EFFECTS OF RESTRICTED DIET AND INTESTINAL FLORA ON THE LIFE SPAN OF SMALL INTESTINE EPITHELIAL CELLS IN MICE

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Summary Previous data have shown that the life span of small intestine epithelial cells in germ-free (GF) mice was 4.3 days, while that in conventional (CV) mice was 2.1 days, under ad libitum feeding. On the other hand, in the author's laboratory, it was also found that feeding conditions affected the cells' life span. That is, in CV mice the life span of the cells lengthened under restricted feeding (2.6 days), compared with under ad libitum feeding (1.8 days).

In the present experiment the life span of small intestine epithelial cells was investigated using radioautography, under controlled feeding (setting it equal to ad libitum feeding) and restricted feeding, in both CV and GF mice. Small intestine samples were taken from the middle part of duodenum, jejunum and ileum. Body weight changes, organ wet weights and intestine were also measured.

In the lower part of the small intestine the effects of a restricted diet on epithelial cell life span prolongation appeared clearly in CV mice, but this effect was reduced in GF mice. This may be partly because the restricted group had slightly shorter villi in the case of GF mice.

Keywords life span, restricted diet, intestinal flora, intestinal epithelial cells

The intestinal epithelium is constantly renewed through cryptal proliferation with extremely short cell cycles, followed by cell migration to the villus and then completing their life span by extrusion from the villus tip.

The life span of intestinal epithelial cells is influenced by a number of factors, and the intestinal milieu is an important controlling factor. It has been reported that the migration of epithelial cells from the crypt to the tip of the villus (i.e., the life span of the cells) takes twice as long in GF mice as in CV mice—4.3 vs. 2.1

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days (1); although cholic acid fed to GF mice decreases the turnover time of intestinal epithelial cells to same rate as CV mice (2, 3). Furthermore, it was found that feeding conditions affected the life span of the cells (4, 5); in CV mice the life span of the cells lengthened under restricted feeding (2.6 days), compared with under ad libitum feeding (1.8 days). However, it is not known whether the effect of restricted feeding on life span prolongation of the cells is connected with the presence of intestinal flora or not.

Therefore, in this experiment, the life span of epithelial cells was investigated under controlled feeding (the same as ad libitum feeding) and restricted feeding in both CV and GF mice, in order to clarify the role of intestinal flora in life span prolongation under restricted feeding.

METHODS

1. Animals and diet. ICR/JCL male mice, aged from 80 to 90 days, weighing about 32 g were used in this experiment. Eighteen CV and 18 GF mice were kept in a single rearing cage, and were equally divided into controlled and restricted (38% restriction) feeding groups, respectively. That is, four experimental groups were set up; CV-control (CV-C), CV-restricted (CV-R), GF-control (GF-C) and GF-restricted (GF-R) feeding groups.

GF mice were maintained in a Trexler-type flexible film isolator under a standard germ-free environment. In order to ascertain that a GF environment was being maintained, bacterial cultures of the isolator, the bedding and the animals’ feces were taken every week.

Both CV and GF mice were given sterilized drinking water (autoclaved at 121°C, for 30 min) ad libitum, and fed a sterilized commercial diet (NIHON CLEA’s CL-2, solid diet for GF animals). If the restricted diet was fed once a day, animals were especially damaged by this short-time feeding (6). Three meals per day of equal amounts are desirable, so mice were fed equal amounts three times per day, at 06:00, 14:00 and 22:00. In the controlled diet, mice were fed a total of 4.8 g daily (1.6 g × 3) which was determined by the author’s preliminary experiment. In the case of the restricted diet, mice were fed a total of 3.0 g daily (1.0 g × 3) (38% restriction).

2. Autoradiography procedure. Microautoradiography of mice small intestinal epithelium was undertaken according to the method of Matsuzawa (7). Twenty μCi of sterile thymidine-methyl-3H (The Radiochemical Center Ltd., England) in 0.2 ml of physiological saline solution was administered i.p. to each mouse. Injections of GF mice were performed in the isolator by admitting sterile thymidine vials through a germicidal trap. Animals were only removed from the isolator immediately before sacrifice. Each mouse was sacrificed an appropriate time after an isotope injection. When the mice were sacrificed, body weight and wet weights of liver, kidney, heart and spleen were measured. The wet weights of the stomach and caecum without contents were also measured. Moreover, the lengths of small intestine, caecum and large intestine were measured.
One cm segments of duodenum, jejunum and ileum were then removed, and these were immediately fixed in a solution of 10% neutral formalin. Each gut specimen was embedded in paraffin, and sectioned at 5 μm. The slides were then deparaffinized and coated with SAKURA NR-M2 nuclear track emulsion with the dipping method. The slides were stored in black boxes and maintained in a dark room at 4°C. After 4 weeks of exposure, the slides were developed and then stained with hematoxylin-eosin. After the slides were mounted, 10 ideally longitudinally-sectioned villi were selected for quantitation from each animal. At 600 × magnification the total number of cells lining one side of the section of each single-file column was counted from crypt bottom to villus tip. Thus villus length and crypt depth were measured. The number of cells behind the leading edge of the labeled epithelium was also counted. Thus, an average villus height and an average labeled height, in terms of epithelial cells in a single-file column, were determined for each animal. The percentage value of total cells of the villi labeled was then calculated. Thereafter, the life span of epithelial cells was determined by the migration chase method. Migrating speed was expressed as the number of migrating epithelial cells per hour.

