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Dynamics of Epithelial Cells in the Corpus of the Mouse Stomach

I. IDENTIFICATION OF PROLIFERATIVE CELL TYPES AND PINPOINTING OF THE STEM CELL

SHERIF M. KARAM AND CHARLES PHILIPPE LEBLOND
Department of Anatomy, McGill University, Montreal, Quebec, Canada

ABSTRACT **In a recent study of the corpus epithelium in the mouse stomach, eleven cell types have been identified and enumerated (Karam and Leblond: Anat. Rec. 232:231–246, 1992). The dynamics of these cells will be examined in a series of five articles, of which this is the first. This article focuses on the proliferative ability of the cells, as measured by the labeling index in radioautographs from mice sacrificed 30 min after an intravenous injection of ^{3}H-thymidine. Furthermore, the ultrastructure of the cells found to be proliferative was examined in the hope of finding features characteristic of stem cells.**

On the basis of their labeling index, the epithelial cells have been classified into four groups. The first includes three cell types which do not take up any label and accordingly are non-dividing: *parietal* or oxyntic cells, cells named *pre-parietal* as they are immature cells suspected of being parietal cell precursors, and the rare *caveolated* or brush cells. The second group is composed of three cell types which are only rarely labeled and, therefore, divide only occasionally: *zymogenic* or chief cells, *entero-endocrine* cells, and cells named *pre-zymogenic* cells as they are suspected of being zymogenic cell precursors. The third group includes two cell types which are always labeled at a low degree and, therefore, divide regularly, but at a low rate: surface mucous cells, herein called *pit* cells, whose labeling index is 0.8%, and mucous neck cells, simply known as *neck* cells, 1.8%. The final group consists of three immature cell types with high labeling indices indicating a high rate of division: *granule-free* cells, which are devoid of secretory granules and have the highest labeling index, 32.4%, *pre-pit* cells, which possess a few dense secretory granules similar to, but smaller than, those in pit cells, 24.6%, and *pre-neck* cells, with a small number of secretory granules similar to, but smaller than, those in neck cells, 11.3%. These three cell types, as well as pre-parietal cells, are rapidly renewed, with the turnover times estimated at 3.0 days for pre-neck and pre-parietal cells and less than 2.6 days for granule-free and pre-pit cells.

Ultrastructural studies of granule-free cells reveal that they may be subdivided into three subtypes according to their Golgi features: subtype I, which consists of undifferentiated cells in which the Golgi trans face exhibits no prosecretory vesicles; subtype II, named *pre-pit cell precursors* because the Golgi trans face shows prosecretory vesicles similar to those in pre-pit cells; and subtype III, named *pre-neck cell precursors,* whose prosecretory vesicles are similar to those in pre-neck cells. On the other hand, pre-parietal cells include three variants that could each arise from a different granule-free subtype: variant I, which has no mucous secretory

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Address reprint requests to Dr. C.P. Leblond, Department of Anatomy, McGill University, 3640 University St., Montreal, Quebec, Canada H3A 2B2.

Sherif M. Karam's present address is Department of Molecular and Cellular Biology, 245 Life Sciences Addition, University of California, Berkeley, CA 94720.

granules, could arise from the undifferentiated cells; variant II, which possesses dense mucous granules similar to those in pre-pit cells, could come from pre-pit cell precursors; and variant III, which has cored granules as in pre-neck cells, could come from pre-neck cell precursors.

Only the undifferentiated granule-free cells have the features expected from stem cells and, therefore, are considered to be the stem cells of the epithelium. A model based on the radioautographic and morphological data (Fig. 17) summarises the filiation of the other immature cell types as follows. The undifferentiated granule-free cells as stem cells reproduce themselves and give rise to three other cell types: 1) the pre-parietal cells lacking secretory granules (i.e., variant I); 2) the pre-pit cell precursors, which mainly give rise to pre-pit cells, but also yield the variant II pre-parietal cells; 3) the pre-neck cell precursors, which mainly give rise to pre-neck cells, but also yield the variant III pre-parietal cells. Further differentiation of these immature cell types into the other cells of the corpus epithelium is examined in the succeeding articles.

