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

II. Changes in Cellular Migration Rates

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ABSTRACT

The effect of protein-free and starvation diets on the migration of cells from the crypts onto and up the villi of the rat ileum was studied. Rats starved for 3, 7, or 10 days or fed a protein-free diet (PFD) for 3, 7, or 11 wk were injected with thymidine-^{3}H and sacrificed at timed intervals. The time required for the labeled cells to first appear on the villi of experimental animals was longer than in the controls. This was the result of an elongated cycle in the protein-depleted animals and a lengthening of the maturation period in both the starved and protein-depleted animals. Determination of the distance which labeled cells had migrated up the villi in control and experimental animals, after thymidine-^{3}H injection, indicated that cells in animals starved for 7 days migrated more rapidly than those in the fed controls, while those of 10-day starved animals moved more slowly. The cells of animals fed PFD for 3 wk migrated up the villi more rapidly, those of animals depleted for 7 wk migrated at the same time rate, and those of 11-wk PFD animals migrated more slowly than the fed controls. There is apparently no correlation between the cell cycle time in the crypt cells and the rate of migration of cells up the villus.

INTRODUCTION

Renewal of the intestinal epithelium involves two primary processes: the production of new cells in the crypts of Lieberkühn and the movement of these cells from the crypts onto and up the villi. We have previously reported that rats fed a protein-free diet showed a progressive increase in the length of the total cycle time (GT) of the crypt cells primarily due to a lengthening of the synthetic phase (S), and that rats starved for as long as 10 days did not show a significant increase in GT, although S and the premitotic phase (G_2) did increase significantly (1, 2). The present investigation seeks to elucidate in detail the effect of a protein-free or starvation diet on cellular migration in the rat ileum.

The rate of migration of cells up the villi has not been studied in detail although several authors have made some general observations. Bélanger (3) studied rats and saw labeled cells at a midvillus during the second day after the injection of methionine-^{35}S. On the fourth day postinjection, labeled cells were at the villus tip. In the mouse, Walker and Leblond (4) saw labeled cells in the lower third of the villus 24 hr after the injection of thymidine-^{14}C. At 72 hr postinjection these cells were in the upper two-thirds of the villi. By 72 hr postinjection Leblond and Messier (5) saw labeled cells throughout the villi and in the gut lumen. They estimated that complete renewal ofthe intestine of the mouse took place in 3 days, while Bertalanffy

(6) estimated that complete renewal of the rat ileum took 1.4 days.

Dietary stress has been shown to affect the rate of cellular migration along the villi. Brown, Levine, and Lipkin (7) observed a slowing in cell migration up the villi of mice starved for 48 hr. Deo and Ramalingaswami (8) observed that a low-protein diet increased the villus transit time in monkeys. In contrast, Hooper (9) saw increased rates of migration in rats which had been starved for 5 days. She suggested that increased motility of the intestine in starved rats might result in more cells being shed from the villus tip and that this might influence the migration rate.

In this study, migration was examined in two phases: the rate of movement of the cells onto the villi and then their rate of movement up the villi. Since the cell cycle is modified in response to dietary stress (2), the time required for the cells to reach the base of the villi might also be changed. In addition, before the cells can move onto the villi they must differentiate from proliferative cells to ones capable of elaborating digestive enzymes and absorbing nutrients. This period of change or maturation phase (10) begins shortly after the cell divides (11) and is accompanied by active protein synthesis. Since it was believed that starvation or protein depletion might affect this differentiation process, we sought to determine whether those dietary stresses affected the duration of the phase.

Finally, the migration of the epithelial cells up the villi was studied. As we have already indicated, somewhat conflicting reports on the effect of starvation on this movement have been published, and little is known about the effect of protein-free diet. Further, there is some question whether the rate of this movement is determined solely by the pressure of new cells being formed in the crypts or whether other factors are involved. This investigation, therefore, examined the effect of diet on the migration rates, and by inference, sought to determine whether this migration is at all independent of the rates of cellular proliferation.

MATERIALS AND METHODS

Male Wistar rats which were approximately 60 days of age at the onset of the experiment and weighed 200–250 g were used. Control animals and ones which had been fed PFD (12) for 3, 7, or 11 wk, or were starved for 10 days, were studied to determine the effect of dietary stress on the rate of movement of cells from the crypts onto the villi. The animals were injected with thymidine-^{3}H (70 μCi/100 g of body weight) and were sacrificed at 10, 12, 14, 16, and 18 hr after the injection. The pieces of intestine extracted were fixed in acetic alcohol (1:3), hydrolyzed in 1 N HCl, and stained in Feulgen's (1, 2). The tissues were then embedded in paraffin and sectioned at 6 μ. Radioautographs were made with Kodak NTB liquid emulsion. The slides were stored in the dark for approximately 3 wk, and then were developed with Kodak D19 developer and Kodak Acid Fixer. The radioautographic sections were examined for the presence or absence of labeled cells on the villi. More than 50 villi at each time interval were scored. Only villi with a clear crypt-villus junction were used. The per cent of villi containing labeled cells was plotted against time after thymidine-^{3}H injection.