RESULTS

1. Body weight and organ wet weights

Body weight changes after feeding the experimental diet are shown in Fig. 1. In general, CV mice body weights were lower than GF mice body weights. Each restricted group, both CV and GF, was about 15% lower than each control group in final body weights.

Final body weights and wet weights of liver, kidney, heart and spleen are shown in Table 1. As in previous reports (8, 9), in this experiment liver weights of the GF-C group were significantly lighter (p<0.01) than those of the CV-C group, and spleen weights of the GF-C group were significantly lighter (p<0.01) than those of the CV-C group.

![Fig. 1. Body weight changes in CV and GF mice after being fed a controlled or restricted diet (each point indicates the mean value of 7* or 9 mice).](image-url)
Table 1. Final body weights and organ wet weights.

|                      | Conventional | Germ-free |
|----------------------|--------------|-----------|
|                      | Control      | Restricted| Control | Restricted |
| Number of mice       | 9            | 7         | 9       | 9           |
| Body weight (g)      | 28.9±1.8*    | 24.6±2.0  | 34.4±3.3| 28.9±2.8    |
| Wet weight of organs (mg/g BW.) |             |            |         |             |
| Liver                | 49.3±1.7     | 51.5±6.1  | 39.5±1.9**| 36.1±3.4    |
| Kidney               | 15.0±1.3     | 13.7±5.3  | 13.1±1.0| 13.0±0.8    |
| Heart                | 4.37±0.48    | 4.54±0.33 | 3.54±0.24| 3.99±0.33   |
| Spleen               | 2.54±0.57    | 3.46±1.85 | 1.84±0.30| 1.82±1.12   |

* Mean ± SD
** Significantly lighter (p<0.01) than that of CV-C group.

weights of the GF-C group were also lighter than those of the CV-C group, although not significantly. The effects of restricted diet on the wet weights of these organs were not observed in either CV or GF mice.

2. Measurements of the gastrointestinal tract

The lengths of small intestine, caecum and large intestine, and the wet weights of stomach and caecum per g of body weight are shown in Table 2. As in previous reports GF-C mice had significantly longer caecums (p<0.001) than CV-C mice, and the restricted diet had almost no effect on the length of the intestine in either CV
or GF mice. GF-C mice had significantly smaller stomachs than CV-C mice ($p<0.01$). The restricted diet had no effect on stomach wet weights. No difference was observed in the wet weights of the caecum between CV-C and GF-C groups or between control and restricted groups of both CV and GF mice.

3. The size of small intestine epithelium

The lengths of the villus expressed as the number of epithelial cells are shown in Fig. 2. The GF-C group had significantly longer villi ($p<0.001$) than the CV-C group in the duodenum and jejunum; but not significantly in the ileum. No difference was observed in the width of the villus measured at the mid height of the epithelium in the CV-C and GF-C groups, or between the control and restricted groups of both CV and GF mice.

Crypt depths, expressed as the number of epithelial cells, are shown in Fig. 3. Crypt depths of GF mice were shallower than those of CV mice as in previous data (10). Almost no difference in the size of small intestine epithelium was observed between control and restricted groups of both CV and GF mice, except for a slightly

![Fig. 2. Duodenal, jejunal and ileal villus length expressed as number of epithelial cells (mean ± SD).](image)

![Fig. 3. Duodenal, jejunal and ileal crypt depth expressed as number of epithelial cells (mean ± SD).](image)
longer villus in the GF-C group than in the GF-R group. Besides the above, villus length expressed in μm was also measured, and it was in proportion to that expressed by the number of epithelial cells.