Key words: Stomach, Radioautography, ^{3}H-thymidine, Stem cell, Granule-free cell

In the 1980s, a systematic investigation of cell dynamics in the mouse gastric epithelium was initiated in the pyloric antrum, (Lee et al., 1982; Lee, 1985a,b; Lee and Leblond, 1985a,b). In the corpus, the information available on cell-dynamics (Stevens and Leblond, 1953; Hattori and Fujita, 1976; Kataoka, 1970; Wattel and Geuze, 1977; Kataoka et al., 1986) is rather limited and, therefore, the present and following articles (Karam, 1993; Karam and Leblond, 1993a,b,c) will examine the origin, differentiation, migration (if any), and eventual fate of the eleven cell types previously defined in the corpus (Karam and Leblond, 1992).

The corpus epithelium is made up of one layer of cells which invaginate into the lamina propria to form blind tubules, referred to as "units" (Lee et al., 1982). According to the localization of the different cell types, the units are divided into four regions: the most distal region close to the gastric lumen is the foveola or pit; next is the isthmus, the neck follows, and the most proximal region or base ends in a cul-de-sac (Fig. 1). Most of the epithelial cells populating the four regions have been well characterized in human (Rubin et al., 1968) and rodents (Helander, 1962, 1981; Ito, 1987). Thus, the pit region is mainly composed of cells that accumulate dense mucous granules in the apex and are called surface mucous cells or herein pit cells (Karam and Leblond, 1992; Lee, 1985b), but it also includes a few parietal cells in the mouse (Fig. 1). The neck region is characterized by mucous neck cells, simply called neck cells, which exhibit marbled, frequently cored mucous granules (Karam and Leblond, 1992). This region also comprises some parietal cells. The base region is mainly composed of zymogenic cells but includes parietal cells as well as another cell type which has not been as well characterized in the past as the cells listed so far; this cell type produces secretory granules intermediate in appearance between those of neck cells and zymogenic cells (Sato and Spicer, 1980; Kataoka et al., 1990; Suzuki et al., 1983; Cornaggia et al., 1986) and has been named pre-zymogenic cell (Karam and Leblond, 1992). Finally, the isthmus region includes, besides parietal cells, four rather small, immature cell types, recently described in some detail (Karam and Leblond, 1992) and sketched in Figure 2. One type is devoid of secretory granules and accordingly named granule-free cell. Another includes a few small, dense granules similar to those in pit cells and has been tentatively called pre-pit cell. A third has a few small cored granules similar to those in neck cells and has been tentatively designated pre-neck cell. The last one has long apical microvilli as in parietal cells and may also have an incipient canaliculus; it has been tentatively referred to as pre-parietal cell. The last two other

Fig. 1. Drawing of a unit from the corpus of the mouse stomach, as seen after glutaraldehyde-formaldehyde fixation, osmium tetroxide postfixation, and toluidine blue staining using 0.5-μm thick sections. The unit is divided into four regions listed at left. 1) The pit region is lined by pit cells in which secretory granules accumulate at the apex; also included are a few parietal cells displaying perinuclear and peripheral grayish, dot-like mitochondria between which is a light space corresponding to the canaliculi. The number of apical granules in pit cells increases in the direction of the gastric lumen (GL), but decreases at the free surface. The increase along the pit wall is usually gradual, as depicted on the right side of the pit, but may be irregular, as depicted on the left side. The limits of the pit region are arbitrarily defined as follows: at the surface, by a point along the gastric lumen (GL) halfway to the opening of the next pit (arrow) and, on the isthmus side, by the first cell in which secretory granules (indicated by black dots) are lined up along the apical cell membrane. 2) The isthmus region is characterized by the presence of four rather small cell types: pre-pit cells containing a small number of secretory granules, similar to those in pit cells but scattered within the cytoplasm instead of being lined up along the apical membrane; granule-free cells which, as the name indicates, are devoid of secretory granules; pre-parietal cells, characterized by a light microvillar area at the apex or a pale region within the cytoplasm corresponding to a small canaliculus or both; and pre-neck cells containing a small number of pale secretory granules. Mitoses (M) are frequently encountered in the isthmus. 3) The neck region is taken to begin at the first neck cell which, like the other neck cells of the region, is characterized by the presence of numerous pale secretory granules. 4) The base region extends from the first zymogenic cell to the blind end of the unit. Zymogenic cells are characterized by distinct, intensely stained apical secretory granules and a smoothly basophilic basal cytoplasm. Close to the neck border, pre-zymogenic cells contain secretory granules intermediate in density between those of neck cells and zymogenic cells. Finally, lightly stained entero-endocrine cells with finely granular cytoplasm are mainly seen in the base. (Of the 11 cell types present in the units, only the rare caveolated cell is not depicted in this drawing). ×1,000.

