POPULATION DYNAMICS OF INTESTINAL EPITHELIA IN THE RAT TWO MONTHS AFTER PARTIAL RESECTION OF THE ILEUM

M. R. LORAN, Ph.D., and T. T. CROCKER, M.D.

From the Gastrointestinal Research Laboratory, Department of Medicine, and the Cancer Research Institute, University of California Medical Center, San Francisco

ABSTRACT

Sprague-Dawley rats that had been subjected 2 months previously to partial resection (10 per cent) of the small intestine and an equal number of control rats were injected with tritiated thymidine and sacrificed at intervals during the subsequent 16 hours. Segments of duodenum, jejunum and ileum were prestained by the Feulgen technique and radioautographed. The proportion of crypt cells bearing labeled nuclei, the percentage of labeled crypt cells in mitosis and the appearance of labeled crypt cells on the villi were determined. Comparison of control and resected rats showed that (a) the proportion of intestinal crypt cells incorporating thymidine was considerably greater and uniformly high throughout the shortened intestine, (b) the life cycle of crypt cells was slightly reduced, and was uniform throughout the shortened intestine, and (c) the time during which cells were retained in crypts was markedly reduced. On the basis of persistent, generalized increase in the production of crypt cells, and on prior evidence that the epithelial cells of shortened intestine continue to have a brief life span and evidence of metabolic immaturity, the existence of a humoral factor, tentatively called "intestinal epithelial growth hormone," is postulated.

As reported previously (1), resection of 10 per cent of the small intestine (removal of 10 cm of ileum) in rats was followed by a pronounced increase in the rate of migration of the epithelial cells covering the villi in the remaining portion of small intestine. Two months after resection, the distance traveled by mucosal cells examined 2 and 24 hours after labeling with tritiated thymidine was increased by approximately 23 per cent in the duodenum, 114 per cent in the jejunum, and 141 per cent in the ileum. The "life span" of epithelial cells in the intestinal mucosa, as estimated from the rate of migration and the height of villi, was decreased to 82, 46, and 42 per cent of normal for duodenum, jejunum, and ileum, respectively.

The present study demonstrates that the rate of appearance of new cells on the intestinal villi is accelerated in proportion to the increased rate of cell migration. Proliferation in intestinal crypts is also increased throughout the small intestine, but this increase is not proportional to the increase in the rate of migration. It is proposed that a growth factor, provisionally called intestinal epithelial growth hormone (IEGH), is released in response to intestinal resection and that this factor accelerates the rate of epithelial proliferation throughout the small bowel.

MATERIALS AND METHODS

In a first experiment, the experimental animals consisted of 16 male Sprague-Dawley rats, weighing 300 to 350 gm. A 10 cm portion of the lower ileum, representing 10 per cent of the length of the small intestine, was removed from each at a point about 20 cm

cephalad of the ileocecal valve, and an end-to-end entero-enterostomy was performed. Animals operated upon in this way will be referred to hereafter as "resected" animals, and the intestine remaining after resection will be termed "shortened" intestine.

Two months after resection, each of these animals was given an intraperitoneal injection of 1.5 μc of. tritiated thymidine (specific activity, 1.9 c/mM) per gm of body weight. At that time an equal number of non-operated (control) animals of the same weight and birth date were given similar injections. One animal each from the resected and control groups was sacrificed at ½ hour, at hourly intervals from 1 to 14 hours, and at 16 hours. In a second experiment two animals each from a group of 10 resected and a group of 10 control animals were sacrificed 8, 9, 10, 11, and 12 hours after labeling.

Sections of ileum, jejunum, and duodenum approximately 1 cm long were fixed for 6 hours in each of three changes of a solution of 70 per cent ethanol, neutral formalin, and glacial acetic acid (20:2:1). Three serial sections, 3 μ thick, were cut transverse to the axis of each segment of intestine at two levels. The two sets of sections were fixed to slides with gelatin chromic acid, prestained by the Feulgen technique for radioautography by the stripping-film method (2) and developed after 10 days' exposure. This method yielded labeled nuclei over which there was a minimum of 15 grains and non-labeled nuclei over which there was a maximum of 2 grains.

