Cell Proliferation Kinetics in the Gastrointestinal Tract of Man. IV. Cell Renewal in the Intestinalized Gastric Mucosa [1, 2]

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SUMMARY—The proliferation of intestinal and gastric cells in atrophic intestinalized gastric mucosa adjacent to a gastric carcinoma was studied. Measurements were made after injection of thymidine-methyl-^{3}H (^{3}H-TDR) and preparation of microautoradiographs from the patient's mucosal biopsy specimens. Gastric mucous cells and intestinal principal, goblet, and Paneth cells incorporated ^{3}H-TDR into DNA and entered into mitosis. Cell-cycle-phase durations were similar to those of normal gastric and intestinal cells of man. Maturing gastric and intestinal cells also continued to synthesize DNA, as they migrated to the luminal surface of the gastric mucosa.—J Nat Cancer Inst 42: 9–17, 1969.

ATROPHY and intestinalization of the stomach are usually present in certain diseases of man including gastric ulcer, gastric cancer, and pernicious anemia (*1–5*). The intestinalization is characterized by the presence in the stomach of all cell types normally present only in the small intestine. A recent study demonstrated rapid renewal of these gastric and intestinal cells in a patient with pernicious anemia (*6*). However, there were too few intestinal cells to permit detailed measurements of their proliferation kinetics.

In the present study, the kinetics of cell proliferation were analyzed in the intestinalized gastric mucosa of a patient with cancer of the stomach and large numbers of intestinal cells. Measurements were made of proliferative rates, durations of proliferative cell-cycle phases, and spatial localization of gastric and intestinal cells in stomach mucosa. The findings confirmed the rapid renewal observed previously in the atrophic stomach and revealed cell-cycle-phase durations similar to those previously seen in normal gastric and intestinal cells of man (*6, 7*).

An abnormality in the spatial distribution of DNA-synthesizing cells in the gastric mucosa was also observed. As gastric and intestinal epithelial cells migrated to the luminal surface of the mucosa and developed some of the morphologic characteristics of the mature surface cells, they continued to incorporate thymidine into DNA. A similar observation has been made in premalignant colonic and rectal cells of man (*8, 9*). The continued synthesis of DNA in gastric and intestinal epithelial cells during their entire lifespan is of interest, since carcinoma may arise from

[1] Received June 11, 1968; accepted October 30, 1968.

[2] This work was supported by Public Health Service research grant CA 08921 and fellowships award 1-F3-CA 31497 from the National Cancer Institute; and by research career program award K3-AM 4468 from the National Institute of Arthritis and Metabolic Diseases.

[3] Mrs. Helen Fuss provided valuable technical assistance.

either of these cell types in the atrophic stomach (*10*).

MATERIALS AND METHODS

The patient studied, referred to as patient A, was a 77-year-old white male with histologically proved adenocarcinoma of the stomach that had metastasized to regional lymph nodes and liver. He had received no chemotherapy or radiation therapy. Ten millicuries of thymidine-methyl-^{3}H (^{3}H-TDR, specific activity 10.6 Ci/mM) were rapidly injected intravenously. Biopsies were then taken with a hydraulic biopsy tube from the body of the stomach adjacent to the tumor, at frequent intervals from 1–48 hours and 4 days after injection of ^{3}H-TDR. The specimens were fixed in 10% buffered formalin, dehydrated, oriented in paraffin to obtain longitudinal sections through the gastric pits, and sectioned at 3 μ. Sections were dipped in Kodak NTB liquid emulsion in preparation for microautoradiography. They were exposed in the dark with Drierite for 4 months, developed in Kodak D-19 solution, and stained with hematoxylin and eosin.

Morphologic observations of the degree of atrophy and intestinalization in each section were made. The number of gastric, intestinal principal, goblet, and Paneth cells in all well-oriented gastric pits and glands was recorded. The frequency of each cell type and of labeled and mitotic figures of each cell type was tabulated. At each period, the ratio of the number of ^{3}H-TDR-labeled mitotic figures to the total number of all mitotic figures was plotted separately for gastric and intestinal principal cells. For each cell type, the ratios of labeled cells per total cells and mitoses per total cells were also noted. For these mitotic indexes 4,117 gastric, 4,949 intestinal, and 981 goblet cells were counted. For labeling indexes 1,802 gastric, 4,146 intestinal, and 394 goblet cells were counted. In each well-oriented gastric pit, the position of the cell adjacent to the surface was designated as position 1, and the ^{3}H-TDR-labeled epithelial cell nearest the surface of the mucosa was designated as the labeled leading-edge cell. The number of cell positions below the surface of all labeled leading-edge cells was recorded. To obtain an approximation of epithelial-cell migration rates, the position of the average labeled leading-edge cell in the mucosa was plotted against time. Regression lines were then calculated by the least-square formula to record the change in position of the average labeled leading-edge cell in time.

