Cellular Proliferation of Intestinal Epithelia in the Rat Two Months after Partial Resection of the Ileum*

By M. R. LORAN, Ph.D., and T. L. ALTHAUSEN, M.D.

(*From the Gastrointestinal Research Laboratory of the Department of Medicine, University of California School of Medicine, San Francisco*)

PLATES 327 TO 330

(Received for publication, November 4, 1959)

ABSTRACT

Sprague-Dawley rats subjected 2 months previously to partial resection (10 per cent) of the small intestine and their controls were injected with tritiated thymidine and sacrificed at 2 and 23 hours. Segments of the duodenum, jejunum, and ileum were autoradiographed, and the migration of the labelled cells during the period between 2 and 23 hours was measured with an eyepiece micrometer. The cells had migrated 35, 42, and 34 per cent of the total distance from the crypts to the tips of the villi in the control segments of duodenum, ileum, and jejunum respectively, and 43, 90, and 82 per cent, respectively, in similar segments from resected animals. The rate of migration in the portion of the intestine remaining after resection was approximately three times the normal rate in the ileum, twice the normal rate in the jejunum, and showed an increase of one-third in the duodenum. These results demonstrate that the rate of cell renewal is considerably greater in the remaining portion of the intestine of resected animals than in normal intestine. The increased rate of migration after resection, together with the increase in the height of the villi, resulted in an increase in the rate of cell renewal amounting to 141 per cent in the ileum, 114 per cent in the jejunum, and 23 per cent in the duodenum when compared with control segments.

This study is the fourth in a series of reports on the effects of "partial" (10 per cent) resection of the small intestine. The first three dealt with intestinal absorption, enzymatic activity, hypertrophy, and metabolism in the resected rat (1–3); the present study is concerned with the effects of resection on cellular dynamics. As Leblond (4) has stated: "Many tissues are in a state of flux, their (cell) population size varying chiefly with the rates of reproduction, on the one hand, and with the rates of conversion to non-reproductive units or of destruction, on the other hand. The state of a tissue fluctuates with its physical and chemical environment—changes in space, nutrients, hormones. For limited supplies it may have to compete with other tissues in the same pool. Accordingly, growth, maintenance, adaptive change, regeneration, hyper- and hypoplastic states, and even neoplasia of tissues cannot be properly understood, except in terms of the dynamics of labile (cell) populations in interplay with their environment."

The intestine, because it is characterized by extremely rapid renewal of cells, is particularly adapted to such studies. According to Leblond (4), the epithelium of the small intestine has a higher rate of synthesis and a shorter turnover time than any other tissue in the body; in fact, only a few rapidly proliferating tumors grow faster than intestinal epithelium. In addition, he found that the rate of turnover (estimated in intestinal tissue by the time the cells take to move from the crypt to the tip of the villus) is less than 2 days in the duodenum and 3 days in the ileum; the

* Supported in part by a grant (Physiol. A-910) from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and funds from the Wine Advisory Board, California State Department of Agriculture, and the Springer Fund (No. 162), allocated by the Committee on Research, University of California School of Medicine.

mean time between cell divisions in the crypts is approximately 12 hours.

In a recent report (3), we described studies performed on excised, isolated segments of jejunum and ileum taken from rats 6 weeks after they had been subjected to partial resection of the small intestine. Our observations showed that energy for "active" transport of vitamin A across the intestine (in both jejunum and ileum) was supplied by anaerobic glycolytic phosphorylation instead of by oxidative phosphorylation as in the normal intestine. In other words, anaerobic glycolytic phosphorylation is not rate-limiting in the intestine remaining after resection. In addition, Blecher and White (5) have shown that lymphosarcoma cells, in contrast to thymic lymphocytes, incorporate a significant amount of radioactivity from glycine-2-C^{14} into their proteins and nucleic acids under anaerobic conditions when glucose is added to the incubation medium. Villee and Hagerman (6), in experiments on liver slices from fetal and adult rats, demonstrated a higher rate of glycogen deposition in the fetal liver and a higher rate of activity of one or more glycolytic enzymes, which are rate-limiting in the adult. Fetal liver slices also showed a higher rate of lipogenesis which was evident anaerobically as well as aerobically.