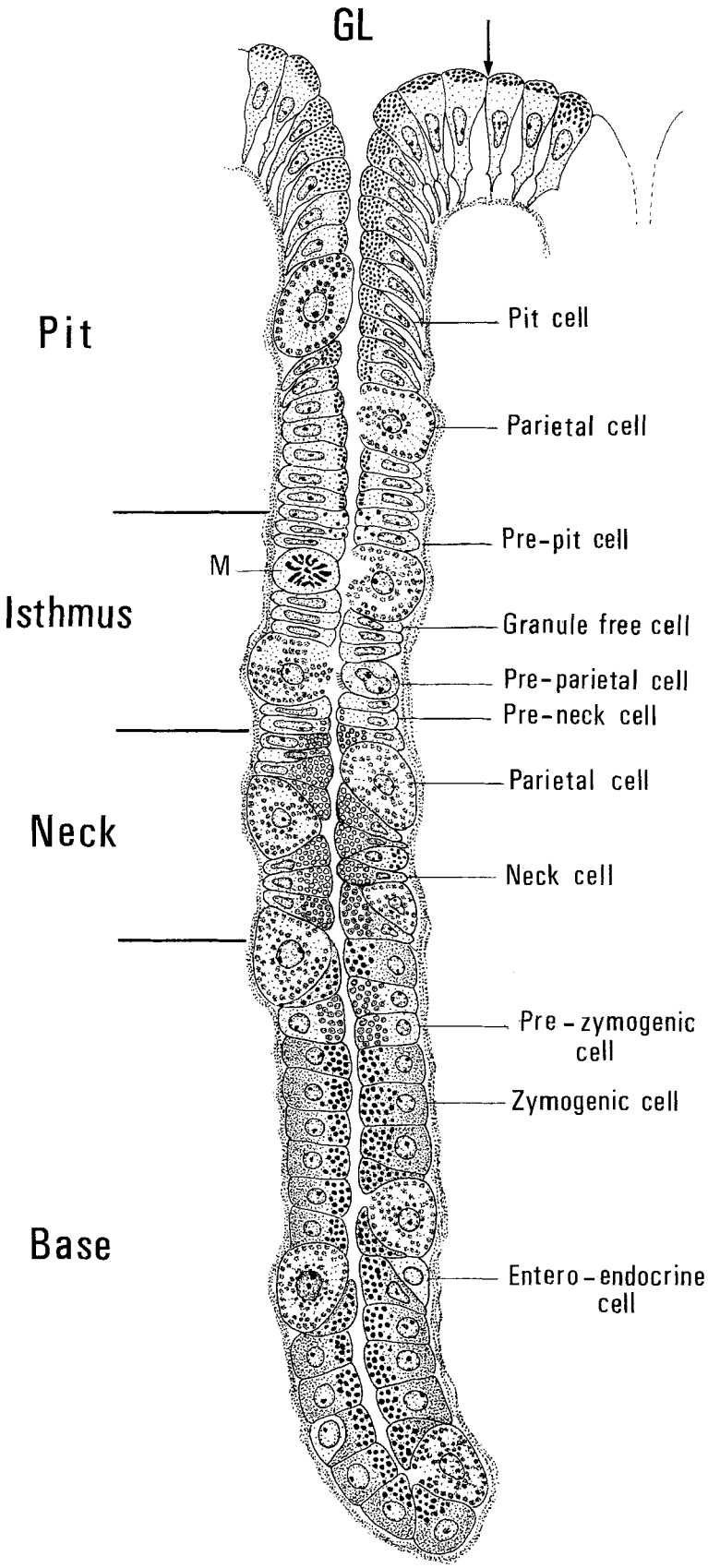

GL
Pit
Isthmus
Neck
Base
M
Pit cell
Parietal cell
Pre-pit cell
Granule free cell
Pre-parietal cell
Pre-neck cell
Parietal cell
Neck cell
Pre-zymogenic cell
Zymogenic cell
Entero-endocrine cell

Fig. 1.