Proportion of Labeled Crypt Cell Nuclei

The percentage of labeled crypt cell nuclei was determined by counting the number of labeled and unlabeled nuclei in each of 10 to 23 crypts of intestine from resected and control animals sacrificed at ½, 4, 5, 6, 7, 8, and 9 hours after the injection of tritiated thymidine. Each crypt was identified by its position at the base of an intervillous space, and was usually seen to communicate with that space. The upper limit of the crypt was identified by the slight fold or elevation commonly present where the crypt wall joins the surfaces of the two adjacent villi which it supplies. From 1,500 to 2,000 crypt cell nuclei were counted per segment of ileum, jejunum, and duodenum of each animal sacrificed at ½ hour, and approximately 800 crypt cell nuclei per segment of intestine from animals sacrificed at subsequent hours.

Rate of Appearance of Labeled Cells on Villi

Two sets of three serial sections each of duodenum, jejunum, and ileum from one animal in each group sacrificed ½, 1, 2, 3, 4, 6, 7, and 8 hours after injection of tritiated thymidine were prepared as described and examined. The proportion of villi bearing labeled cells at or above the base was recorded as a function of time to give the estimated rate of movement of

cells from crypt to villus. Since the sections were serial rather than random, the same villus was probably scored more than once. Although data derived in this way arise from a smaller group of villi than if the sections were random, and therefore may be less representative of the entire segment of intestine, such data permit recognition of more of the cells that emerge onto a given villus from the several crypts which supply it. In this way a more complete picture of a given crypt and villus is obtained.

Time of Appearance of Labeled Crypt Cells in Mitosis

The proportions of labeled mitotic figures in the duodenum, jejunum, and ileum of the resected and control animals were recorded for each time of sacrifice after the injection of tritiated thymidine. From 500 to 1,000 mitoses were counted per section. From these data graphs were drawn, representing the appearance and disappearance of labeled mitoses through approximately 1½ cycles of cell division.

RESULTS

Cell Production in Crypts

The mean number of crypt cells ranged from 51 to 83. In general, no difference was found between the number of crypt cells in a given segment of intestine from the resected and control animals nor between the number in any one segment compared with another (Tables I, II, and III). Thus, resection did not result in a change in the size of the crypt population even when the height of the villi was increased.

Within the first ½ hour after the injection of tritiated thymidine the proportion of crypt cells with labeled nuclei in duodenum, jejunum, and ileum was 62, 69, and 65 per cent, respectively, in the resected animals, and 33, 37, and 44 per cent, respectively, in the control animals. The proportion of crypt cells in the phase of deoxyribonucleic acid (DNA) synthesis was higher in the resected animals, indicating that more crypt cells were involved in proliferation in the resected than in the control animals.

In the resected animals the proportion of crypt cell nuclei labeled after the first ½ hour remained unchanged at about 70 per cent during the ensuing time periods studied (Tables I, II, and III). In the control animals the proportions of labeled crypt cell nuclei were significantly lower than the values for the resected animals for 3 hours in the duodenum (Table I), 6 hours in the jejunum

TABLE I

Proportion of Labeled Crypt Cell Nuclei in the Duodenum of Resected and Control Animals at Indicated Times after Injection of Tritiated Thymidine

Hours after labeling	Experimental group	Number of crypts	Number of crypt cells			Number of labeled crypt cells			Per cent labeled crypt cells
			Mean	t	P*	Mean	t	P*	
½	Resected	23	68	0.12	NS	42	4.48	<0.001	62
	Control	23	69			23			33
3	Resected	10	66	0.29	NS	46	3.33	<0.01	69
	Control	10	67			34			52
4	Resected	10	70	0.62	NS	50	0.48	NS	72
	Control	10	73			48			66

* NS indicates no statistically significant difference between the means under comparison.

TABLE II

Proportion of Labeled Crypt Cell Nuclei in the Jejunum of Resected and Control Animals at Indicated Times after Injection of Tritiated Thymidine

Hours after labeling	Experimental group	Number of crypts	Number of crypt cells			Number of labeled crypt cells			Per cent labeled crypt cells
			Mean	t	P*	Mean	t	P*	
½	Resected	22	62	2.3	<0.05	43	3.93	<0.001	69
	Control	22	67			29			37
4	Resected	10	72	0.67	NS	50	5.77	<0.001	69
	Control	10	68			25			36
5	Resected	12	70	0	NS	51	5.68	<0.001	72
	Control	12	70			26			38
6	Resected	12	81	2.5	<0.05	54	4.94	<0.001	66
	Control	10	64			33			52
7	Resected	11	72	0.89	NS	46	1.53	NS	68
	Control	11	79			55			70
8	Resected	10	75	1.14	NS	51	1.27	NS	68
	Control	10	83			57			69
9	Resected	10	64	1.21	NS	48	1.2	NS	67
	Control	10	71			42			66

* NS indicates no statistically significant difference between the means under comparison.