RESULTS

Morphologic Observations

The gastric mucosa of this patient had a markedly reduced number of glands containing parietal and chief cells, increased numbers of mononuclear cells in the lamina propria, and large numbers of glands composed of intestinal principal, goblet, and Paneth cells. These intestinalized glands occurred singly and in groups. In this patient's gastric mucosa, the degree of intestinalization was extensive, and intestinal cells and well-defined intestinal crypts were present in all biopsy specimens. Areas of focal degeneration of epithelial cells and increased infiltration of mononuclear cells in the epithelial layer were also seen.

Surface epithelial cells were not uniformly aligned as in normal mucosa. Areas of hyperplasia were present, size and shape of surface cells differed, and nuclei showed variation in size and shape as well as loss of basal orientation. The surface cells, however, demonstrated changes characteristic of gastric cells that migrated from the neck region to the gastric lumen and intestinal cells that migrated onto villi. These surface cells became more columnar, nuclei were larger and paler, and nucleoli became more prominent. Cells in the neck region of the gastric glands were low columnar, nuclei had a more dense chromatin pattern, and nucleoli were less prominent than in the surface cells. The cells in the neck region could not be distinguished from those of normal specimens (fig. 1).

Frequency of Cell Types in Intestinalized Gastric Mucosa

The frequencies of cell types were obtained from all well-oriented columns in each of the biopsy specimens. Gastric cells and intestinal principal cells were present in approximately equal num-

bers and together comprised 90% of the total epithelial-cell population (table 1). Goblet cells were less frequent and Paneth cells were rare. Gastric mucous, intestinal principal, goblet, and Paneth cells were labeled with ^{3}H-TDR 1 hour after injection. Labeled mitoses of each of these cell types were subsequently seen. Examples of labeled cells and mitoses observed at intervals after ^{3}H-TDR injection are shown in figure 2. The frequencies of mitoses and labeled cells after injection of ^{3}H-TDR are recorded in table 2 for each cell type at the indicated time periods. These frequencies were greater than those previously observed in gastric mucosa of man and were close to previous observations made in intestinal crypt-cell populations of man (*6, 11*). Of the 173 Paneth cells seen in all specimens, 20 were labeled, 5 were in mitosis, and 2 of the mitoses were labeled with ^{3}H-TDR. The number of labeled gastric, intestinal principal, and goblet cells approximately doubled in 48 hours.

TABLE 1.—Frequency of cell types in intestinalized stomach of Patient A

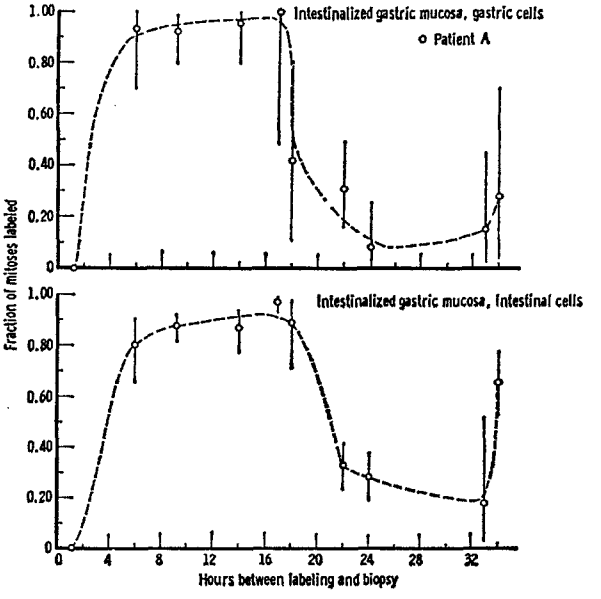
Cell type	Number of cells counted	Percent
Gastric	4, 117	40. 9
Intestinal principal	4, 949	49. 1
Goblet	981	9. 7
Paneth	34	0. 3
Total cells counted	10, 081	100. 0

TABLE 2.—Labeling and mitotic indexes of cell types in the intestinalized stomach of Patient A