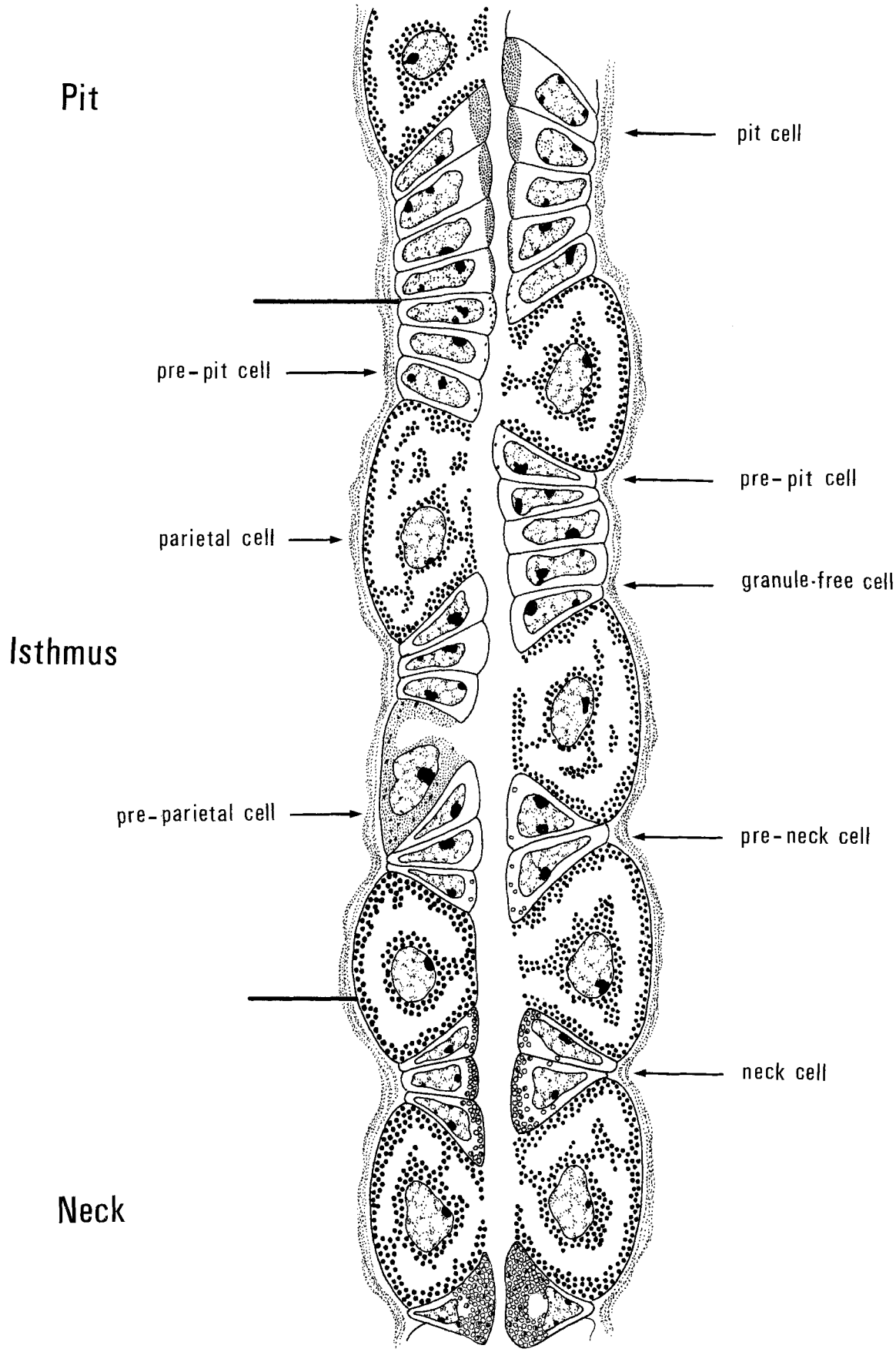

Pit
pit cell
pre-pit cell
pre-pit cell
parietal cell
granule-free cell
Isthmus
pre-parietal cell
pre-neck cell
neck cell
Neck