(Table II), and 4 hours in the ileum (Table III). After reaching about 70 per cent, the proportion of labeled crypt cell nuclei in either group did not rise further. A rising proportion of labeled crypt cell nuclei in normal mice was also observed by Fry, Lesher, and Kohn (3). This rise is caused by mitotic divisions of cells labeled during the original pulse of label, followed by retention within the crypt of the resultant daughter cells. An upper limit of 70 per cent of labeled crypt cells was also

TABLE III

Proportion of Labeled Crypt Cell Nuclei in the Ileum of Resected and Control Animals at Indicated Times after Injection of Tritiated Thymidine

Hours after labeling	Experimental group	Number of crypts	Number of crypt cells			Number of labeled crypt cells			Per cent labeled crypt cells
			Mean	t	P*	Mean	t	P*	
½	Resected	22	80	0.12	NS	52	3.36	<0.01	65
	Control	22	79			35			44
4	Resected	10	65	0.54	NS	44	3.26	<0.01	68
	Control	10	69			25			36
5	Resected	10	51	1.81	NS	35	1.81	NS	68
	Control	10	61			36			58
6	Resected	10	73	0.74	NS	52	1.05	NS	72
	Control	10	69			46			67

* NS indicates no statistically significant difference between the means under comparison.

TABLE IV

Crypt Transit Time: The Time of Appearance of Labeled Cells on Villi after Injection of Tritiated Thymidine

Hours after labeling	Percentage of labeled villi					
	Duodenum		Jejunum		Ileum	
	Control	Resected	Control	Resected	Control	Resected
½	0	12	0	41	0	46
1	0	30	0	79	0	64
2	12	39	0	100	0	82
3	23	54	34		37	100
4	66	80	54		53	
5	85	100			64	
6	100		76		73	
7			95		81	
8			100		100	
Slope (regression coefficient)	23.8± 2.77	18.5± 0.73	14.76± 3.38	60.0± 18.78	11.65± 0.77	20.8± 1.41
t	1.83		2.65		5.72	
P*	NS		<0.05		<0.001	

* NS indicates no statistically significant difference between the means under comparison.

reported by Fry et al. (3), and was correlated with the observation that the remaining 30 per cent of crypt cells are not proliferative.

These data demonstrate that in the control animals some progeny of the initially labeled cells remained in the crypt for as long as 3 to 7 hours. A similar retention did not occur in the resected animals. Had new cells been retained in the crypts of the intestine of the resected animals, the proportion of labeled nuclei would also have risen with time. Since the maximum proportion of crypt cells was labeled initially and the proportion did not rise, even though labeled cells were dividing, the newly formed daughter cells must have left the crypts as rapidly as they were produced. This phenomenon was not more pronounced in
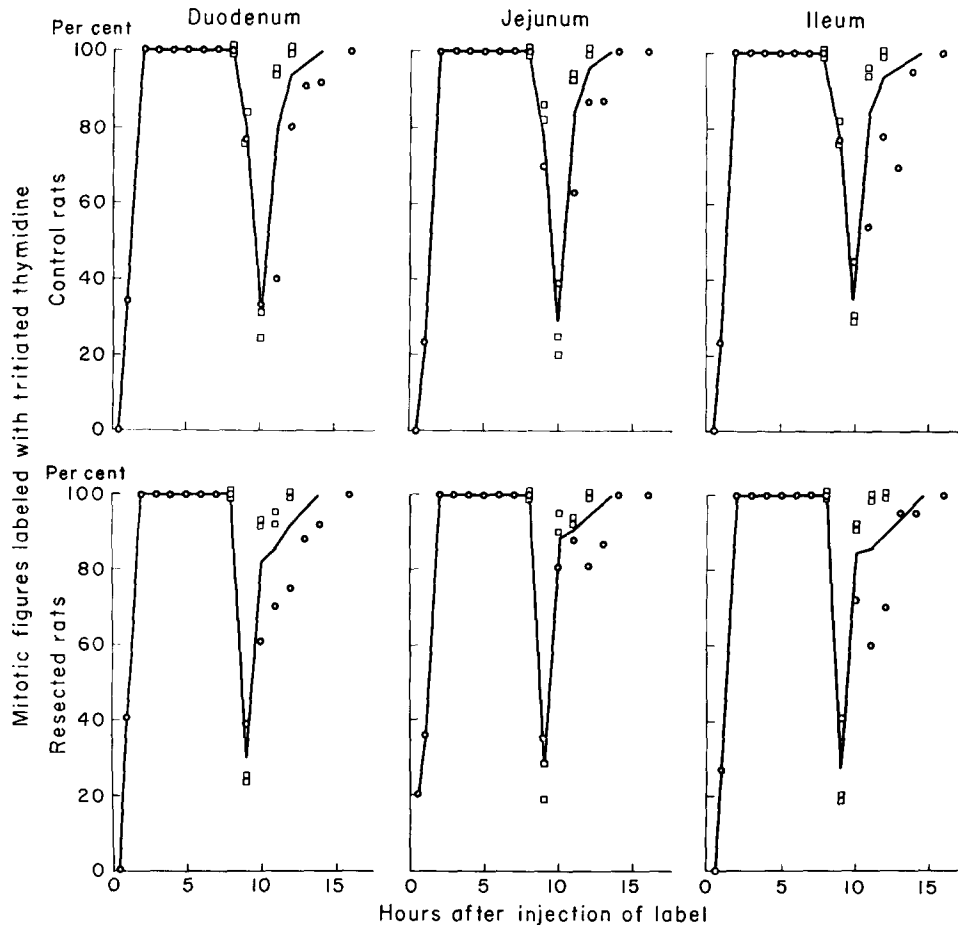
Figure 1 Percentage of labeled mitotic figures in intestinal crypts at times after injection of tritiated thymidine are shown for resected and control rats from two experiments. Circles (experiment 1) and squares (experiment 2) represent single animals.