Cell type	Mitoses/ 100 cells (1–48 hr)	Labeled cells/100 cells		
		1–6 hr	24 hr	48 hr
Gastric	1. 4	14	22	26
Intestinal principal	1. 0	19	37	42
Goblet	2. 0	18	31	36

Durations of Proliferative Cell-Cycle Phases

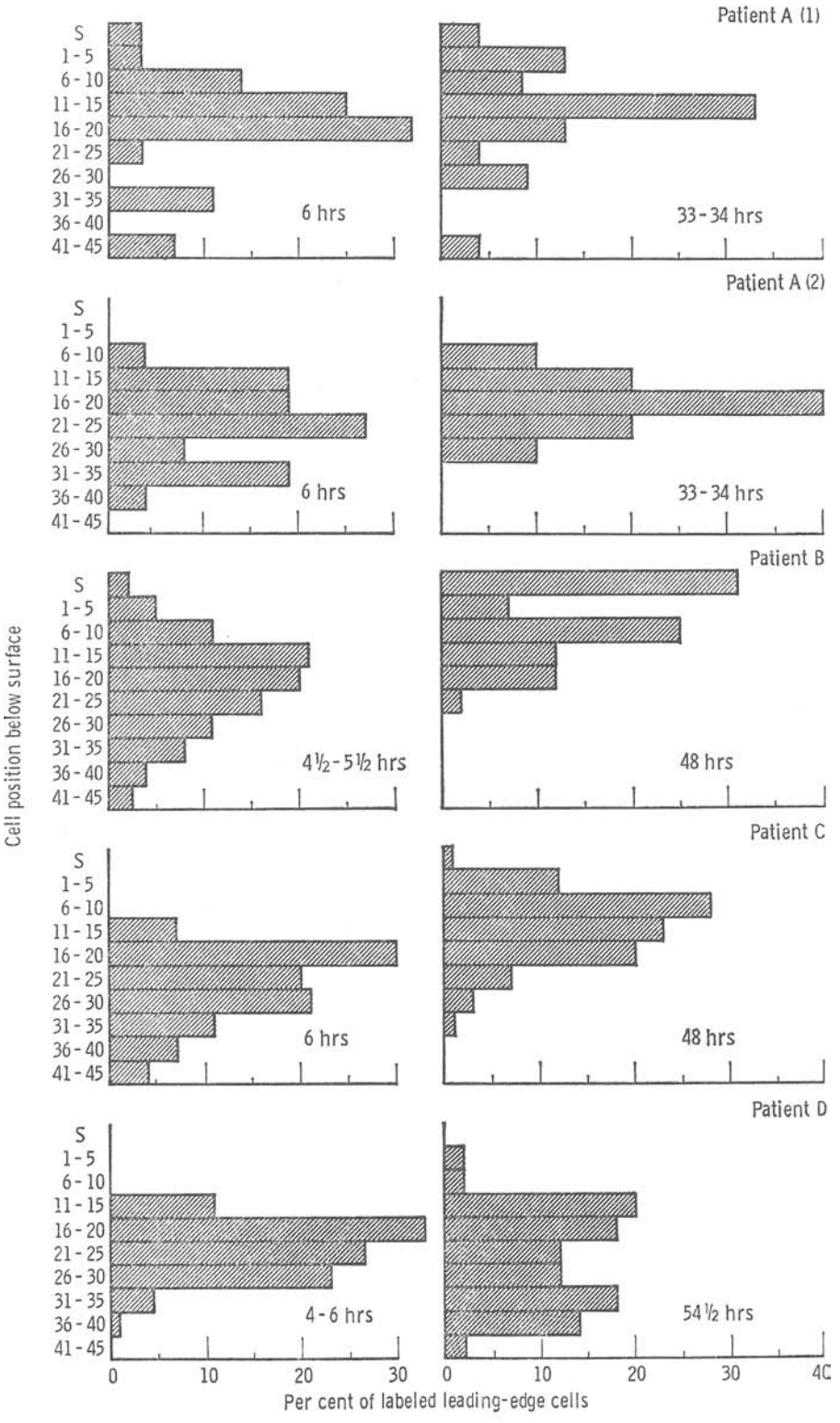
The fractions of mitoses labeled at intervals after ^{3}H-TDR (*12*) are plotted separately for gastric and intestinal principal cells in text-figure 1. The location in time of the beginning and end of the ascending limbs of the labeled mitosis curves and analysis of the areas under the curves indicate that the minimum duration of the G_2 premitotic phase of the proliferative cell cycle is greater than 1 hour, the maximum duration of the G_2 phase extends to 6 or more hours in each cell type, and the duration of the DNA synthesis phase is approximately 14–18 hours for each cell type. The beginning of an ascending limb of a second wave of labeled mitoses at 34 hours in the intestinal and possibly in the gastric cell populations suggests a proliferative cell cycle close to 30 hours.