To estimate the duration of the maturation phase the duration of one-half S plus G_2 plus M was substracted from the time required for 50% of the villi to have labeled cells on them (10). The length of the maturation phase was calculated for controls, 10-day starved animals, and 3-, 7-, and 11-wk PFD animals.

To determine the rate of movement of cells up the villi, control animals and ones which had been fed experimental diets were injected with thymidine-^{3}H and were sacrificed 24, 48, 54, and 60 hr later. The extracted pieces of intestine were treated as previously outlined, embedded, and sectioned. Radioautographs were made. In these experiments the distance to which the labeled cells had migrated up the villi was measured. The entire length of the villus was also measured. Again, only villi with a clear crypt-villus junction were counted. Approximately 60 villi were examined in each group at each time period. To avoid differences in villus and labeled cell height resulting from preparative procedures, comparisons among the groups were made on the basis of the per cent of the villus which had been traversed by labeled cells.

Control, 3-, 7-, and 11-wk PFD, and 3-, 7-, and 10-day starved animals were studied. In addition, to determine whether starvation had an immediate effect on migration rates, animals which had not been starved previously were injected with thymidine-^{3}H and were subsequently fasted (control-fast). They were sacrified at 24, 48, 54, and 60 hr postinjection, and their tissues were treated and examined in the same manner as described above.

RESULTS

Early Migration

Both starvation and protein-free diets appeared to slow the rate of movement of cells from the crypts onto the villi (Table I). Although at 10 hr postinjection the animals which had been starved for 10 days had a few more villi with labeled cells

TABLE I

The Effect of Starvation and Protein-Free Diet on the Movement of Cells from the Crypts onto the Villi

Treatment	Time after injection of thymidine-^{3}H				
	10 hr	12 hr	14 hr	16 hr	18 hr
Control	6.67 ± 3.21*	27.77 ± 7.46	85.10 ± 5.79	100.0 ± 0	100.0 ± 0
Starved 10 days	10.66 ± 3.57	19.23 ± 4.46	29.41 ± 6.38‡	46.53 ± 4.96‡	
PFD					
3 wk	0.01 ± 0.13‡	5.26 ± 2.57‡	25.00 ± 8.84‡	59.01 ± 6.30‡	
7 wk	8.53 ± 3.08	8.51 ± 4.07‡	23.07 ± 6.75‡	55.76 ± 6.89‡	74.63 ± 5.32‡
11 wk	5.00 ± 2.81	9.09 ± 3.54‡	18.00 ± 5.43‡	14.85 ± 3.54‡ (38.00 ± 4.83‡)	58.33 ± 7.12‡

* Results expressed as per cent of villi with labeled cells on them ±SE.

‡ Significantly different from the control at the 1% confidence level.

on them than the controls, the difference was not significant. However, at 12 hr and thereafter a smaller per cent of villi of these animals had labeled cells than the controls. The difference became significant at 14 hr. The controls showed 28% of their villi labeled at 14 hr, 85% at 14 hr, and all labeled at 16 hr. 10-day starved animals had only 19% of their villi labeled at 12 hr, 29% at 14 hr, and 47% at 16 hr.

As the period of protein depletion increased, the movement of cells from the crypts onto the villi was progressively slowed. No significant differences were seen among the 7- and 11-wk PFD groups at 10 hr postinjection. The animals sacrificed at that time after being fed a protein-free diet for 3 wk had very few labeled cells on the villi and were significantly different from the controls. By 12 hr and at every time period thereafter, all three protein-depleted groups showed a significantly smaller per cent of villi with labeled cells on them. 3-wk PFD animals had 5% labeled villi at 12 hr, 25% at 14 hr and 59% at 16 hr. The 7-wk PFD animals has 9% labeled villi at 12 hr, 23% at 14 hr, 56% at 16 hr, and 75% at 18 hr. Finally, 11-wk PFD animals had 9% at 12 hr, 18% at 14 hr, 15% at 16 hr, and 58% at 18 hr.

The times at which 50% of the villi were labeled (Table II) were 12.6 hr for the controls, 16.3 hr for the animals starved for 10 days, and 15.5 hr, 15.6 hr, and 17.2 hr for animals fed a protein-free diet for 3, 7, and 11 wk, respectively. Since the maturation period begins shortly after the cell divides and before it enters G_1 of the next cycle (11), these times represent the average time for cells to pass through an S, a G_2, and a maturation period.