the ileum and less pronounced in the duodenum, as would have been expected from total transit times (1), but occurred at all levels of the shortened small intestine.

Crypt Transit Time: The Time of Appearance of Labeled Cells on Villi

In the control rats no labeled cells appeared on or above the bases of villi before 2 hours (duodenum) or 3 hours (jejunum and ileum) after the injection of tritiated thymidine. Thereafter, the proportion of villi bearing labeled cells advanced at the rates described by the slopes of Table IV. As shown, the time of emergence of labeled cells onto the villi was earliest in the duodenum and latest in the ileum; also, the rate at

which 100 per cent of villi acquired labeled cells was highest in the duodenum and lowest in the ileum.

In the resected rats, some villi in all segments of intestine bore labeled cells as early as ½ hour after administration of tritiated thymidine; in jejunum and ileum 100 per cent of villi bore labeled cells by 2 or 3 hours (Table IV). The rates of appearance of labeled cells on villi of the jejunum and ileum were significantly greater in the resected animals than in the controls; in the duodenum, the rate was less altered by resection. Although the appearance of labeled cells on the duodenal villi was earlier in the resected than in the control animals (½ hour compared with 2 hours), the subsequent rate of acquisition of

labeled cells was the same in both groups (Table IV).

These data on normal rat intestine may be compared with the extensive data of Lesher *et al.* on mouse duodenum (4, 5), jejunum (6) and ileum (3), and on mouse small intestine in general (7). These authors found that the time at which labeled cells first appeared on the bases of villi and the time elapsing before 50 per cent of villi bore labeled cells was shortest in the duodenum and increased toward the ileum. The similarity of the data on their normal mice and our control rats serves to identify a normal pattern of cell movement from crypt to villus.

Resection resulted in an increase in the rate of appearance of labeled cells on villi throughout the remaining portion of small intestine. This effect was statistically significant in the jejunum and ileum, but not in the duodenum. Resection also resulted in accelerated migration, a decrease in total transit time, and shorter residence of cells on villi (1). These effects were less marked in the duodenum than in the jejunum and ileum. Cell movement onto villi, as well as up villi, was therefore accelerated most in segments nearest the site of resection.

