CELL POPULATION CHANGES IN THE INTESTINAL MUCOSA OF PROTEIN-DEPLETED OR STARVED RATS

I. Changes in Mitotic Cycle Time

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ABSTRACT

The effect of a protein-free diet and starvation on the duration of the rat ileal crypt cell cycle time was studied by Quastler's technique of labeled mitoses. Rats were fed a protein-free diet for 3, 7, or 11 wk or were starved for 7 or 10 days. Progressive protein depletion resulted in a progressive lengthening of the cycle time (GT), due primarily to a lengthening of the synthetic phase (S) of the cycle. The presynthetic gap (G_1) was the same as the control value after 3 wk and lower, but not significantly so, due to the large variability, after 11 wk. The duration of the postsynthetic gap (G_2) plus mitotic phase (M) was not affected by the diet. As the dietary stress became more severe, the cell cycle also became more variable. Although the GT of rats starved for as long as 10 days was only slightly different from the control, the relative duration of the components of the cycle changed significantly. S and G_2 were longer in the starved animals while G_1 was of shorter duration.

INTRODUCTION

The intestinal epithelium constantly undergoes renewal, cells being produced in the crypts and subsequently moving onto and up the villi. They are eventually lost from the tips of the villi. Many factors such as age (1–3), partial resection of the intestine (4), various hormones (5), intestinal flora (6), and irradiation (7–9) have been shown to affect the rate of renewal.

That the nutritional state of the animal affects intestinal renewal has also been demonstrated. There is generalized atrophy of both the muscular and mucosal layers of the intestine in response to a low-protein, protein-free (10–12), or starvation diet (13). Denuded villi are sometimes seen in protein-deficient pigs, indicating that renewal has not kept up with desquamation (10). A straightening of the nuclear line is seen in crypts of protein-depleted humans (14). Since crypt cells move out of line when they undergo mitosis, this would suggest a decrease in the amount of division. An increased labeling index in the crypts of the monkey jejunum indicates that protein deprivation may lead to a lengthening of the cell cycle and a slower migration of the cells up the villi (15). 1–6 days of starvation results in a decrease in the mitotic activity, impaired cellular differentiation, and decreased migration rates in the crypts and villi of rats and mice (16–19). An S period of 5–6 hr in the intestinal crypts of newborn chicks increases to 7 hr in chicks starved for 3.5 days (20).

In an attempt to determine the effect of progressive degrees of starvation and protein depletion on intestinal mucosal cell renewal, Hopper et al. (21) found that the crypt cell population de-

clined steadily through 10 days of starvation and 11 wk of protein-free diet. However, the number of cells per crypt synthesizing DNA and dividing remained constant. Since the cell population was falling, this resulted in increased labeling and mitotic indices. When an explanation was sought for the fact that the cell population was falling even though the same number of cells was dividing, two possibilities presented themselves. There could be a change in the crypt cell cycle time and/or a change in the rate of migration of cells from the crypts onto and up the villi.

This study was undertaken to determine the effect of starvation and protein depletion on the duration of the crypt cell cycle and its component parts.

MATERIALS AND METHODS

Male Wistar rats from the Camm Research Institute in Wayne, N. J., were used in these studies. At the beginning of the experiments they were approximately 60 days old and weighed 200–250 g each. Groups of 25 rats were starved for 7 or 10 days or were fed a protein-free diet (PFD) (22) for 3, 7, or 11 wk. Control rats were fed an 18% casein agar gel diet (22) *ad libitum*. All animals were provided with as much water as they desired.

After the animals had been fed the diet for the stated period, they were injected intraperitoneally with tritiated thymidine (thymidine-^{3}H) (70 μCi/100 g of body weight). All injections were made between 8:00 and 9:00 a.m. Initially, the rats were sacrificed at hourly intervals for 12 hr and at 14, 16, and 18 hr postinjection In later experiments animals were not sacrificed between 4 and 7 hours since 100% of the mitotic figures were always labeled at that time.

A small piece of intestine approximately 1 cm from the ileocecal junction was removed, fixed in cold acetic alcohol (1:3), hydrolyzed in 1 N HCl, and stained by the Feulgen procedure. Individual crypts were dissected free and squashes were made (23). Radioautographs were made with Kodak nuclear NTB liquid emulsion. A minimum of 30, and usually more than 50, mitotic figures were scored, as labeled or unlabeled, for each animal. A plot of the per cent labeled mitoses vs. time after injection of thymidine-^{3}H was used to determine the length of the various stages of the cell cycle (24, 25, 26). From such a plot (Fig. 1) the total cycle time (GT) is the time elapsed between the point where 50% of the mitoses are labeled on the first ascending limb of the curve to a point where 50% are labeled on the second ascending limb. The duration of the postsynthetic gap (G_2) plus one-half of the mitosis (1/2 M) is the time elapsed between the time of injection and the time when 50% of the mitotic figures are labeled on the first ascending limb. Using 1 hr as the duration of mitosis (27), the time of G_2 may be calculated. The duration of the synthetic phase (S) is determined from the curve as the time from the point at which 50% of the mitoses are labeled on the first ascending limb to the time at which 50% are labeled on the first descending limb. The presynthetic gap (G_1) may then be calculated by subtracting the durations of S, G_2, and M from that of GT.