Fig. 2.

cell types, entero-endocrine cells (Solcia et al., 1987) and the rare cells called fibrillo-vesicular, brush, or caveolated (Karam and Leblond, 1992; Helander, 1981; Hammond and LaDeur, 1969) are, like parietal cells, scattered through the four regions of the unit (Karam and Leblond, 1992).

Morphological observations have led to the following hypothesis on the dynamic interactions between the eleven cell types (Karam and Leblond, 1992). The granule-free cells would give rise to 1) pre-pit cells developing into pit cells, 2) pre-neck cells becoming neck cells which, through a pre-zymogenic stage, would develop into zymogenic cells, 3) pre-parietal cells becoming parietal cells, and 4) immature entero-endocrine and caveolated cells developing into entero-endocrine and caveolated cells, respectively.

It was decided to test this hypothesis systematically and, as a first step, to measure the ability of the various cell types to undergo mitosis in the hope of eventually identifying the stem cells of the epithelium. The literature on the ability of the corpus cells to proliferate was limited and controversial. Thus, some authors reported that parietal cells could divide (Harms, 1910) or take up ^{3}H-thymidine (Chen and Withers, 1975; Tamura and Fujita, 1983), but others saw no indication of mitotic ability in these cells (Hunt and Hunt, 1962; Willems et al., 1972; MacDonald et al., 1964). Similarly, some reported ^{3}H-thymidine labeling of zymogenic cells (Chen and Withers, 1975; Tamura and Fujita, 1983; Willems et al., 1972), but others did not observe any (Hunt and Hunt, 1962; Creamer et al., 1961). There was, however, general agreement that the cells in the isthmus region could undergo mitosis (Stevens and Leblond, 1953; Plenk, 1932) and take up label soon after a ^{3}H-thymidine injection (Messier and Leblond, 1960; Kataoka et al., 1989).

To examine which cells had the ability to proliferate, radioautographs were prepared 30 min after an intravenous injection of ^{3}H-thymidine; the labeling index of the various cell types was estimated and considered to be a measure of their mitotic activity. Since label was mainly found in the small isthmal cells, the next step was to combine radioautographic and morphologic data to examine the relationship between the four isthmal cell types, the rate at which they turned over, and their eventual fate. One of these cell types was identified as the stem cell of the epithelium.

MATERIALS AND METHODS

The present investigation was facilitated by previous technical findings. Methods had been devised to visualize the eleven cell types—that is, for the light micro-