These data led to the following questions: (*a*) What would be the effect of partial surgical removal and subsequent regeneration, with its interplay of hormonal and other regenerative processes, on the cellular dynamics of the intestine? (*b*) Does anaerobic glycolytic phosphorylation, when it is not rate-limiting, indicate or accompany an increased rate of cellular proliferation and a shortening of the life span of the individual cells? To explore these questions, we undertook to study the rate of cell renewal in the mucosa of the small intestine of rats after partial resection of the ileum.

Materials and Methods

The experimental animals consisted of 8 male Sprague-Dawley rats, weighing 300 to 350 gm. A portion of the lower ileum amounting to 10 per cent of the small intestine was removed from each, and an end-to-end enteroenterostomy was performed.

Two months after resection each of these animals and an equal number of controls of the same weight and birth date were given an intraperitoneal injection of 1.5 μc. of tritiated (H^3) thymidine per gm. of body weight.[1] Thymidine was chosen for the label because it is incorporated exclusively into the deoxyribonucleic acid (DNA) of the cell (7). The turnover rate is estimated by measuring the migration of the labelled cells, and incorporation of thymidine is assumed to occur only on behalf of cell renewal and not on behalf of renewal of cellular constituents. All animals were allowed free access to food and water during the experiment.

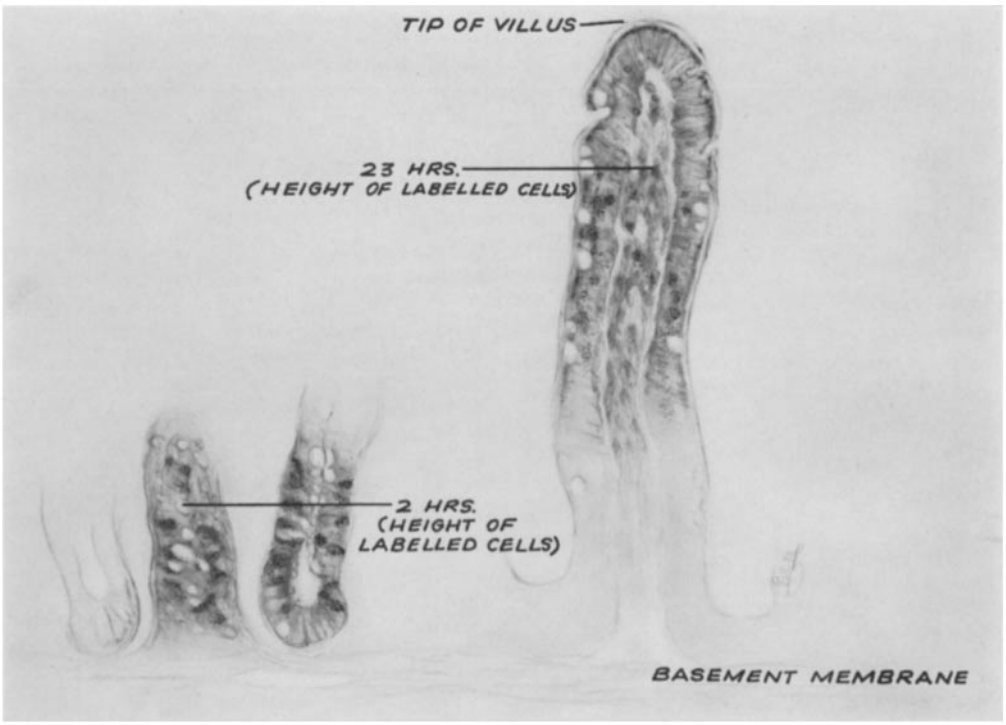
Four animals from the "resected" group and 4 of the controls were sacrificed 2 hours after the injection. Two similar groups of 4 rats each were sacrificed 23 hours after injection. These two time intervals were selected on the basis of results obtained in a preliminary experiment on tissue from normal and resected rats. The 2-hour period was used to define the probable point of demarcation between the villus and the site of cell multiplication which furnishes new cells for the villus. Cells which were synthesizing DNA during this 2-hour period were assumed to have incorporated H^3 thymidine into nuclear DNA in the course of cell regeneration. The 23-hour period was chosen because at this time the labelled cells had migrated well out of the crypts but had not reached the tips of the villi. A longer interval would allow the cells to reach the tips at an undetermined time, making it impossible to calculate the rate of migration accurately. Use of these two time intervals also allowed adequate comparison of the distances traveled by the labelled cells in the intestine of the resected and control animals.