RESULTS

From the labeled mitoses curve (Fig. 1) the times in hours of the total cell cycle and its individual phases in the fully fed rats were determined to be: GT, 11.6; G_2 ½ M, 0.8; G_2, 0.3; S, 8.2; G_1, 2.3.

Starvation had little effect on the total cell cycle time (Table I). However, there were significant changes in the lengths of the different phases of the cell cycle. The first ascending limb of the curve after 7 days of starvation rose more gradually shan that of the controls, the effect being even more pronounced following 10 days of starvation (Fig. 1). Although M is believed to last for 1 hr in the controls, an accurate estimate of the effect of starvation or protein depletion on M and G_2 was difficult to obtain. Therefore, the data have been expressed in terms of G_2 plus one-half M. 10 days of starvation resulted in a significant increase in the length of this phase to 1.12 hr. The duration of S also increased in response to starvation, being significantly different after 10 days. After 7 and 10 days starvation, S was 8.5 hr and 10.1 hr, respectively. In contrast to these changes, the duration of G_1 fell to 1.2 hr and 0.4 hr following 7 and 10 days starvation, respectively.

Protein depletion resulted in a significant lengthening of the total cycle time (Fig. 2). After the animals had been fed the diet for only 3 wk, the cycle was significantly increased from 11.6 to 13.5 hr (Table I). The crypt cells of animals which had been deprived of protein for 11 wk showed another significant increase in total cycle time to approximately 16.2 hr. After 3 wk of protein-free diet, the duration of G_1 rose slightly but, as in the starved animals, the duration of the G_1 phase ultimately dropped. However, because of the high variability of G_1, the changes were not significant. The greatest change in the mitotic cycle occurred in the duration of the S phase. After only 3 wk of protein depletion, this phase had increased to 9.9 hr, after 7 wk to 11.7 hr, and after 11 wk to 13.4 hr; all values were significantly different from the control value of 8.2

TABLE I

The Effect of Starvation and Protein-Free Diet on the Duration (in Hours) of the Mitotic Cycle of Crypt Cells

Treatment	GT	G_1	S	$G_2 + \frac{1}{2}M$
Control	11.6 ± 0.86*	2.3 ± 0.76	8.2 ± 0.40	0.80 ± 0.01
Starved				
7 days	10.8 ± 0.38	1.2 ± 0.29	8.5 ± 0.25	0.80 ± 0.01
10 days	11.9 ± 0.50	0.4 ± 0.40‡	10.1 ± 0.25‡	1.12 ± 0.15‡
PFD				
3 wk	13.5 ± 0.59‡	2.5 ± 0.54	9.9 ± 0.22‡	0.70 ± 0.10
7 wk	12.9§	—	11.7‡,§	0.90 ± 0.05
11 wk	16.2 ± 2.15‡	1.7 ± 1.73	13.4 − 1.26‡	0.80 − 0.15

* Results are expressed as mean ± SE of the mean.
‡ Significantly different from the control at the 5% confidence level.
§ The SE could not be computed.

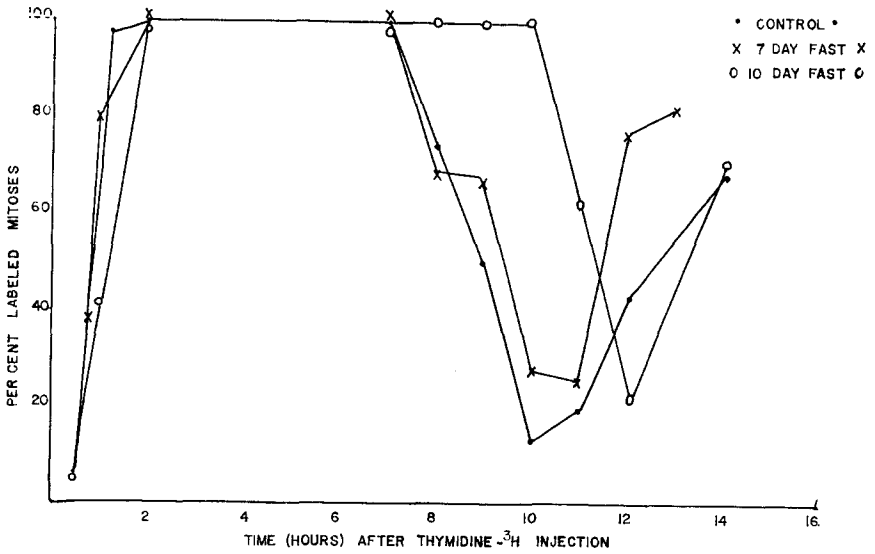

FIGURE 1 The effect of starvation on crypt cell cycle time.

hr. The duration of G_2 plus one-half M was not altered over the course of protein depletion.

