mice, indicating that intestinal bacteria may modify the length of intestine in some way.

Marked differences were observed in the responses of villus length to graded levels of cellulose between CV and GF mice. That is, in CV mice there was a gradual increase in villus length as cellulose content increased, whereas in GF mice there was a marked decrease in villus length.

In the CV mice, graded levels of dietary cellulose had no effect on the epithelial cell turnover. On the other hand, in the GF mice it was observed that the greater the amount of dietary cellulose, the faster the turnover rate becomes. As a result, dietary cellulose would enhance the turnover rates of jejunal epithelial cells only in the absence of intestinal bacteria.

Keywords cellulose, intestinal bacteria, alimentary tract, epithelial cell turnover

The level and quality of luminal nutrition have a strong influence on the physiology of the alimentary tract. For example, excess luminal nutrition caused by hyperphagia, overfeeding or intermittent starvation induces hypertrophy of the
intestine (1). Many papers have commented on the morphological responses of the alimentary tract to high bulk feeding. Most reports suggest that there is an increase in length and/or hypertrophy of the intestine in animals fed on bulky diets (2-6). Cellulose, a component of dietary fiber slightly fermentable in the animal gut, has long been known to aid intestinal elimination by providing bulk to the diet. In a dietary cellulose study, Takehisa et al. showed that the sizes of small intestine and caecum were not affected by cellulose intake but that those of stomach and large intestine (colon + rectum) were affected (7). In the above investigations, a problem yet to be solved concerns the role of intestinal microorganisms in alimentary tract responses to various luminal nutritional conditions.

The rate of cell renewal in the intestinal epithelium is a matter of great functional significance to nutrition. There is evidence that the level of luminal nutrition can control the mucosal cell population by way of direct or indirect actions on crypt mitosis (8). In the case of dietary restriction, the epithelial cell turnover of small intestine is reduced in CV mice (9-11). It has been hypothesized that bulky food is also a luminal factor responsible for changes in epithelial cell turnover (12). That is, in the small intestinal epithelium, increased cell loss from the villus tip is brought about when animals were fed large amounts of bulky food. However, definitive results have not been obtained until now.

Thus, the present experiment was performed in order to clarify the above hypothesis. Moreover, in this experiment the role of normal intestinal flora in the gastrointestinal responses to a graded cellulose intake was also investigated. GF mice were used to exclude any effect of changes in intestinal bacterial flora.

METHODS

1. Animals and diets. Male ICR/JCL conventional (CV) and germ-free (GF) mice, initial body weight 33-38 g were used. Mice were 60 days old when experimental diets were first given, and after a 26-28 day feeding period the mice were killed (86-88 days of age). The mice were housed in individual cages with wire-mesh floors, thus eliminating contact with bedding materials. GF mice reproduced in our laboratory were maintained in a Trexler-type flexible film isolator in a standard germ-free state. GF check was undertaken as reported previously (11).

Details of the diets fed are shown in Table 1. Semi-synthetic basal diet (B) served as our fiber-free control. Cellulose powder was added to the basal diet to final concentrations of 5, 15, and 30%. Thus, eight experimental groups (CV-B, CV-5C, CV-15C, CV-30C, GF-B, GF-5C, GF-15C, and GF-30C) were compared. Both CV and GF mice were given sterilized (autoclaved at 121°C, for 30 min) food and drinking water ad libitum throughout the study. After 8-10 day feeding, food intake was recorded for 3 days.

2. Tissue sampling procedures. After feeding for 26-28 days, the mice were killed by cervical dislocation. Immediately after death, stomach, small intestine,
Table 1. Composition of experimental diets.

| Diet Type                        | Basal diet (B) | Cellulose powder |
|----------------------------------|----------------|------------------|
| Basal diet (B)                   | 20.0           | 5.0              |
| Potato starch                    | 68.0           |                  |
| Corn oil                         | 5.0            |                  |
| Salt mix                         | 4.8            |                  |
| Vitamin mix<sup>a</sup>          | 2.0            |                  |
| D,L-Methionine                   | 0.2            |                  |
| 5% cellulose diet (5C)           | 95.0           |                  |
| Basal diet                       | 85.0           |                  |
| Cellulose powder<sup>b</sup>     | 15.0           |                  |
| 15% cellulose diet (15C)         | 70.0           |                  |
| Basal diet                       | 70.0           |                  |
| Cellulose powder                 | 30.0           |                  |
| 30% cellulose diet (30C)         |                |                  |

<sup>a</sup> Extra amounts of vitamins are added to the vitamin mix, considering vitamin decomposition due to autoclaving at 121°C, for 30 min, as previously reported (13).