Sections of tissue were removed from the duodenum, jejunum, and ileum, immersed for 1 hour in acetic acid:alcohol (30:70), fixed for 48 hours in buffered formalin (with one rinsing at 24 hours), then imbedded in paraffin and sectioned. Autoradiographs were made with stripping film as described by Pelc (8) and developed after 20 days' exposure. After development the sections were stained with methylene blue–eosin.

Measurement of Height of Villi and Labelled Cells:

Measurements were made on 10 villi in each segment of intestine with an eyepiece micrometer, 20 mm. in diameter having 100 divisions, at 430 magnifications. Only villi which were vertically oriented from the basement membrane of the lowest point in the crypt were selected for this purpose. The distance between the basement membrane and the tips of the villi in the 23-hour specimens and between the basement membrane of the crypt and the labelled cells in the 2-hour specimens were measured (Text-fig. 1). The height of the villi was calculated by subtracting the 2-hour measurements from the 23-hour measurements. Rate of cell migration was estimated by measuring the

[1] H^3 thymidine, having a specific gravity of 1.6 curies per mM, was obtained from the Schwarz Laboratories, Mount Vernon, New York.



TEXT-FIG. 1. Schematic drawing of intestinal villi showing method of determining height of villus and distance traveled by labelled cells.

distance from the basement membrane of the crypt to the leading edge of the labelled cells. Subtraction of the 2-hour measurements from the 23-hour measurements gave the distance the labelled cells had traveled up the villus in a 21-hour period. The rate of cell turnover was obtained from the ratio of the distance the cells migrated in 21 hours to the height of the villi. The Student *t* test was used to assess the significance of the data.

RESULTS

The results of the measurements are listed in Table I. As shown, in the experimental animals the villi along the entire length of the intestine had increased in height. The greatest increase, amounting to 63 per cent, occurred in the ileum, the portion of the intestine nearest the site of resection. The villi of the upper jejunum showed a 36 per cent increase in height and those in the duodenum a 4.9 per cent increase. These figures correlate well with the average increase of 37 per cent for the entire intestine calculated on the basis of weight per cm. of length (1).

Autoradiographs of sections of ileum taken from a normal and a resected animal 2 hours after injection of thymidine are shown in Figs. 1 and 2. At this time the labelled cells in both sections appeared to be in the lower two-thirds of the crypts. The undifferentiated chief cells of the crypts subsequently differentiate into columnar absorptive cells at the bases of the villi. At 23 hours, most of the labelled cells in a control segment of jejunum (Fig. 3) had advanced approximately one-third of the distance up the villi, whereas in similar tissue from a resected animal (Fig. 4) the cells had advanced about nine-tenths of the total distance. In the normal ileum at 23 hours (Fig. 5) the cells had migrated approximately one-fourth of the length of the villi, while in segments of ileum taken from the resected animal (Fig. 6) they had migrated approximately three-fourths of the total distance.

The distances traveled by the labelled cells in 21 hours (23 minus 2 hours) were greater in the experimental than in the control segments of intestine (Table I). The increase in the rate of cell migration was inversely related to the distance of the segment from the site of resection. In the

TABLE I

Increase in Height of Villi and Rate of Cellular Migration and Cellular Proliferation in the Small Intestine of Rats Subjected to Partial Resection of the Terminal Ileum 2 Months Prior to Sacrifice

	No. of villi	Duodenum	*t*	P	Jejunum	*t*	P	Ileum	*t*	P
Height of villi (microns)										
Controls	40	366	2.44	<0.02	258	5.69	<0.001	213	6.19	<0.001
Resected	40	384			351			348		
Increase		4.9%			36.0%			63.0%		
Distance traveled by labelled cells at 21 hours (H^3 thymidine)										
Controls	40	129	4.3	<0.001	108	9.9	<0.001	72	7.8	<0.001
Resected	40	165			315			285		
Increase		27.9%			192.0%			296.0%		
Rate of turnover (distance migrated/height of villus)										
Controls		0.35			0.42			0.34		
Resected		0.43			0.90			0.82		
Increase		23.0%			114.0%			141.0%		

resected rats the rate of migration of the mucosal cells in the remaining intestine was approximately three times the normal rate in the ileum, two times the normal rate in the jejunum, and increased one-third in the duodenum. Calculation of the ratio of the distance traveled by the cells in 21 hours to the height of the villi gave an estimate of the rate of turnover in normal and experimental segments. The figures in Table I show an increase in the rate of cell turnover of 23 per cent in the duodenum, 114 per cent in the jejunum, and 141 per cent in the ileum of the experimental segments of intestine.