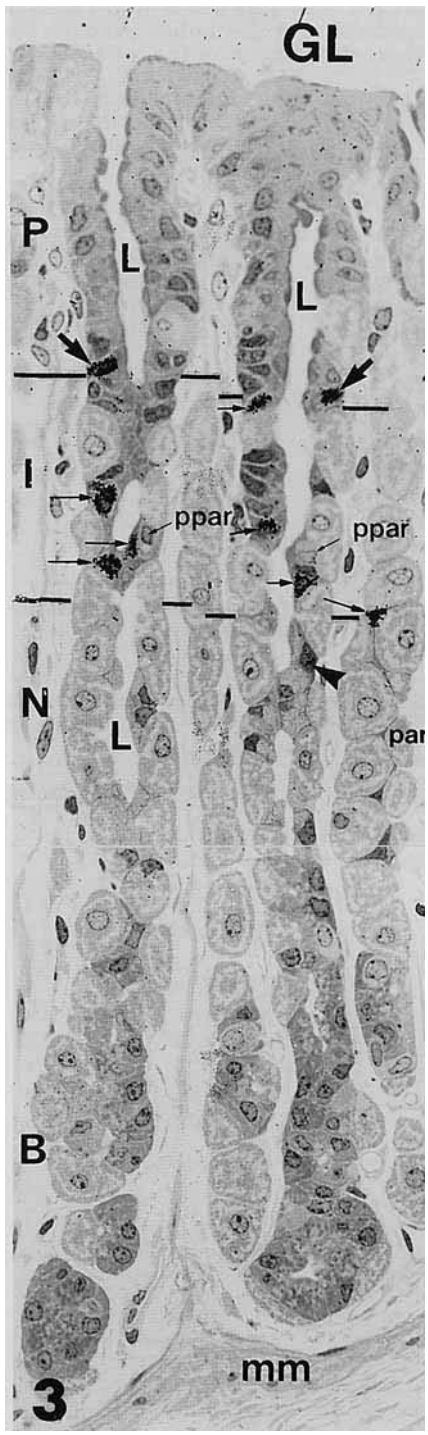


Fig. 3. Montage of a light microscopic radioautograph including two units, 30 min after a ^{3}H-thymidine injection. The unit at left shows its lumen (L) opening into the gastric lumen (GL), while the opening of the unit at right is not in the plane of section. Both units have their four regions at about the same level. The isthmus (I), delimited by black lines, includes several labeled cells (horizontal arrows) and an unlabeled pre-parietal cell (ppar) in the left unit. This cell is identified by its more or less spherical shape and the presence of an infranuclear light area representing a small canaliculus. The isthmus region at right includes three labeled cells (horizontal arrows) and an unlabeled pre-parietal cell (ppar). The parietal cells (par) scattered throughout the four regions are not labeled. In the pit region (P) pit cells are not labeled except for one in each unit located next to the isthmus border (Large arrows). In the neck region (N), the only labeled cell (arrowhead) is located fairly close to the isthmus. The numerous zymogenic cells in the base region (B) are not labeled. mm, muscularis mucosa. ×400.

Fig. 2. Diagrammatic representation of the isthmus delimited by black lines on the left. Part of the pit is at the top and part of the neck is below. From the top down, the pit includes part of a parietal cell at upper left and, on both sides, several pit cells in which the apical group of secretory granules decreases gradually. The isthmus comprises pre-pit cells (next to the pit border) whose cytoplasm includes few apical secretory granules shown as small dots (mitochondrial nodules are not depicted), large parietal cells with central nucleus and extensive pale canaliculus, then several granule-free cells, on the left a pre-parietal cell shaded in contrast to the small pale canaliculus and showing mitochondria as dense dots smaller than in parietal cells, and pre-neck cells with few pale secretory granules located close to the neck border. Finally, the neck region includes neck cells which show an increasing number of pale secretory granules; a minute dense particle may be distinguished within some of the granules. ×2,500.

TABLE 1. Labeling index (%) of cells in the corpus epithelium of the mouse stomach 30 min after a ^{3}H-thymidine injection (Experiment II; N = 4)

	No. of cells counted in four animals	Labeling index ± s.e. (%)
Pit		
Pit cells	1,577	0.8 ± 0.27
Isthmus		
Pre-pit cells	175	24.6 ± 3.2
Granule-free cells	328	32.4 ± 3.1
Pre-parietal cells	160	0
Pre-neck cells	246	11.3 ± 3.3
Neck		
Neck cells	545	1.8 ± 0.47
Base		
Pre-zymogenic cells	208	0.5
Zymogenic cells	1,327	0.2
All regions		
Parietal cells	2320	0
Entero-endocrine cells[1]	750	0
Caveolated cells	10	0

[1]In other series, an occasional entero-endocrine cell has been labeled 30 min after a ^{3}H-thymidine injection.

TABLE 2. Labeling index (%) of immature isthmal cells from 3 h to 8 days after a ^{3}H-thymidine injection (Experiment II; N = 2)

	Time after a ^{3}H-thymidine injection						
Cell type	3 h	6 h	12 h	1 d	2 d	4 d	8 d
Granule-free	33.3	51.0	46.1	22.4	7.9	0	0
	47.4	41.0	44.4	18.1	6.9	3.6	0
Mean	40.4	46.0	45.3	20.3	7.4	1.8	
Pre-pit	32.5	35.1	21.9	20.9	13.2	0	0
	26.9	31.1	34.9	25.0	17.5	1.5	0
Mean	29.7	33.1	28.4	23.0	15.4	0.8	
Pre-neck	15.0	32.8	20.0	15.8	8.8	2.5	0
	18.0	29.3	22.2	20.0	5.9	4.1	0
Mean	16.5	31.1	21.1	17.9	7.4	3.3	
Pre-parietal	0	0	0	16.7	36.7	3.2	0
	0	0	0	13.3	43.9	2.0	0
Mean				15.0	40.3	2.6	