TEXT-FIGURE 1.—Fraction of mitoses labeled in microautoradiographs prepared from gastric biopsies of intestinalized mucosa obtained 1 hour to 34 hours after injection of ^{3}H-TDR; gastric cells are shown in *upper diagram* and intestinal cells in *lower diagram*.

Spatial Distribution of DNA-Synthesizing Cells in Normal and Intestinalized Mucosa

Text-figure 2 shows the cell position below the surface occupied by labeled leading-edge cells at early and late periods after injection of ^{3}H-TDR. The data are grouped according to the percent of total labeled leading-edge cells occupying units of 5 cell positions. Text-figure 2 records data derived from the patient described in the present study, referred to as Patient A. Data are similarly recorded



for three other patients—patients B, C, and D—to compare the spatial distribution of ^{3}H-TDR-labeled leading-edge cells in the gastric mucosa of Patient A with similar data from patients with both normal and abnormal gastric mucosae. Patient B had pernicious anemia and intestinalized gastric mucosa, and had had a previous subtotal gastric resection for carcinoma and benign polyps (*6*). Patient C had an obstructing carcinoma of the distal esophagus and normal gastric mucosa, biopsy specimens of which were obtained through a gastrostomy (*11*). Patient D had an inoperable carcinoma of the nasopharynx and a feeding gastrostomy through which biopsy specimens of normal gastric mucosa were obtained (*6*).

Following ^{3}H-TDR injection of Patients C and D with normal mucosae, labeled leading-edge gastric cells were located 11 or more cell positions below the mucosal surface. These labeled epithelial cells were separated from the luminal surface by unlabeled maturing cells. Labeled leading-edge cells reached the surface 48–54 hours after injection of ^{3}H-TDR into Patients C and D. In contrast, after injection of ^{3}H-TDR into Patients A and B who had intestinalized mucosae, labeled leading-edge cells extended from the neck region of gastric glands and from the crypts of intestinalized glands to the luminal surface of the mucosa (text-fig. 2 and fig. 3). The distinct zone of unlabeled maturing cells normally separating proliferative cells from luminal surface was absent. In Patients A and B, the ^{3}H-TDR-labeled cells in the neck region had morphologic characteristics of normal neck cells, and the labeled epithelial cells at the luminal surface of the mucosa had morphologic characteristics of well-differentiated surface cells. In addition, epithelial cells in various stages of morphologic transition from neck to surface cells were also labeled immediately after injection of ^{3}H-TDR, in Patients A and B.

Rate of Migration of ^{3}H-TDR-Labeled Cells in Intestinalized and Normal Mucosa

Migration rates of gastric epithelial cells were estimated from the change in position of the average labeled leading-edge cell plotted against time. Regression lines were calculated from the data by the least-square formula. The data for the four patients are summarized in table 3. The number of time periods examined for each patient is N; the slope of the least-square line k is the change of cell position per unit change of time (cell positions per hour); the intercept b is the average leading-edge cell position below the surface at time zero. In Patients A, B, and C, the migration rate varied from 0.19–0.32 cell positions per hour, while in Patient D this rate appeared to be much slower. The correlation coefficient r, and its level of significance p, may be taken as a measure of the goodness of fit of the points to the line. The level of confidence at which we accept k is indicated by the p value for r: p is significant or borderline significant in Patients A, B, and C; p is not significant in Patient D, and k is not significantly different from zero. The average labeled leading-edge cell position below the surface, b, was slightly greater in the normal compared to the intestinalized mucosae.

The data, therefore, indicate that cells moved toward the luminal surface at a rate approximating 1 cell position in 3–5 hours in the intestinalized mucosa of two patients (A and B) and in the normal mucosa of one patient (C). In Patient D, the position of labeled leading-edge cells in different biopsy specimens varied greatly and, therefore, significant migration was not measurable. However, migration can be presumed to have taken place in some areas of mucosa, since labeled cells were seen at the luminal surface in the last biopsy specimen obtained 54 hours after injection of ^{3}H-TDR.