In the autoradiograph shown in Fig. 7, a higher magnification of the upper end of the villus seen in Fig. 4, the silver grains on the film are directly above the nuclei of the columnar absorptive cells. One of the principal advantages of using tritiated thymidine for autoradiography is the high resolution resulting from its short-range beta radiation. The maximum range of beta rays is only 6 microns, and half the rays travel less than 1 micron. The net distances of these tracks from origin to terminus are even shorter due to the frequent changes in the direction of travel, so that most of the activated silver grains lie within 1 micron of their source (9).

DISCUSSION

In a previous publication (1) we reported that in rats subjected to partial resection of the small intestine, the remaining portion regained its weight, while all coats increased in thickness. This increase resulted from shortening of the intestine, the net result being that the original weight was distributed in a considerably shorter length of intestine. The results of resection in one group of rats were compared to the results of simple transection and of sham operation in two other groups. Simple transection was not followed by a change in either the length or thickness of the intestine, whereas sham operation resulted in a significant thinning of the intestine and a decrease in weight without any alteration in length. Our interpretation of the data was that the non-specific stress of abdominal operation led to some loss of intestinal tissue. Actual removal of a section of intestine, on the other hand, released a mechanism for restoration of lost tissue which compensated for the deleterious effects of the operation and, in addition, resulted in true hypertrophy of the remaining small intestine. The effects of simple transection were intermediate between those of the sham operation and resection. Transection apparently resulted in a partial release of the tissue-restoring mechanism, since the weight of the intestine was maintained

but no hypertrophy occurred. Thus, the restoration of organ weight and surface area appear to be fundamental end-products of regeneration. Whatever the complex of neural, hormonal, humoral and, in the case of the intestine, structural or mechanical influences involved, the end result is a restoration of lost weight and surface area.

Our present data, based on actual measurements, confirm that the height of the villi is increased throughout the remaining intestine and that this increase is inversely related to the distance of the measured segment from the site of resection. In addition, we found that the rate of turnover of the intestinal epithelium was also greatly intensified, not only in the portion nearest the site of anastomosis in the lower ileum but also in the jejunum at a point approximately two-thirds of the way up the intestine from the site of resection. Even the duodenal epithelium at the opposite end of the intestine showed a significant increase in the rate of cellular proliferation. It is of interest that the increase in the rate of cellular proliferation was considerably greater than the increase in the height of the villi. Our observation that the increase in turnover rate of the mucosa was not restricted to the site of resection but occurred along all segments of the intestine indicates that the entire organ is involved in the regenerative response. This phenomenon may be an example of "physiologic overshoot," which could result from one or more of several factors, such as decreased sensitivity of the cells to growth inhibitors, increased sensitivity to growth stimulators, or disequilibrium between pituitary and adrenal cortical hormones. Whatever the cause of the change, a new equilibrium was established between renewal and death of the epithelial cells, which was on a markedly different level than that in the normal intestine.

In addition, on the basis of our study it appears that anaerobic glycolytic phosphorylation, when it is not rate-limiting, is associated with an augmented rate of cell renewal and migration in the mucosa of the small intestine. Because of the simultaneous occurrence of these phenomena after partial resection, the intestinal mucosa in the postregenerative state appears to have acquired some of the characteristics of neoplastic tissue. However, a third criterion of neoplastic tissue, namely inability to maintain organ equilibrium in terms of weight, is lacking.

From a therapeutic standpoint it can be assumed that tissues dependent on anaerobic mechanisms for energy requirements have a better chance of survival under certain conditions that might be lethal to normal tissue. Conversely, by interfering with anaerobic glycolysis it might be possible to destroy such tissues without at the same time causing serious damage to adjacent normal tissue. In addition, because of its altered metabolism and increased rate of cellular proliferation, this type of tissue might respond differently to drugs than the normal intestine. Comparative studies on the effect of drugs on the two types of tissue might lead to a better understanding of the influence of altered environment on the mechanism of action of drugs. Finally, the small intestine which has been subjected to partial resection may prove a valuable investigative tool for the purpose of following the transition from normal to abnormal growth patterns and metabolic processes.

