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

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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
portion of the lower ileum amounting to 10 per cent of
an increased rate of cellular proliferation and a
shortening of the life span of the individual cells?
To explore these questions, we undertook to
of the ileum.
the small intestine was removed from each, and an
equal number of controls of the same weight
and birth date were given an intraperitoneal injection
curies per raM, was obtained from the Schwarz Labora-
tories, Mount Vernon, New York.

(b) Does anaerobic glycolytic phosphorylation,
which is rate-limiting in the intestine of rats after partial resection
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?

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
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,
jejenum, 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 de-
veloped after 20 days' exposure. After development the
sections were stained with methylene blue-eosin.

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 p. of tritiated (H3) 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 esti-
mated 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
H3 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
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1 H3 thymidine, having a specific gravity of 1.6
curies per mCi, was obtained from the Schwarz Labora-
tories, Mount Vernon, New York.
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 mi-
grated 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
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.

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REFERENCES
EXPLANATION OF PLATES

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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)
Fig. 3. A section of jejunum from a normal animal sacrificed 23 hours after the injection of tritiated thymidine. Stained with methylene blue-eosin. X 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. X 250.
(Loran and Althausen: Intestinal epithelia after partial ileum resection)
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.
PLATE 330

Fig. 7. The upper end of the villus shown in Fig. 4, at higher magnification. X 340.
(Loran and Althausen: Intestinal epithelia after partial ileum resection)