<sup>b</sup> Avicel® PH-101, mean particle size 40μm (Oriental Yeast Co., Ltd., Tokyo).

and caecum were removed. After measuring the length of intestine, the tissues were blotted dry on filter paper and weighed.

3. Autoradiographic techniques. Microautoradiography of jejunum was undertaken according to the method of Matsuzawa (14). After feeding the experimental diets for 23–25 days, 20μCi of sterile thymidine-methyl-3H (The Radiochemical Centre, Ltd., England; specific activity, 24 Ci/mmol) in 0.2 ml of physiological saline solutions was administered i.p. to each mouse. In the CV mice, 3 mice were killed at each of 3 intervals after 3H-thymidine injection: 12.4–16.0 hr (I), 28.5–31.7 hr (II), and 48.2–52.3 hr (III). In the GF mice, again, 3 mice were sacrificed at each of 3 intervals: 24.3–27.1 hr (I), 60.1–63.3 hr (II), and 95.5–98.8 hr (III) after injection, except the 5C and 15C diet groups at period (I) (2 mice each). One cm segment of mid-jejunum was then removed, and this was immediately fixed in a solution of 10% neutral formalin (pH 7.1). Each gut specimen was embedded in paraffin, and sectioned at 4μm. The following procedures were performed as the authors have previously reported (11). The autoradiographs were developed in KONIDOL-X for 5 min at 20°C, after 4 weeks of exposure, and then stained with hematoxylin and eosin. In the autoradiographs, cells were scored as labeled if three or more silver grains were present over their nuclei.

4. Quantitative evaluation. After the slides were mounted, 10 ideally longitudinally-sectioned villi were selected for quantitation from each animal. Measurements were made of villus height, crypt depth in terms of the number of
epithelial cells, and the position on the villus covered by radioactive grains visualized over nuclei. Details are discussed elsewhere (11). The life span, i.e., the time from birth in cryptal proliferation to death in villus tip extrusion, is equal to the sum of crypt transit time plus villus transit time. This was determined by a migration chase method (9).

5. **Statistical analysis.** Almost all results were expressed as the mean ± SD. Statistical analysis was carried out using Student's t-test. The difference between means was considered significant if $p<0.05$.

**RESULTS**

1. **Body weight gains and food intakes**

   Despite feeding for a period as short as 26-28 days, significant increases of body weight of the adult mice were observed in CV-5C, GF-5C, and in GF-15C diet groups, compared with each basal diet group (Table 2). In both CV and GF states there was a tendency for body weights not to increase as much in non-cellulose (B) or high-cellulose (30C) group. The greater the cellulose content, the larger was the amount of food intake. Energy intake was almost equal among the four groups in both CV and GF mice.

   **Table 2.** Body weight gains and food and energy intakes in the four dietary groups of CV and GF mice.

| No. of mice | Weight gain (%/22 days) | Food intake (g/day) | Energy intake (kcal/day) |
|-------------|--------------------------|---------------------|--------------------------|
| CV-B        | 9                        | +2.7                | 4.3±0.3                  | 17.1                      |
| CV-5C       | 9                        | +6.1*               | 4.6±0.5                  | 17.3                      |
| CV-15C      | 9                        | +3.7                | 4.8±0.7                  | 16.2                      |
| CV-30C      | 9                        | +0.8                | 6.5±0.2                  | 18.1                      |
| GF-B        | 9                        | +0.9                | 3.9±0.4                  | 15.5                      |
| GF-5C       | 8                        | +4.9**              | 4.6±0.4                  | 17.3                      |
| GF-15C      | 8                        | +6.1**              | 4.8±0.5                  | 16.2                      |
| GF-30C      | 9                        | +0.3                | 5.9±0.8                  | 16.4                      |

Results are means and means±SD. *$p<0.02$, **$p<0.01$, compared with group B.

2. **Measurements of the gastrointestinal tract**

   Wet weights of empty stomach and caecum are shown in Fig. 1. Stomach weight was influenced by intestinal bacteria, because significant responses to the graded fiber intake were observed in the presence of intestinal bacteria, but not in the absence thereof. Responses of caecum wet weight to the graded cellulose intake in CV mice were similar to those in GF mice. In both groups the large amount of cellulose intake resulted in increased caecum weight. Notably, the fiber-free basal

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Fig. 1. Wet weights of stomach and caecum in four dietary groups of CV and GF mice. Results are means ± SD. *p < 0.05, **p < 0.001 compared with 0% cellulose group (B).