The authors wish to express their appreciation to Drs. S. R. Pelc and T. T. Crocker for their help in the interpretation of the experiments. The technical assistance of Mr. W. I. Goldstein, Miss Julie Micou, and Mr. Thomas McClure is acknowledged.

References

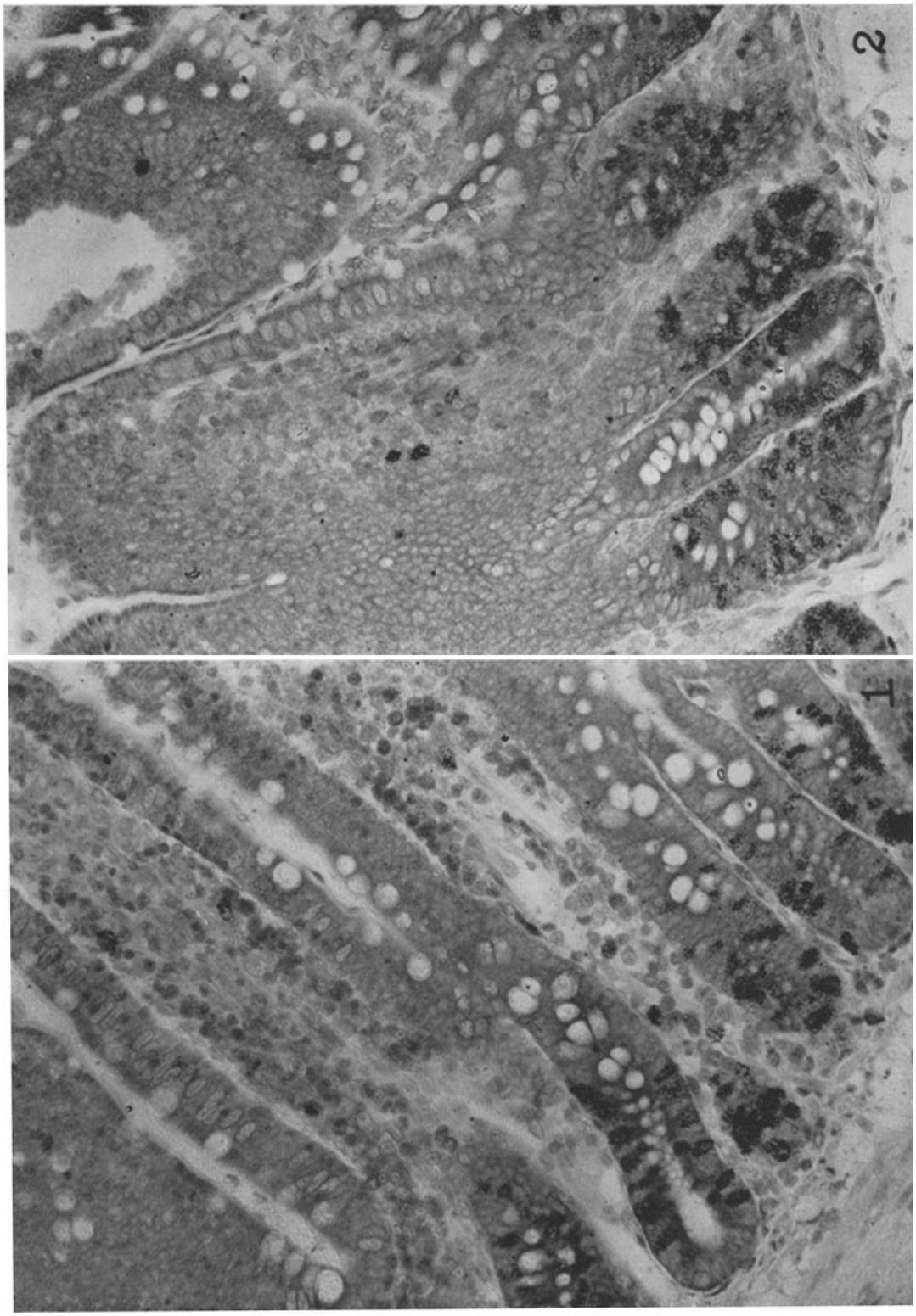
1. Loran, M. R., and Althausen, T. L., *Gastroenterology*, 1956, **31,** 717.
2. Loran, M. R., and Althausen, T. L., *Am. J. Physiol.*, 1958, **193,** 516.
3. Loran, M. R., and Althausen, T. L., *Am. J. Physiol.*, 1959, **197,** 1933.
4. Leblond, C. P., *in* Conference on Dynamics of Proliferating Tissues, (D. Price, editor), Chicago, University of Chicago Press, 1958.
5. Blecher, M., and White, A., *J. Biol. Chem.*, 1958, **233,** 1161.
6. Villee, C. A., and Hagerman, D. D., *Am. J. Physiol.*, 1958, **194,** 457.
7. Amano, M., Messier, B., and Leblond, C. P., *J. Histochem. and Cytochem.*, 1959, **7,** 153.
8. Pelc, S. R., *Nature*, 1947, **160,** 749.
9. Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., and Sherman, F. G., *Proc. Nat. Acad. Sc.*, 1958, **44,** 476.