TEXT-FIGURE 2.—Cell position below the surface occupied by labeled leading-edge cells at early and late time periods after injection of ^{3}H-TDR, in 2 patients with intestinalized gastric mucosa (A and B), and 2 with normal gastric mucosa (C and D). In Patient A, 1) records gastric cell positions and 2) records intestinal cell positions. In Patients B, C, and D only gastric cells are recorded.

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TABLE 3.—Migration of labeled leading-edge cells in intestinalized gastric mucosa from Patients A and B, and normal gastric mucosa from Patients C and D*

Patient	Cell type	N	k	b	r	p
A	Gastric	9	0.19	20.1	0.86	$0.01 > p > 0.001$
	Intestinal	6	.22	26.0	.92	$.01 > p > .001$
B	Gastric	6	.32	24.9	.75	$0.1 > p > .05$
C	Gastric	7	.23	27.1	.80	$.05 > p > .02$
D	Gastric	9	.06	32.3	.07	$p > 0.1$

*N, number of time periods examined; k, slope of least-square line indicating cell positions per hour; b, average leading-edge cell position below the surface at time zero; r, correlation coefficient.

DISCUSSION

In this study, the intestinalized gastric mucosa contained populations of rapidly proliferating gastric and intestinal cells, with normal cell-cycle phases, each capable of self-renewal. Gastric mucous, intestinal principal, goblet, and Paneth cells all incorporated ^{3}H-TDR into DNA and then entered into mitosis. The proliferative cell-cycle-phase durations of gastric mucous and intestinal principal cells were similar to those measured in the normal stomach and intestine of man. The frequencies of labeling and mitosis in gastric mucous, intestinal principal, and goblet cells were slightly higher than other measurements made in the stomach, and were comparable to measurements made in the small intestine of man (*11*, *13*, *14*). Although there were fewer Paneth cells than other cell types, metaphase figures and interphase cells were observed labeled with ^{3}H-TDR, indicating renewal of Paneth elements. Paneth-cell labeling and mitosis have been observed in the small intestine of rodent (*15*) and man (*16*) where these elements appear to undergo renewal at a slower rate than the epithelial principal cells.

Incorporation of thymidine into cells at or near the luminal surface of the atrophic gastrointestinal mucosa in the present study represents a deviation from normal. In normal gastric mucosa, proliferative cells are separated from the luminal surface of the stomach by other maturing, nonproliferating epithelial cells (*11*). The latter cease DNA synthesis during maturation, as they migrate to the surface. In other normal cell systems within the body, rapid cell division and specialization also occur at different periods during the life of the cell. In the maturation of epidermal and hematopoietic as well as gastrointestinal cells (*17–20*), in fibrogenesis of the lens (*21*), and chondrogenesis (*22*), proliferative functions cease during specialization.

However, in a few normal cell systems *in vitro*, cells undergoing specialization have been observed to divide and express their cytodifferentiative characteristics simultaneously; examples are cultured retinal pigment cells, beating heart cells, and cartilage cells making matrix (*23–25*). DNA synthesis has also been observed in maturing epidermal cells of viral-induced skin papillomas in rabbits, and it has been suggested that viral DNA enters the epidermal cells and replicates (*26*). The cellular control mechanisms operating under abnormal conditions that enable some of these cells to make DNA during their entire lifespan have not been clarified.

Whether continued incorporation of thymidine into DNA of the gastric and intestinal cells observed here relates to the development of carcinoma also has not been clarified. Carcinomas arise in these tissues with increased frequency (*1*, *27*) and may spread along the surface of the mucosa (*10*, *28*). Cells exfoliated from the surface of the atrophic intestinalized stomachs also have increase and variation in nuclear size, variation in size and distribution of nuclear chromatin, and appear cytologically similar to exfoliated carcinoma cells (*3*, *29*, *30*).