TABLE 3. Labeling index (% ± s.e.) of immature isthmal cells after continuous ^{3}H-thymidine infusion for 1–4 days (Experiment III: N = 3 at 1 and 4 days: N = 4 at 2 and 3 days)[1]

	Infusion days			
Cell type	1	2	3	4
Granule-free	67.4 ± 1.4	96.9 ± 0.6	97.4 ± 0.1	99.1 ± 0.5
Pre-pit	58.5 ± 2.3	97.3 ± 0.7	97.8 ± 0.2	98.7 ± 0.7
Pre-neck	22.2 ± 1.9	64.8 ± 2.7	89.3 ± 1.3	98.3 ± 0.9
Pre-parietal	0.9 ± 0.9	24.4 ± 2.0	68.2 ± 5.3	95.1 ± 3.1

[1]At times later than 4 days, the labeling index of the four immature cell types is 100%.

TABLE 4. Surface area of nucleoli in immature isthmal cells (Experiment IV)[1,2]

Cell type	Mean ± s.e. (μm^2)
Granule-free	2.11 ± 0.42
Pre-pit	2.45 ± 0.23
Pre-neck	1.90 ± 0.16
Pre-parietal	1.32 ± 0.09

[1]The largest nucleoli were selected for measurement.
[2]Nucleolar area does not differ significantly, except in pre-parietal cells in which it is significantly smaller than in pre-pit and pre-neck cells.

TABLE 5. Mitochondrial width and nodule diameter in immature isthmal cells (Experiment IV)

Cell type	Mitochondrial width ± s.e. (nm)[1]	Nodule diameter ± s.e. (nm)[2]
Granule-free	333 ± 1.9	288 ± 6.7
Pre-pit	299 ± 12.6	244 ± 23.7
Pre-neck	374 ± 19.0	232 ± 11.2
Pre-parietal[3]	374 ± 23.0	

[1]Mitochondrial width does not differ significantly except in pre-pit cells where it is significantly smaller than in the other cells.
[2]The nodule diameter is significantly greater in granule-free cells than other cells. (Nodules are infrequent in pre-parietal cells and have not been recorded.)
[3]The pre-parietal value was taken from the immature parietal cells described as stage I in article IV (Table 6; Karam, 1993).

scope (LM), fixation in mixed aldehydes, postfixation in osmium tetroxide, and staining of 0.5-μm thick sections in toluidine blue; and for the electron microscope (EM), addition of tannic acid to the mixed aldehyde fixative and postfixation in osmium partly reduced by ferrocyanide (Karam and Leblond, 1992). The EM procedure emphasized membranes and, as a result, plasma membrane and glycocalyx were prominent, while organelles were sharply outlined.

Four experiments made use of 2-month-old male C57BL/6 mice weighing 26 gm on the average. The mice were kept in cages provided with water and food ad libitum until the time of sacrifice.

Experiment I: Cell Survey in the LM

Two mice under anesthesia were perfused through the left ventricle with lactated Ringer's solution for about 15 seconds, followed by a mixture of 2.5% glu-

Fig. 4. Subtype I granule-free cell referred to as undifferentiated. This cell is characterized by a Golgi stack (G) devoid of prosecretory vesicles and, therefore, considered not to be involved in secretory granule production. The inset at upper right shows a similar Golgi stack from another subtype I cell at a higher magnification; the stack is only composed of parallel saccules; at their left is part of the nucleus (N_3) and at their right is a rER cisterna and a mitochondrion containing a nodule (n). The other organelles of the main cell show typical features of immaturity. Thus, diffuse chromatin predominates in the nucleus (N_1); and one of the two nucleoli (nu) comprises many light fibrillar centers (c) associated with the dark fibrillar component (f), outside of which is the granular component (g). The cytoplasm is rich in free ribosomes (r) with few rER cisternae. There are several mitochondria (m), some of which (mn) include a dense spherical nodule. The apical plasmalemma shows a few short microvilli (mv) coated with a glycocalyx. The parietal cell at left displays long microvilli without distinct glycocalyx (MV) and large mitochondria (M). av, apical vesicles; J, junctional complex; N_2, dividing nucleus at right in a pre-pit cell characterized by a dense secretory granule (sg). (This micrograph is an enlargement of Fig. 11 in Karam and Leblond [1992].) ×19,175. **Inset:** ×30,400.