Explanation of Plates

Plate 327

Fig. 1. A section of ileum from a normal animal sacrificed 2 hours after the injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.

Fig. 2. A section of ileum from a resected animal sacrificed 2 hours after injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.

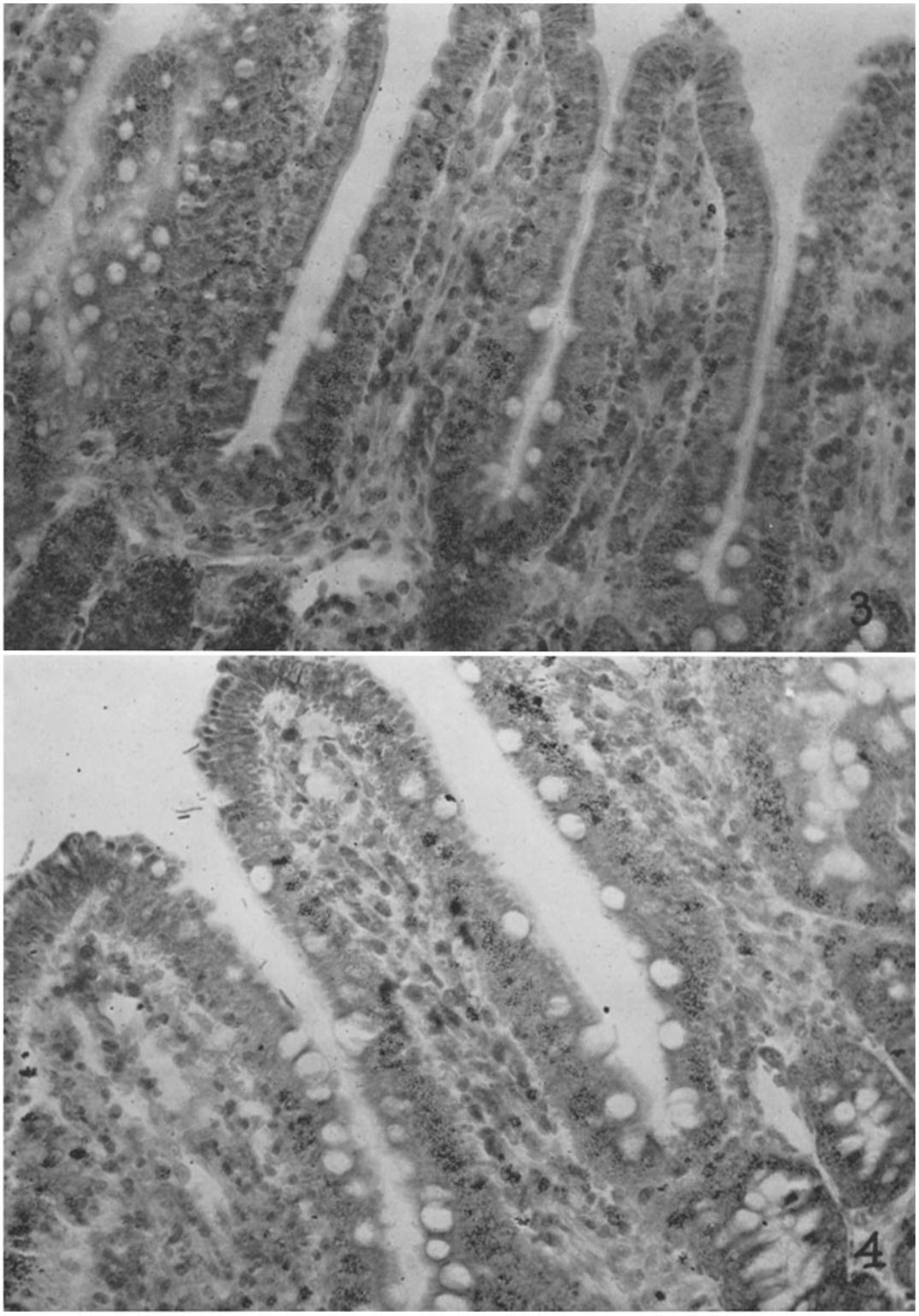


(Loran and Althausen: Intestinal epithelia after partial ileum resection)

PLATE 328

FIG. 3. A section of jejunum from a normal animal sacrificed 23 hours after the injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.

FIG. 4. A section of jejunum from a resected animal sacrificed 23 hours after the injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.

