DNA SYNTHESIS AND PROLIFERATION BY
VILLOUS EPITHELIAL CELLS IN FETAL RATS

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INTRODUCTION

Radioautographic studies with the labeled desoxy-ribonucleic acid (DNA) precursor, tritiated thymidine, have shown that cell proliferation in mammalian small intestinal epithelium normally is confined to the crypts (1). Following their formation in the crypts, most epithelial cells migrate onto the villi where they lose their capacity to proliferate as they mature into functional absorptive cells. Such restriction of cell proliferation to the crypts has been demonstrated in both adult rats (1, 2) and in young animals 7 days of age (3) and older (4).

In fetal rats, formation of villi begins at the 17th day of gestation, and tall, well-developed villi have formed in the proximal intestine by the 19th day of gestation. In contrast, discrete, well-defined crypts do not develop until shortly after birth (5, 6, 7). In this report, we present evidence that in the duodenum of fetal rats, DNA synthesis and cell proliferation occur in epithelial cells along the entire length of the villi and that this unique pattern of cell renewal changes shortly before birth.

MATERIAL AND METHODS

Fetal and newborn rats from timed pregnant Sprague-Dawley albino rats were studied from the 19th day of gestation through 1 day after birth. Newborn animals were born on the afternoon of the 22nd day of gestation and were injected immediately after birth. For the study of fetal animals, a dose of 225–250 μCi of thymidine-^{3}H (SA 6.7 Ci/mmole; New England Nuclear Corp., Boston, Mass.) was injected into the tail vein of the pregnant females; for the newborn rats, a dose of 15–20 μCi of thymidine-^{3}H was injected intraperitoneally. Segments of duodenum were removed from fetal and newborn animals 1.5 hr later. After fixation in chilled chrome osmium fixative (8), the tissue was embedded in epoxy resin and sections 1 μ thick were mounted on glass slides. These were dipped in Ilford K-5 emulsion (Ilford Ltd., Ilford, Essex, England), exposed for 4–8 wk, developed, and stained with toluidine blue (9, 10). In addition, thin sections were cut and stained with heavy metals for electron microscope studies (11).

RESULTS

Radioautographs of duodenum obtained from 19- and 20-day fetuses and from one litter of 21-day fetuses 1.5 hr after thymidine-^{3}H injection revealed a predominance of labeled nuclei in epithelial cells located between villi and at the extreme base of the villi (Figs. 1 and 2), that is, at the site of future crypts. However, there was also significant labeling of nuclei in epithelial cells along the entire length of the villi (Figs. 1 and 3). Mitoses occurred along the length of the villi. In striking contrast, in duodenum obtained from a different litter of 21-day fetuses, 22-day fetuses, and from newborn rats and 1-day-old suckling
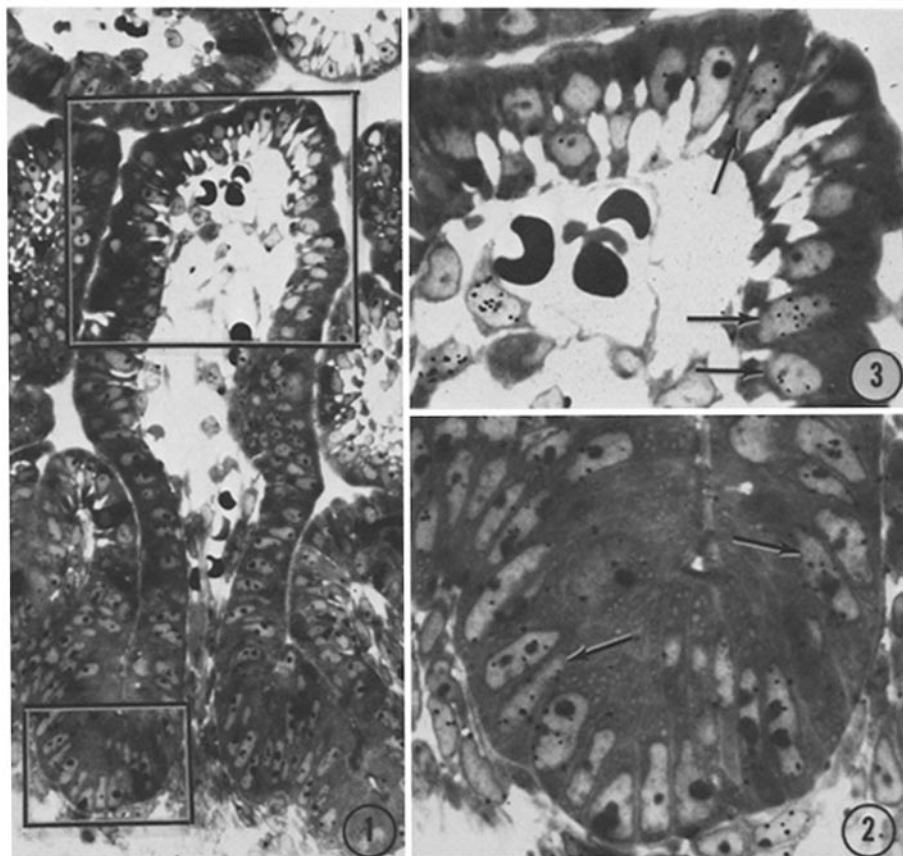
FIGURE 1 A representative tall villus lined by columnar epithelium from the duodenum of a 21-day-old fetal rat. Boxes indicate the areas used for quantitation of labeled cells. × 125.