Fig. 2. Length of small intestine, colon, and caecum in four dietary groups of CV and GF mice. Results are means ± SD. a, p < 0.05; b, p < 0.02; c, p < 0.01; d, p < 0.001 compared with 0% cellulose group (B).

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diet had a unique effect on caecum weight in both CV and GF mice and on stomach weight in CV mice.

The intestine was longer in GF mice than in CV mice (Fig. 2). There was a tendency for the small intestine and colon to the relatively longer in the basal and 30C diet groups in the presence of intestinal bacteria (CV state). These responses were not observed in GF mice. In contrast, the length of caecum was influenced by a graded cellulose intake only in the absence of intestinal bacteria (GF state); large intake of cellulose reduced the length of caecum in GF mice. Responses to a graded cellulose intake were not observed in CV mice.

3. Mucosal morphology of mid-jejunum

Villus length and crypt depth in jejunal epithelium are shown in Fig. 3. No difference was observed in crypt depth irrespective of the cellulose content in both CV and GF mice. Regarding the villus length, in CV mice there was a gradual increase as cellulose content increased. In contrast, in GF mice there was a marked decrease as cellulose content increased.

4. Epithelial cell turnover of mid-jejunum

Migration of labeled epithelial cells after administration of tritiated thymidine is shown in Fig. 4A, B. Figure 4A shows the results of the CV state. This figure suggests that almost no differences exist in the turnover rates among the four groups. Therefore, in the presence of normal bacterial flora (CV state), graded
cellulose intake had no effect on the epithelial cell turnover. On the other hand, in GF mice marked influence of graded cellulose intake was observed (Fig. 4B), i.e., the greater the quantity of dietary cellulose, the faster the turnover rate becomes. This is understandable in the light of the fact that high cellulose intake would enhance the turnover rate of jejunal epithelial cells only in the absence of intestinal bacteria (GF state).

By extrapolation from the above data (Fig. 4), the life span and crypt transit time of epithelial cells were determined (Table 3). Almost no response in crypt transit time, life span, or villus migrating speed to graded cellulose intake were observed in CV mice. On the other hand, in GF mice the life span was gradually
Table 3. Crypt and villus transit time, villus migrating speed, and life span of jejunal epithelial cells in four dietary groups of CV and GF mice.

| Group | Transit time (hr) | Villus migrating speed\(^a\) (cells/hr) | Life span (hr) |
|-------|------------------|----------------------------------------|---------------|
|       | Crypt            | Villus                                 |               |
| CV-B  | 11.9             | 80.6                                   | 1.04          | 92.5          |
| CV-5C | 14.0             | 87.0                                   | 0.98          | 101.0         |
| CV-15C| 13.7             | 77.4                                   | 1.18          | 91.1          |
| CV-30C| 11.2             | 94.5                                   | 0.95          | 105.7         |
| GF-B  | 21.5             | 105.9                                  | 1.08          | 127.4         |
| GF-5C | 18.0             | 98.8                                   | 1.11          | 116.8         |
| GF-15C| 12.7             | 91.8                                   | 1.11          | 104.5         |
| GF-30C| 18.4             | 64.8                                   | 1.44          | 83.2          |

\(^a\) Villus migrating speed = villus length (cell No.)/villus transit time (hr).

shortened as cellulose content increased. In the 30C group, this was 44.2 hr longer than in the B group. Regarding crypt transit time almost no responses were observed, although those of the 15C group appear somewhat anomalous. In GF mice, the villus migrating speed was markedly increased by high-cellulose intake, \textit{i.e.}, this was much faster in the 30C group than the B group.

**DISCUSSION**

It has been reported that the length and weight of stomach increased significantly with a 30% cellulose diet, and the length and weight of large intestine (colon + rectum) also showed a tendency to increase following increasing cellulose content when weanling CV mice were given diets containing 0 to 30% cellulose for 9 weeks (7). Though in the present experiment adult CV mice were fed on graded levels of cellulose diet (0 to 30\%\_\_\_) for about 4 weeks, increases in stomach weight were also observed with the 30\% cellulose diet. However, the length of colon did not respond to the high-cellulose feeding compared with basal diet (0\% cellulose) feeding. This is probably because the feeding period is shorter in the present experiment, and the quality of cellulose powder is also different from that of the previous case; \textit{i.e.}, we used Avicel\textsuperscript{\textregistered} PH-101, as opposed to the previous use of powdered filter paper as cellulose powder. Notwithstanding, it is notable that the gastrointestinal responses to graded cellulose intake were observed even in our short-term feeding. It is thought that more attention must be paid to the unique responses of the intestine to non-cellulose diet feeding.