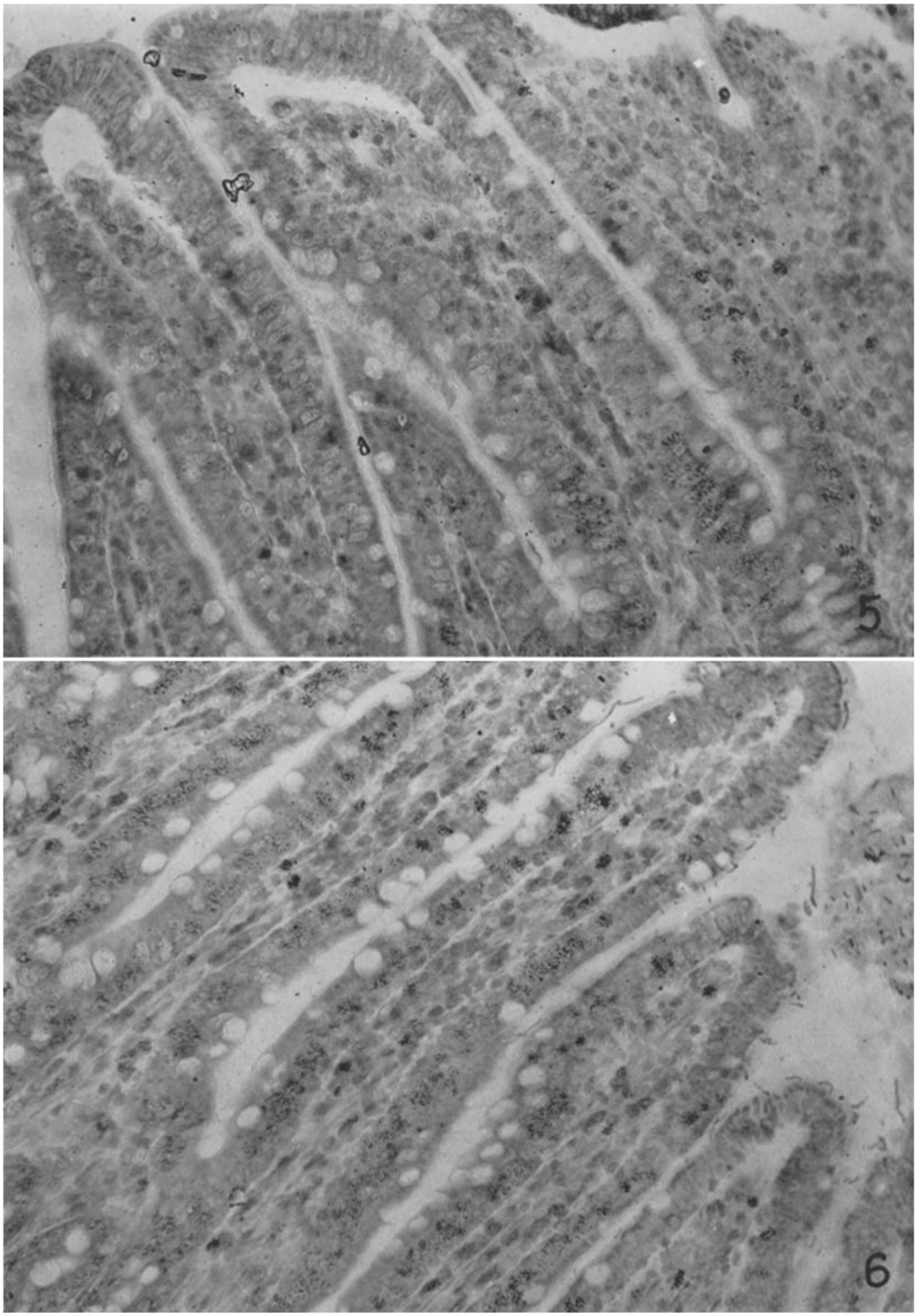


(Loran and Althausen: Intestinal epithelia after partial ileum resection)

PLATE 329

FIG. 5. A section of ileum from a normal animal sacrificed 23 hours after the injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.

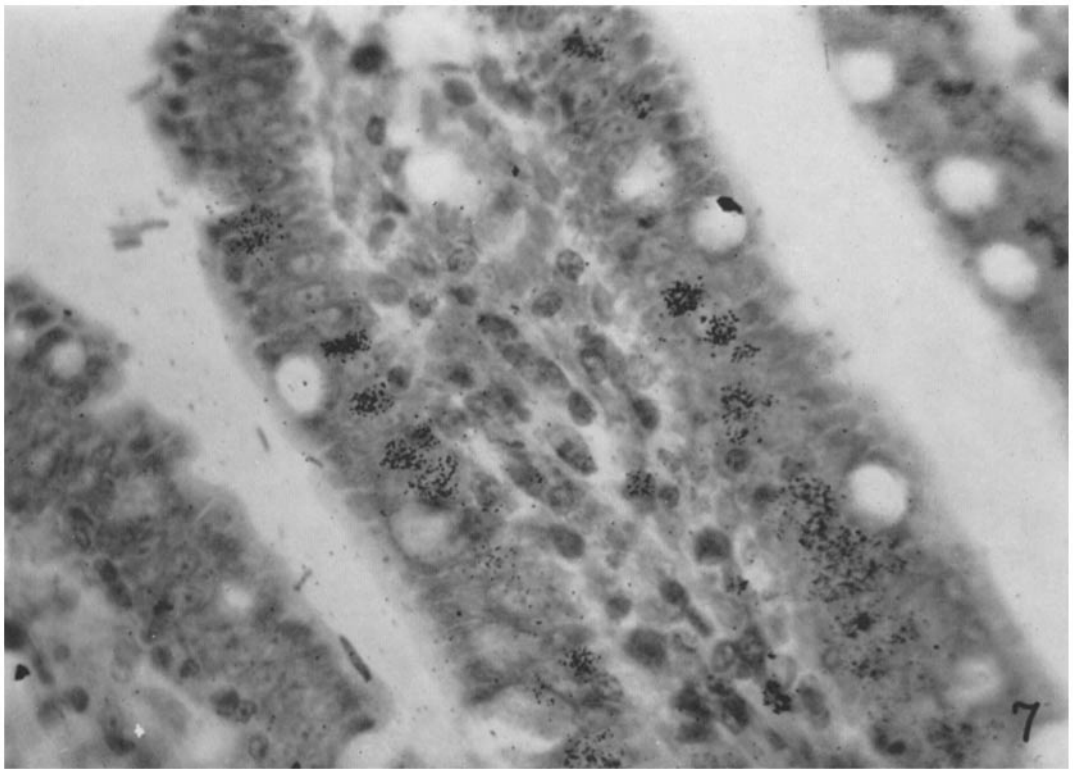
FIG. 6. A section of ileum from a resected animal sacrificed 23 hours after injection of tritiated thymidine. Stained with methylene blue–eosin. × 250.



(Loran and Althausen: Intestinal epithelia after partial ileum resection)

PLATE 330

FIG. 7. The upper end of the villus shown in Fig. 4, at higher magnification. ×340.



(Loran and Althausen: Intestinal epithelia after partial ileum resection)