DISCUSSION

Loran and Crocker (4) reported that the rat ileum crypt cell cycle was 9.4 hr with an S phase of 8.2 hr. The present determination of the crypt cell cycle time in the rat ileum (control animals) indicated a GT of 11.6 hr. G_1 was 2.3 hr, S was 8.2 hr, and G_2 plus one-half M was 0.8 hr. The discrepancy between the two studies may lie in the determination of G_1 and/or G_2 and could also be the result of strain differences (Sprague-Dawley rats in the earlier work and Wistar rats in this study). Differences in crypt cell cycle times have been observed in mice of two different strains (28).

Most investigators have suggested that the G_1 phase is the most variable component of the cell cycle (2, 27, 29). On the other hand, it has been shown that low doses of chronic irradiation lead to a change in the G_1 and/or S phase of the mouse duodenal crypt cells (8). Additionally, germ-free mice have a longer crypt cell cycle time than conventional mice, and the changes are in S and G_1 (6). Changes in both G_1 and S have also been observed in rats infected with nematodes (30). In the

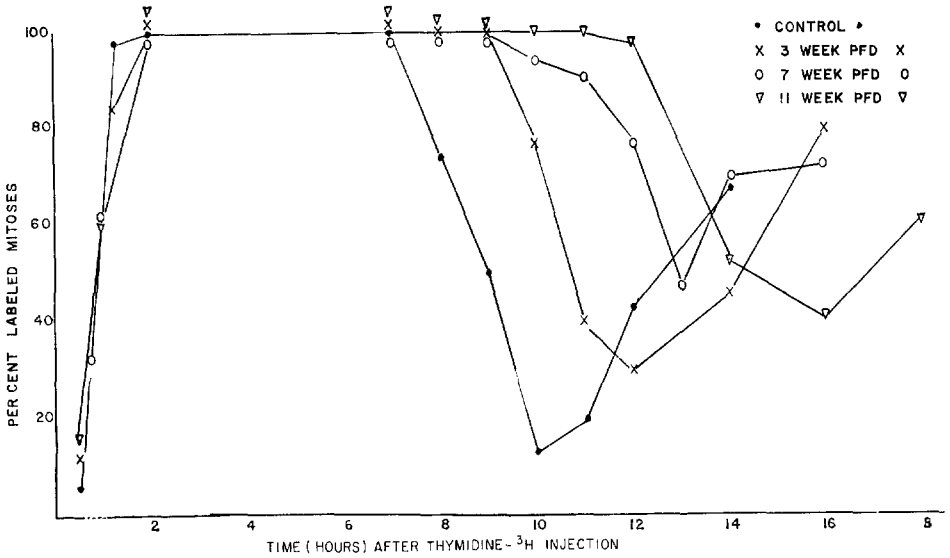

FIGURE 2 The effect of protein-free diet on crypt cell cycle time.

present paper, it has been shown that starvation and PFD will result in significant changes in both the S and G_1 phases of the cell cycle.

As the dietary stress became more severe, the duration of the cycle time became more and more variable among the cells. If all the cells had exactly the same GT, the labeled mitoses plot would remain at 0% labeled for a period (after the isotope injection) equal to G_2 and M, rise abruptly from 0% to 100% labeled, remain at 100% labeled for the duration of S, and then fall abruptly to 0% labeled (31). Individual cell variations modify this ideal curve, causing it to rise and fall more and more gradually as the variability of the different phases is compounded (25). The increased variability observed in these experiments was particularly pronounced in the protein-depleted animals. The curves rose and fell more gradually as the animals were depleted for 3, 7, and 11 wk. In addition, the minimum point on the descending limb was progressively higher as the duration of protein depletion increased.

The causes for the changes in the cycle times and its component periods are a matter of speculation. Some of the processes occurring before a cell can undergo mitosis include the reproduction of centrioles, mobilization of the mitotic apparatus, and duplication of DNA and chromosomes (32). Experiments with human amnion cells (33) have suggested that at least some new protein molecules must be synthesized in G_2 before a cell can enter mitosis. Mobilization of enzymes must also take place before DNA can be synthesized. Since a full complement of DNA must be synthesized before a cell can divide, an increase in the duration of S might indicate a decrease in the rate of synthesis of DNA. This could result if enzyme levels were lowered or if energy requirements could only be met more slowly. Inhibition of oxidative phosphorylation can also prevent a cell from entering into the synthetic or mitotic phases of the cell cycle (34).

The observations in these experiments do indicate that part of the decline in crypt cell population of the protein-depleted animals is related to the increased cell cycle time. The increased labeling index in crypts, noted in the earlier experiment on protein-deprived rats (21) and monkeys (15), also could be the result of the lengthening of the cell cycle time.

This investigation was supported by United States Public Health Service Research Grants No. AM-04341 and GM 835.

Received for publication 15 December 1970, and in revised form 29 January 1971.

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