In the present investigation, the role of intestinal bacteria in the gastrointestinal responses to graded levels of dietary cellulose was also investigated; therefore...
GF mice were used as well as the CV type. The weight and length of certain portions of the gastrointestinal tract were found to be affected by the presence of intestinal bacteria. For example, the effect of graded levels of cellulose intake on the wet weight of stomach in CV mice was clearly different from those in GF mice, *i.e.*, heavier stomachs were observed with non-cellulose or high-cellulose diets in CV mice, but in GF mice there were no differences among the four groups. It must be noted that despite the large intake of food in the GF-30C group, enlarged stomachs were not observed. This fact indicates that the intestinal bacteria have an important role in enlarging the stomach by high-cellulose intake. On the other hand, no effect of intestinal bacteria was observed at least in caecum weight responses, since the responses of caecum wet weight to the graded cellulose intake in CV mice were similar to those in GF mice. These observations cannot be explained in detail from the present data alone.

Brown *et al.* reported that the length and wet weight of the small bowel were significantly greater in rats fed on a high-fiber (pectin) diet for 12 to 15 weeks than in either pellet- of basal-diet fed rats (6). Although feeding in the present study was short-term, responses of intestinal length to graded cellulose intake were observed in both CV and GF mice. These responses differed between CV and GF mice, indicating that intestinal bacteria may modify the changes of the intestine length in some way.

In a high-fiber diet study, Brown *et al.* reported that a significantly greater crypt depth was observed in the mid-jejunum of the pectin-fed rats compared with controls (6). In a non-fiber diet study, Lehnert showed that the number of cells per crypt in elemental diet-fed mice was reduced by approximately 30% compared with chow-fed controls (15). In the present study, however, the responses of crypt depth to graded cellulose intake showed less variation.

When either CV rats are fed on a high-kaolin (bulk) diet (16) or CV mice are fed on a high-pectin diet (6), villus length in mid-jejunum showed a tendency to increase slightly compared with controls. Our results in CV mice are in accord with those findings. That is, significantly longer villi were observed in high-cellulose feeding (15C and 30C groups) compared with basal control (B group). The increase, however, was not linear relative to the increase in cellulose content. On the other hand, in the absence of intestinal bacteria (GF state) gradual decrease in villus length of mid-jejunum was recorded in proportion to the cellulose content. It must be noted that remarkable differences in the responses of villus length were recorded between CV and GF mice. Dietary cellulose evidently acts to shorten the villus length in mid-jejunum, only when there is no effect of intestinal bacterial flora. The intraluminal degradation of cellulose in the CV mice must also be considered, as reported previously, even in mono-gastric animals (17–19).

Until now it has been thought that cellulose or other bulky substances cause rapid cell extrusion from the villus tip (12). Only in a study of non-residue diet (elemental diet), has reduced epithelial cell turnover actually been found compared with chow-fed controls (15). As a result, in the present investigation no change in
epithelial cell turnover was found due to graded cellulose intake in CV mice. Accordingly, it is concluded that the hypothesis that high-bulk feeding such as high-cellulose diet caused increased epithelial cell turnover is not correct, at least with regard to the CV state.

In GF mice, however, increased epithelial cell turnover was observed due to high-cellulose intake. Results of migrating speed support this finding. These facts indicate that dietary cellulose enhances the turnover rate of jejunal epithelial cells. This increased turnover would not depend on the presence of microbial products such as volatile fatty acids or free bile acids in the lumen. Increased epithelial cell turnover was not found in CV mice, perhaps for the following reasons. Firstly, cellulose degradation by intestinal bacteria occurs, and consequently this has a reduced effect on the cell turnover; secondly, there were changes of intestinal flora by graded cellulose feeding. Another important finding in this study was the marked reduction in epithelial cell turnover due to non-cellulose diet in GF mice. Their life span (127.4 hr) was about 44 hr longer than those of the 30C group. Prolonged life span was markedly reduced when cellulose was gradually added to the diet. This data suggests that greater retardation of epithelial cell turnover could be achieved if GF mice were given a non-residue elemental diet.

Further studies concerning intestinal function, such as digestive or absorptive capacity, should also be undertaken when mice are fed graded levels of cellulose in both CV and GF states.

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