INFLUENCE OF RESTRICTED DIET ON THE CELL CYCLE IN THE CRYPT OF MOUSE SMALL INTESTINE

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Summary Previously we reported that the small intestine of mice adapted to dietary restriction had low mitotic activity in the crypts. In that case there was no marked difference in the villus and crypt cell numbers, but the migrating speed from crypt to villus decreased, consequently the transit time was prolonged. The present study was undertaken in order to clarify whether or not the cell cycle of the proliferative cells in the crypts alters when the mitotic activity decreases. The results obtained in this study indicate that: 1) There are differences in cell cycle and labeling index between the unrestricted animals and restricted animals. 2) The cycle time of the duodenal and jejunal cells, particularly of G1 phase was prolonged under dietary restriction. 3) The prolongation of cycle time was not found in the ileum. It is thought that the proliferative cell number principally controls mitotic activity in the crypts. Still, reduction of the proliferative cell number will be accounted for by the increase of resting cells in G1 phase.

Keywords restricted diet, cell cycle, mouse small intestine, mitotic activity

The components and energy of some foods are factors regulating the renewal of intestinal epithelial cells in mice and rats through oral or intravenous alimentation (1-4). These factors influence one or more of the three elements which regulate cellular renewal: cellularity in the crypts and on the villi, the transit time and the migrating speed from crypts to villi.

In our previous study we found that mice adapted to the dietary restriction showed lower mitotic activity in the crypts of the intestinal epithelium as compared with unrestricted animals. In that case there was no marked difference in the length of villi or crypts, but the migrating speed from crypts to villi decreased, consequently the transit time was prolonged (5, 6).

It is thought that the crypt cell number and the proliferating cell number

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dictate the mitotic activity in the crypt. Further, the cell cycle and number of proliferative cells dictate the mitotic cell number. Thus, the mitotic activity in the crypt may be regulated by three elements: the crypt cell number, the proliferative cell number, and the cell cycle of proliferative cell. It is important to clarify the physiological factors that influence these elements or which element alters most. It is unfortunate, however, that the two elements apart from the crypt cell number were not determined when the mitotic activity was examined.

This study was undertaken to clarify whether or not the cell cycle of the proliferative cells in the crypts alters when the mitotic activity decreases.

MATERIALS AND METHODS

1. Animals and diets. Adult male ddY mice were used in this experiment. The experimental diet consisted of 20% casein, 69% potato starch, 5% soybean oil, 4% salt mixture, 1.8% vitamin mixture and 0.2% choline chloride. All animals were allowed to feed from 10.00 to 16.00 hr. After a two-week training period, the animals were divided into the unrestricted and restricted groups and fed for two weeks. The restricted group received only 60% of the diet taken by the unrestricted group.

2. Cell cycle analysis. The cell cycle was analyzed by the chase method; the labeled mitotic cell index method (7). The animals received a single intraperitoneal injection of 20 µCi/mouse of [methyl-3H]thymidine (specific activity: 20.3 Ci/mmol) in a 0.2-ml volume of 0.9% saline at 10.00 hr on starting to feed. At 0.5–27 hr after the injection, two or three animals were sacrificed by cervical dislocation at one hr intervals for 5 hr, two or three intervals for 27 hr. A one-cm long segment of duodenum, jejunum and ileum was cut open on cardboard and sprayed thoroughly with buffered formalin by syringe, and then fixed for 5–6 hr. The tissues for the microautoradiography were prepared according to the methods given in the previous paper (8).

About 500 mitotic figures were counted per animal. Under 600× magnification, each mitotic figure with three or more grains was considered as a labeled mitosis. The cycle time (Tc) and the durations of the DNA synthetic phase (S), pre-synthetic phase (G1), post-synthetic phase (G2) and mitotic phase (M) were calculated from the fraction of labeled mitoses curve (FLM method) according to the half-height method of Mendelsohn and Takahashi (9). In order to confirm the mitotic activity the initial labeling index at one hr after [3H]thymidine injection was measured and calculated, and the proliferative cell pool size was presumed.