4. The life span of epithelial cells

The results on the life span of the cells are shown in Table 3 with transit times and migrating speeds. In the duodenum the life span of the cells was 78.0 hr in CV-C, 82.8 hr in CV-R, 95.0 hr in GF-C and 98.3 hr in GF-R group. In the GF-C group the life span of the cells was slightly longer than in the CV-C group. The restricted diet had a slight effect on life span prolongation of the cells in both CV and GF mice.

In the jejunum the life span of the cells was 75.6 hr in CV-C, 116.7 hr in CV-R, 109.0 hr in GF-C and 119.2 hr in GF-R mice. In the GF-C group the life span of the cells was about 33 hr longer than in the CV-C group. The restricted diet had a strong effect on the life span prolongation of the cells only in CV mice; in the CV-R group the life span of the cells was about 41 hr longer than in the CV-C group. On the other hand, the restricted diet had a slight effect on life span prolongation of the cells in GF mice.

In the ileum the life span of the cells was 65.2 hr in CV-C, 97.7 hr in CV-R, 94.8 hr in GF-C and 100.1 hr in GF-R mice. In the GF-C group the life span of the cells was about 30 hr longer than in the CV-C group. In the ileum also the restricted diet had a large effect on life span prolongation of the cells only in CV mice; in the CV-R group the life span of the cells was about 33 hr longer than in the CV-C group.

There was tendency for migrating speed to be slower in restricted groups of

|                | Transit time (hr) | Life span (hr) | Migrating speed (cell no./hr) |
|----------------|------------------|----------------|------------------------------|
|                | Crypt | Villus |                   |                             |                             |
| Duodenum       | CV-C   | 12.7 | 65.3 | 78.0 | 1.09 |
|                | CV-R   | 16.1 | 66.7 | 82.8 | 1.13 |
|                | GF-C   | 18.7 | 76.3 | 95.0 | 1.97 |
|                | GF-R   | 21.2 | 77.1 | 98.3 | 1.74 |
| Jejunum        | CV-C   | 14.8 | 60.8 | 75.6 | 1.25 |
|                | CV-R   | 15.8 | 100.9 | 116.7 | 0.77 |
|                | GF-C   | 15.6 | 93.4 | 109.0 | 1.23 |
|                | GF-R   | 16.9 | 102.3 | 119.2 | 1.02 |
| Ileum          | CV-C   | 14.1 | 51.1 | 65.2 | 0.84 |
|                | CV-R   | 13.7 | 84.0 | 97.7 | 0.50 |
|                | GF-C   | 21.0 | 73.8 | 94.8 | 0.70 |
|                | GF-R   | 25.8 | 74.3 | 100.1 | 0.62 |
both CV and GF mice than in control groups. And in the duodenum, GF mice had fast cell migrating speeds because of their longer villi than CV mice.

On the whole, the effect of the restricted diet and intestinal flora on the life span of the cells was more or less similar in the three parts of small intestine. But in the duodenum district differences among the experimental groups were not observed.

**DISCUSSION**

The epithelium of the small intestine is known to be influenced by a wide variety of factors. Until now numerous investigations have been done on the mechanism of cell proliferation in the small intestine epithelium (11, 12, etc.). Experiments on the life span prolongation of small intestine epithelial cells have been done only under *ad libitum* feeding conditions in GF mice (1, 10). This prolongation was also observed when CV mice were given a restricted diet. However, nothing has been known about the role of intestinal flora in life span prolongation under restricted feeding in CV mice. In the present experiment the effects of feeding conditions and intestinal flora on life span were examined.

The difference in body weight change between CV and GF mice was remarkable, although the amount they were fed was equal. The influence of the restricted diet on the wet weights of organs and on the length of intestines was not observed in either CV or GF mice.

The duodenal villus height in each group of CV mice was shorter than that in previous reports (10); this was almost equal to the jejunal villus height in each group of CV mice. The effect of the restricted diet on villus height was much greater in GF mice than in CV mice.

The width of the villi was almost equal among the four groups. Therefore, GF mice had relatively thinner villi in proportion to their lengths.

The effect of the restricted diet and intestinal flora on the life span of epithelial cells was more or less similar in the three parts of the small intestine. However, in the duodenum distinct differences between the CV and GF mice were not observed. This is probably because bacterial conditions are sparse in the duodenum in comparison with the lower part of the intestine.

It has been reported that the mitotic activity in the crypt decreases and the duration of cell cycles is prolonged in restricted animals (13, 14) and GF animals (15, 16). In the present experiment comparable results were obtained on this point. And in the lower part of the small intestine the effect of the restricted diet on life span prolongation appeared clearly in CV mice, but this effect was reduced in GF mice. This may be partly because the restricted GF group had slightly shorter villi. However, further investigation is needed to elucidate this phenomenon.

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