TABLE II

The Effect of Starvation and Protein-Free Diet on the Maturation Period of Crypt Cells

Treatment	Time after injection when 50% of villi are labeled	Change from control values	½ S + G_2 +M of crypt cell cycle time	Average maturation period
	hr	*hr*	*hr*	
Control	12.6	—	5.5	7.1
Starved 10 days	16.3	3.7	6.7	9.6
PFD				
3 wk	15.5	2.9	6.2	9.3
7 wk	15.6	3.0	7.4	8.2
11 wk	17.2	4.6	8.1	9.1

The duration of the maturation period was calculated by subtracting the duration of half of S plus G_2 plus M (2) from the time when 50% of the villi had labeled cells on them (Table II). The average duration of the maturation phase was 7.1 hr for the controls, 9.6 hr for the 10-day starved animals, 9.3 hr for the 3-wk PFD group, 8.2 hr for the 7-wk PFD animals and 9.1 hr for the 11-wk PFD animals.

Later Migration

Over the experimental period during which cell migration was followed, the total height of the villi did not change significantly from control values. Through 7 days of starvation, the cells migrated up the villi more rapidly than in the controls (Table II). Labeled cells in the control-

TABLE III

The Effect of Starvation and Protein-Free Diet on Cellular Migration along the Villi (Data Expressed as Distance [%] Traveled up the Villi)

Treatment	Time of sacrifice after injection of thymidine-^{3}H			
	24 hr	48 hr	54 hr	60 hr
Control	30.66 ± 3.00*	53.55 ± 2.30	68.20 ± 1.91	88.43 ± 1.90
Control-fast	17.88 ± 1.17‡	65.82 ± 2.48‡	73.55 ± 1.10‡	99.36 ± 0.31‡
Starved				
3 days	27.07 ± 1.39	86.25 ± 2.65‡	88.79 ± 1.82‡	89.06 ± 1.53
7 days	18.29 ± 1.23‡	61.75 ± 2.05‡	74.23 ± 2.44§	94.52 ± 1.04‡
10 days	13.57 ± 0.77‡	42.85 ± 2.82‡	69.07 ± 1.97	87.86 ± 1.84
PFD				
3 wk	23.26 ± 0.89‡	68.83 ± 1.49‡	73.89 ± 1.20‡	90.50 ± 1.15§
7 wk	28.68 ± 2.41	49.62 ± 2.06	71.09 ± 1.63	74.31 ± 2.02‡
11 wk	22.05 ± 1.06‡	46.27 ± 1.81‡	57.78 ± 1.58‡	70.28 ± 1.54‡

* Results expressed as mean ± SE of the mean.
‡ Significantly different from the control at the 1% confidence level.
§ Significantly different from the control at the 5% confidence level.

fast animals had traveled a significantly smaller distance than the controls at 24 hr, 18% as opposed to 31%. At 48, 54, and 60 hr, however, they had traveled a significantly greater distance. The cells in the controls had traversed 54% of the villus at 48, 58% at 54 hr, and 85% by 60 hr. The cells of the control-fast group had traveled 65%, 74%, and 99% at the respective times.

The cells in the animals which had been starved for 3 days traveled at an even faster pace. At 24 hr they had traveled slightly less than the controls, or 27%, but the difference was not significant. By 48 hr the cells had traversed 86% of the villus height, by 54 hr 89%, and by 60 hr 89%. These distances at 48 and 54 hr were significantly greater than the controls. At 60 hr there was no significant difference.

While the cells in animals starved for 7 days traveled more slowly than those in animals starved for 3 days, in general they traveled more rapidly than the controls. At 24 hr they had traversed only 18% of the villus height, a distance significantly less than the controls. However, at 48 hr they had traversed 62%, at 54 hr 74%, and at 60 hr 95% These values were all significantly greater than the controls.

Examination of migration in the animals which had been starved for 10 days showed different results. Initially, the cells traveled at a slower rate than the controls. By 24 hr they had traversed only 14% of the villus height and by 48 hr they had traversed 43%. Both these values were significantly less than those of the controls. By 54 and 60 hr, however, the cells had traversed a distance that was not significantly different from the controls, 69% and 88%, respectively.

Protein depletion had similar results to those in starved animals (Table III). While animals deprived of protein for 3 wk had cells which traversed a shorter distance than the controls at 24 hr, 23% as opposed to 31%, at 48, 54, and 60 hr the cells had traversed significantly greater distances. The distances traveled at these points were 69%, 74%, and 91%, respectively.

Rates of cell movement in animals which had been fed a protein-free diet for 7 wk were less than those in animals which had been depleted for only 3 wk. They were now around control values: 29% at 24 hr, 50% at 48 hr, 71% at 54 hr, and 74% at 60 hr. The 60 hr value is the only one significantly different from the controls.