RESULTS

Figure 1 shows the FLM curves for the duodenum from unrestricted and restricted animals. The first labeled mitoses appeared after 0.5 hr in both groups. The ascending limb of both curves were steep in reaching the first peak at 3 hr after
injection. The descending limb of the curve of the unrestricted group was gradual, while the curve of restricted group remained at the maximum value for 7 hr and then declined. The curve of unrestricted group was asymmetric, but that of restricted group was almost symmetric. It is presumed that the cycling cells in the unrestricted animals delay in the latter half of the S phase because of the gently descending curve. Figure 2 shows the FLM curves of unrestricted and restricted groups for the jejunum. The ascending part of the curve in the unrestricted group reached the first peak at 4 hr after injection, it then descended rapidly, parabolically. The ascending part of the curve in the restricted group was steeper than that of the unrestricted group and the first peak was broad. Figure 3 shows
The FLM curves of ileal crypts in the unrestricted (●—●) and restricted (○—○) groups. Each point shows one animal.

**Table 1.** Summary of cell cycle parameters of unrestricted and restricted mice.

| Region  | T_C (hr) | T_S (hr) | T_{G2}+T_M (hr) | T_{G1} (hr) | Initial labeling index (%) |
|---------|----------|----------|-----------------|-------------|--------------------------|
| Duodenum| UR 14.0  | 8.6      | 2.4             | 3.0         | 50.5                     |
|         | R 15.0   | 8.6      | 2.2             | 4.2         | 36.7                     |
| Jejunum | UR 15.2  | 8.0      | 3.2             | 4.0         | 45.6                     |
|         | R 16.6   | 8.4      | 2.8             | 5.4         | 38.5                     |
| Ileum   | UR 17.4  | 8.8      | 3.6             | 5.0         | 44.2                     |
|         | R 17.4   | 9.2      | 3.0             | 5.2         | 28.1                     |

the FLM curves of the unrestricted and restricted groups for the ileum. The first peak was very similar in both groups.

Table 1 shows the summary of cell cycle parameters of the unrestricted and restricted groups for various regions of small intestine. The durations of the G_1 phase of the duodenum and jejunum were prolonged in the restricted group, that of the ileum, however, did not change. As a result, the cycle time of the duodenum and jejunum in the restricted group was longer than that of the unrestricted group. The initial labeling index was also lower in the restricted group than in the unrestricted group.

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DISCUSSION

The results described in the present paper indicate that: 1) There are differences in cell cycle and labeling index between the unrestricted animals and restricted animals. 2) The cycle time was prolonged under dietary restriction and the prolongation was due to that of the G1 phase. 3) Prolongation of cycle time was found in the duodenum and jejunum, but not in the ileum.

Effects of diets on intestinal cell cycle also have been studied. Most of these studies are related to starvation or protein-free diet. Rose et al. reported that the ileal crypt cells in rats fed a protein-free diet showed a progressive increase in length of the TC primarily due to a lengthening of the duration of the S phase. However, they reported that rats starved for ten days did not show a significant increase in TC, although the durations of the S phase and G2 phase increased significantly (10). Their study showed that the duration of the G1 phase was shortened with lengthening of the durations of the S and G2 phases under starvation and protein depletion. Hagemann and Stragand reported the effect of starvation and refeeding on the cell cycle of mouse ileum. The total cycle time was prolonged two-fold by starvation, and the prolongation was due to the increase of the duration of the S and G1 phases. Refeeding led to the shortening of the duration of the G1 and S phases, consequently the TC of the refed animals was shorter than that of the control animals (11). The results of these studies on the influence of undernutrition on the cell cycle in the small intestinal crypt did not consistent.

In our present study, the duration of the G1 phase in duodenal and jejunal crypts was lengthened slightly by dietary restriction. On the other hand, in the ileum, the duration of neither phase nor cycle time were altered. However, the labeling indices of restricted animals decreased over the whole small intestine. This phenomenon suggests that the proliferative cell pool size is reduced rather than that prolongation of the cell cycle occurs. Hagemann and Stragand found that the reduction of the "proliferating compartment" and the prolongation of cycle time occurred simultaneously (11). From these observations we concluded the following: the proliferating cells in all regions of the small intestine decrease and the cell cycle of the duodenal and jejunal proliferating cells prolongs under dietary restriction. This speculation should be clarified by measuring directly the proliferative cell pool size using the cumulative method in which successive labelings with a radioactive precursor is undertaken (12). Strictly speaking, the reduction of the proliferative cell number will be accounted for by the increase of cells resting in G1 phase.

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