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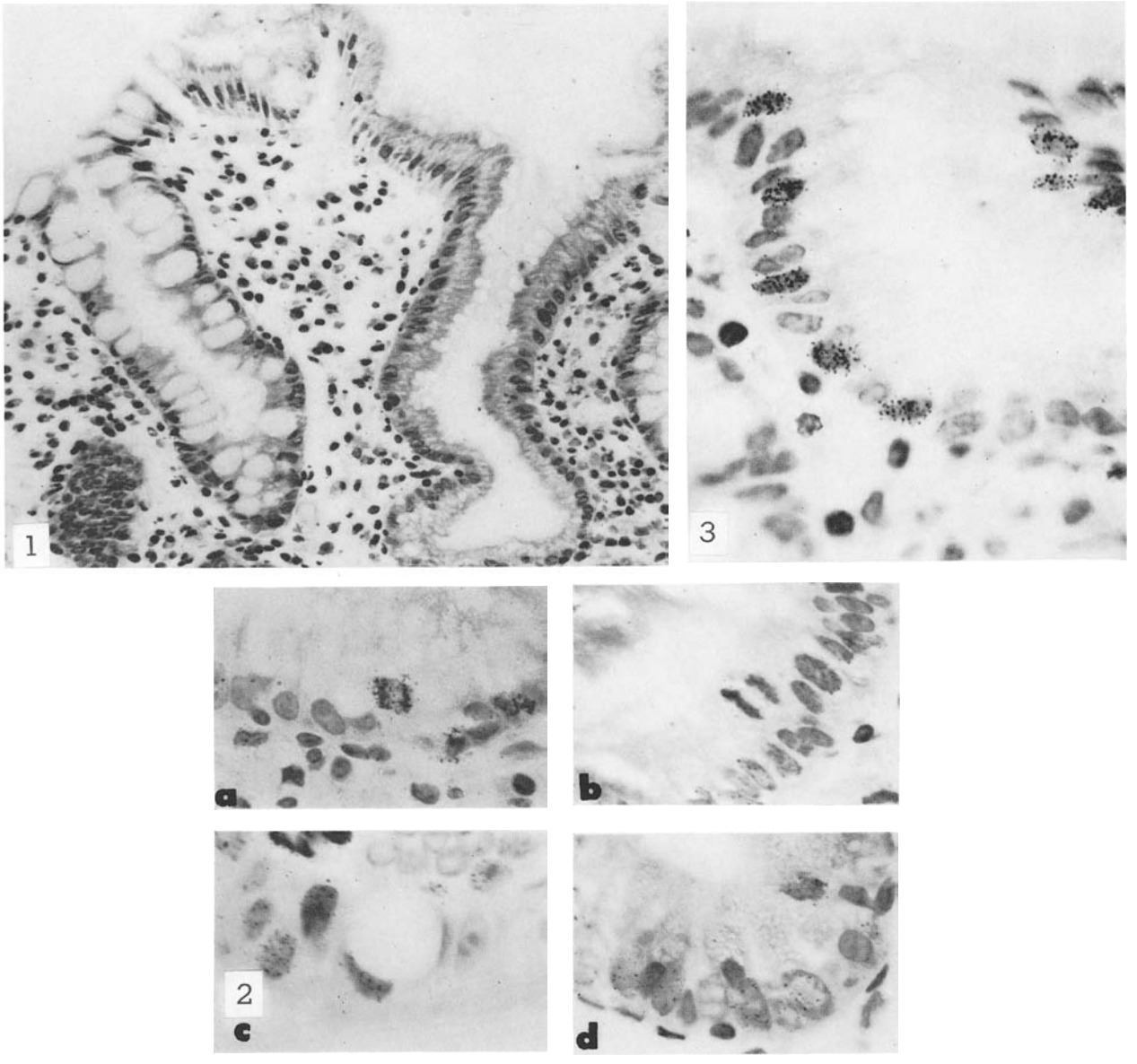

FIGURE 1.—Section from biopsy specimen obtained from stomach of Patient A, showing an intestinalized gland (*left*) with some intestinal principal cells and many goblet cells, and a remaining gastric gland (*right*) with mucus-secreting gastric cells. Extensive intestinalization was seen in all the biopsy specimens, and no parietal and chief cells were seen. Hematoxylin and eosin. × 250

FIGURE 2.—Microradioautographs prepared from gastric biopsies obtained 6–24 hours after injection of ^{3}H-TDR showing: *a*) labeled gastric-cell nuclei in interphase and anaphase; *b*) labeled intestinal principal-cell nuclei in interphase and metaphase; *c*) labeled intestinal goblet-cell nucleus in interphase adjacent to intestinal principal cells; *d*) labeled Paneth-cell nuclei in interphase and prophase. Hematoxylin and eosin. × 500–650

FIGURE 3.—Microradioautographs prepared from gastric biopsy obtained 1 hour after injection of ^{3}H-TDR in Patient A with intestinalized atrophic mucosa. Labeled gastric cells are present at and near the luminal surface. Hematoxylin and eosin. × 550