The slowing of migration continued and was further accentuated in the animals deprived of protein for 11 wk. The values obtained from this group were all significantly less than the controls: 22% at 24 hr, 46% at 48 hr, 58% at 54 hr, and 70% at 60 hr.

DISCUSSION

Early Migration

For several hours after the injection of thymidine-^{3}H, no labeled cells appear on the villi. Quastler (13) observed that it takes 10 hr for thymidine-^{3}H–labeled cells to reach the villus and that it is 11 hr postinjection before cells are moving

up the villi at full speed (14). Messier and Leblond (15) observed that labeled cells begin to migrate up the villi at 12 hr after injection of the label.

We observed labeled cells on the villi of control animals at 10 hr after the injection of thymidine-^{3}H and the average time required for 50% of the villi to become labeled was 12.6 hr. This appeared to be in good agreement with Quastler (13) and Messier and Leblond (15).

The starvation and protein-free diets slowed the movement of cells from the crypts onto the villi. A variety of factors influence the migration of cells from the crypts onto the villi. As mice age, it takes longer for cells to appear on their villi (16). In animals with partial resection of the ileum, the labeled cells are not retained in the crypts and begin to migrate onto the villi shortly after labeling (17). It was suggested that the cells were still functionally immature as they began migrating in resected animals. X-irradiation also results in precocious maturation of crypt cells (11), and the crypts are quickly depleted.

In calculating the duration of the maturation phase, Cairnie, Lamerton, and Steel (10) observed labeled cells at the foot of villi at about 12.5 hr after isotope injection. Since, in their experiments, half of S plus G_2 plus M was 5 hr, the maturation phase lasted about 7.5 hr. This is in good agreement with our estimate of 7.1 hr for the maturing phase in our control animals.

Our starved and protein-depleted animals had a maturing period which was 2–2.5 hr longer than that of the controls. Protein synthesis and energy-demanding reactions take place in the maturation period (18). If these processes were inhibited or took place more slowly due to shortages of high-energy compounds or materials needed for synthesis, it would follow that this period might be extended.

Later Migration

At 24 hr after the injection of thymidine-^{3}H, labeled cells in control animals had migrated farther than those in any of the experimental groups. Since the movement of cells onto the villi was inhibited in the starved and protein-depleted animals, the short distance which labeled cells had migrated up the villi might reflect this delay at 24 hr.

Once the cells were on the villi, however, differences in migration rates became apparent. The cells in animals which had been starved for up to 7 days had a more rapid migration than the controls while the cells of animals which had been starved for 10 days before the injection of the isotope migrated at a slower rate. Animals which had been fed a protein-free diet for 3 wk showed faster migration rates than the controls. The cells in the 7 wk PFD animals migrated at approximately the same rate as the controls, and those of the 11-wk PFD animals migrated more slowly.

These data have some bearing on the causes for differences in rates of migration of cells up the villi. Some previous studies have reported a positive correlation between cell cycle times in the crypts and migration of cells up the villi, suggesting that migration times may be influenced by the pressure of new cells formed in the crypts. Shortened crypt cell cycle times resulting from partial resection of the ileum (17) and infection with nematodes (19) have been accompanied by more rapid migration rates, while elongated crypt cell cycle times in germfree mice (20), aged mice (21), and mice treated with radioprotectors (22, 23) have been associated with slower migration rates. However, strong evidence against this hypothesis is found in radiation experiments where migration of cells up the villi continues at a normal rate despite a sharp decrease in mitotic activity in the crypts (5, 11, 24). The current studies support the conclusion that the rates of migration of cells up the villi are independent of cell renewal processes in the crypts. Cycle times in the crypt cells of starved and PFD rats have been published in a previous paper (2). Increased cycle times and slower migration rates occurred in 10-day starved and 11-wk PFD rats. However, increased rates of migration of cells up the villi were associated with cycle times both the same as (7 day starved) and longer than (3 wk PFD) controls. 7-wk PFD rats had a longer cycle time, and yet their cells moved up the villi at the same rate as the controls.

Our previous data suggested that the decrease in cell number in the crypts of PFD and starved rats was due in part to the increased cell cycle time, particularly in the PFD groups. This resulted in fewer cells being produced per unit time. The present paper shows that the maturation time of these experimental animals is increased by approximately 3 hr over the control value. The cells destined to move onto the villi thus take longer to leave the crypts. This would tend to increase the crypt cell population. Although there was an increased rate of cell migration up the villi, since the cells from the crypt reach the villi more slowly, one

can only conclude that the changes of villus transit time apparently are not concerned with the changes of crypt cell numbers and that the changes of cycle time are the primary cause of the fall in crypt cell population.

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