FIGURE 2 Higher magnification of epithelium at extreme base of the villus in Fig. 1 showing labeling of many cell nuclei (arrows). × 1000.

FIGURE 3 Higher magnification of epithelium at tip of villus in Fig. 1 showing labeling of the nuclei of three columnar epithelial cells (arrows). × 1000.

rats 1.5 hr after thymidine-^{3}H injection, labeled nuclei and mitoses were confined to the epithelial cells in incompletely formed crypts at the extreme base of the villi; none of the epithelial cells on the upper two thirds of the villi contained labeled nuclei.

To quantitate the distribution of proliferating epithelial cells, cells with labeled (three or more grains overlying the nucleus) and unlabeled nuclei were counted (a) on the upper third of well developed villi and (b) in incompletely formed crypts at the extreme base of the villi (see Fig. 1). As shown in Table I, in both fetal and postnatal animals, approximately 50% of the cells at the base of the villi contained labeled nuclei. However, in the 19- and 20-day fetuses and in one litter of

21-day fetal rats, approximately 10% of the cells on the upper third of the villi were labeled. In the other litter of 21-day fetal rats and in the 22-day fetal and all postnatal rats, none of the cells above the base of the villi were labeled.

The epithelial cells of the upper halves of the villi showed significant morphological evidence of differentiation and maturation in the 20- and 21-day animals in which active proliferation was seen on the villi. Typical cells from the crypt region at the base of a villus and from the upper third of a villus are shown in Fig. 4. The cells at the base have irregular short microvilli, many free ribosomes, and little membranous endoplasmic reticulum (Fig. 4A). In contrast, the cells from the upper third of the villi have longer microvilli,

TABLE I

Uptake of Thymidine-³H by Intestinal Epithelium

	No. of animals	Base of villi		Upper third of villi	
Age		Cells counted	Labeled	Cells counted	Labeled
			%		%
Fetal day 19	3	500	47	306	17
20	3	500	51	439	6
21A*	3	500	52	425	11
21B*	3	500	55	400	0
22	4	500	53	400	0
Newborn	3	500	49	400	0
1 day	2	368	40	400	0

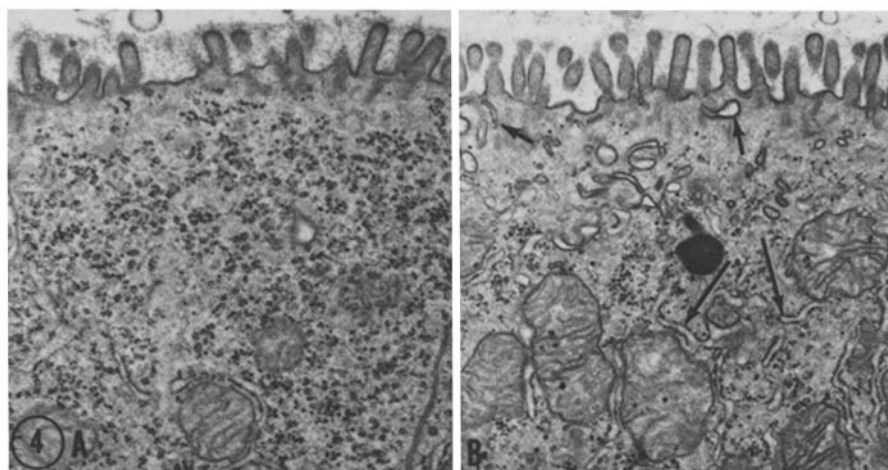* A and B represent animals from separate litters (see text).

FIGURE 4   Electron micrographs of the apical cytoplasm of two duodenal epithelial cells of a 21-day-old fetal rat. 4A, Undifferentiated cell from the extreme base of the villus showing a few short microvilli and many free ribosomes. (X 13,000). B, Absorptive cell from the upper third of the villus showing taller microvilli, pinocytotic vessels (short arrows), and abundant endoplasmic reticulum (long arrows). (X 13,000).

pinocytotic vesicles between microvilli, relatively few free ribosomes, and abundant amounts of membranous endoplasmic reticulum (Fig. 4B).

DISCUSSION

There is a striking change in mucosal morphology in the small intestine of the fetal rat during the final 5 days before birth. The small bowel increases markedly in size. In addition, the epithelium during this period changes from an undifferentiated stratified epithelium without surface specializations to a well-differentiated single layer of columnar epithelium which covers well-developed villi (5, 6, 7). Thus, the demand for expansion of the total epithelial cell population of the gut is extreme. Our studies show that during this period of rapid growth and differentiation, the pattern of epithelial cell proliferation in the duodenum of the fetus differs from that seen in the gut of the adult rat. In the more mature duodenum there is strict compartmentalization of the proliferative zone, and the capacity for DNA synthesis is confined to the epithelial cells of the crypts. In the duodenum of the fetal rat 21 days or younger there

is no well-defined proliferative zone, and DNA synthesis and proliferation occur in epithelial cells along the entire length of the villi. The transition to the adult proliferative pattern occurs during day 21 or day 22 of fetal life.

Further studies are needed to define the factor or factors responsible for the cessation of cell proliferation on the villi of rat small intestine just before birth.

SUMMARY

Radioautographic studies with thymidine-³H show that proliferation of the intestinal epithelium is not limited to the crypts but occurs along the entire length of villi in the duodenum of fetal rats as late as one day before birth. In contrast, proliferating epithelial cells in the duodenum of fetal rats just before birth and of newborn rats are confined to the incompletely formed crypts at the extreme base of the villi.

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