DNA Synthesis and Generation Time of Crypt Cells

Curves describing the appearance and disappearance of labeled mitoses in the crypt cells of the control and resected animals are presented in Fig. 1. Data from 26 resected and 26 control animals are pooled from experiment 1 (circles) and 2 (squares). Each point represents a single animal. The duration of DNA synthesis was arbitrarily taken as beginning at the time 60 per cent of mitotic figures were labeled and ending when the percentage of labeled figures fell below this point. The estimated duration of DNA synthesis in the crypt cells of normal rats was 8.2, 7.7, and 7.8 hours in duodenum, jejunum, and ileum, respectively, which is close to the estimated durations in normal mice (7). In resected rats the duration of DNA synthesis was 7.2, 7.3, and 7.3 hours, respectively. Generation time was estimated by measuring the time between the 60 per cent point on the rising limb of the first curve describing the appearance of labeled mitoses and the same point on the rising limb of the second curve describing the reappearance of these labeled cells in a second cycle of division. The estimated generation

time of crypt cells in control intestine was 9.4, 9.0, and 8.9 hours in duodenum, jejunum, and ileum, respectively, and in shortened intestine it was 8.2, 8.3, and 8.3 hours, respectively. In the resected animals both the duration of DNA synthesis and the generation time of crypt cells became shorter than those in the control animals. In contrast to the slight variation in these values from duodenum toward ileum in control intestine, the duration of DNA synthesis and the generation time were identical at all levels in the shortened intestine.

DISCUSSION

The effects of partial resection of the small intestine in the rat have been described previously (1). Two months after resection, the rate of migration of cells up villi of the shortened intestine was increased and the increase was found to be greatest in segments nearest the site of resection. The life span of an epithelial cell on the villus in the duodenum, jejunum, and ileum, respectively, of resected rats was 82, 46, and 42 per cent of the control values. Our data confirm the findings of the previous study (1).

The increased rate of migration of intestinal crypt cells onto villi in the present study was of the magnitude that would be expected from the previously reported reduction in the life span of epithelial cells in the jejunum and ileum of resected rats (1). In the duodenum of resected rats, the epithelial cells emerge from the crypts and move up the villi at nearly normal rates and have a nearly normal life span. The variation in rates of movement of cells up intestinal villi is not balanced by comparable variation in proliferation of intestinal crypt cells. Instead, 2 months after resection of a portion of ileum, proliferation was increased to the same degree at all levels of the small intestine. The generalized stimulus to proliferation was demonstrated by (*a*) the uniformly high proportion of nuclei labeled at ½ hour in crypts throughout the shortened intestine, and (*b*) by the reduced and uniform duration of the life cycle of a labeled cohort of cells in crypts in all segments of the shortened intestine.

In normal rats, about 35 per cent of crypt cells were labeled at ½ hour after injection of tritiated thymidine. The maximum proportion (70 per cent) of labeled cells was reached 4 to 7 hours later, demonstrating that most of the progeny formed by divisions of the cells that had been

labeled immediately had remained in the crypt for a 4 to 7 hour period before moving onto the villus. In the resected rats, the maximum 70 per cent of crypt cells was labeled immediately, indicating that no retention was occurring. The lack of a period of retention in crypts was equally evident in duodenum, jejunum, and ileum.

The association of three phenomena, rapid cell proliferation, functional immaturity, and anaerobiosis, has been identified in other systems, and is frequently found in fetal and neonatal life. Anaerobiosis has been demonstrated in two studies on the absorptive function of intestine 2 months after resection in the rat (8, 9). The results showed that whereas absorptive competence for vitamin A was not reduced, anaerobic glycolytic phosphorylation associated with vitamin A absorption was no longer rate limiting in the intestine of resected rats. In addition, study of the kinetics of oxygen utilization indicated an imbalance in adenosine triphosphate phosphorylation and dephosphorylation, resulting in a decrease in oxygen consumption and a more anaerobic pattern of metabolism. The association of rapid cell proliferation and anaerobiosis in the intestine of the resected rat strongly suggests that the epithelial cells of the shortened intestine are functionally immature.

The demonstration of morphologically undifferentiated cells on villi following resection would add further support to this conclusion, just as in an analogous situation the demonstration of circulating reticulocytes (immature erythrocytes) serves to prove that erythropoiesis is accelerated.

The increase in the proliferative rate, which occurred equally in all segments of small intestine following resection, persisted long after the total weight of the intestine and its capacity to absorb vitamin A had returned to normal (8, 10). An explanation is offered for the persistence of this effect. A humoral factor, provisionally called "intestinal epithelial growth hormone," may stimulate the crypts of the entire epithelium of the small intestine in response to the initial surgical loss of intestinal tissue. Epithelial cells produced in response to resection are formed at a faster rate and are metabolically deficient (8, 9). A self-propagating state results, in which metabolically deficient cells further stimulate the release of "intestinal epithelial growth hormone," producing persistent, increased, generalized proliferation of intestinal epithelium.

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