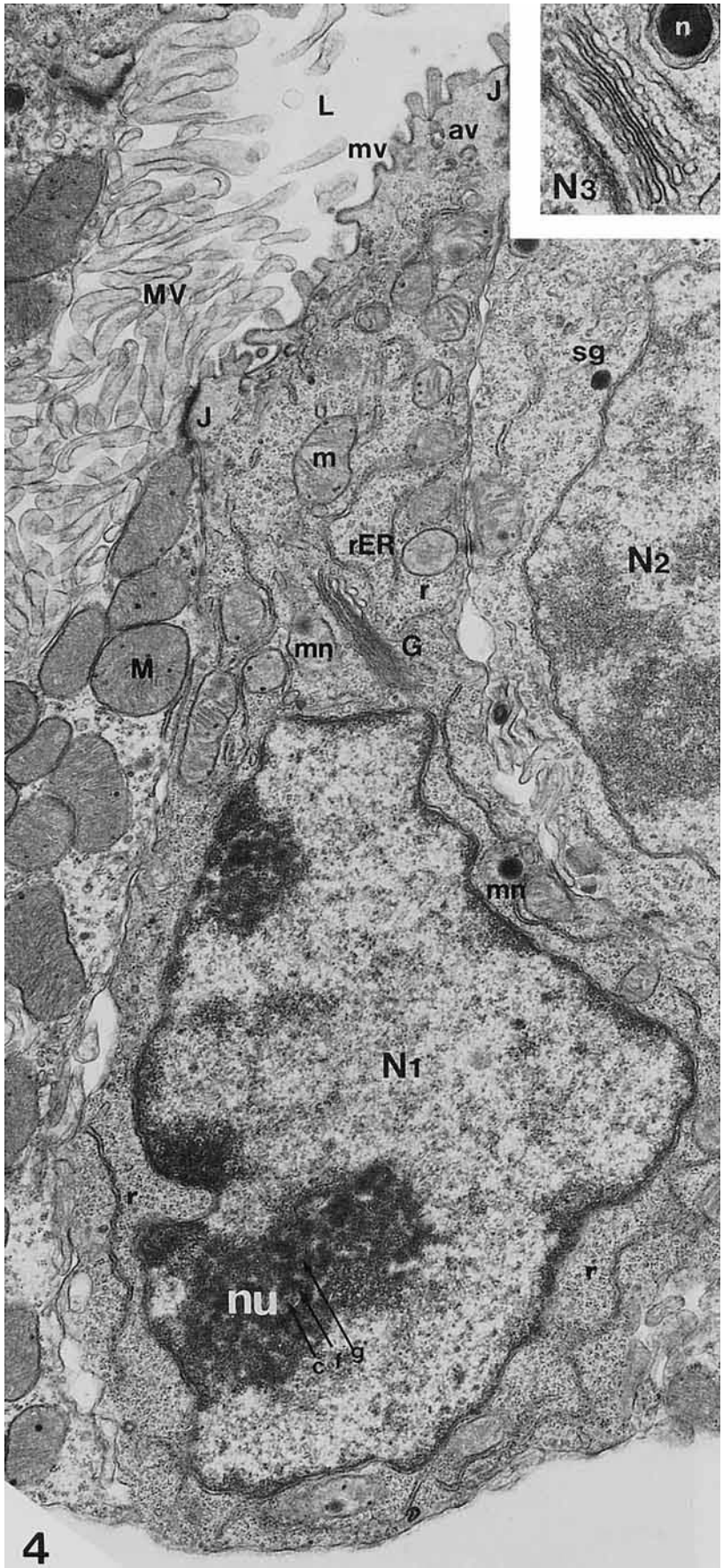
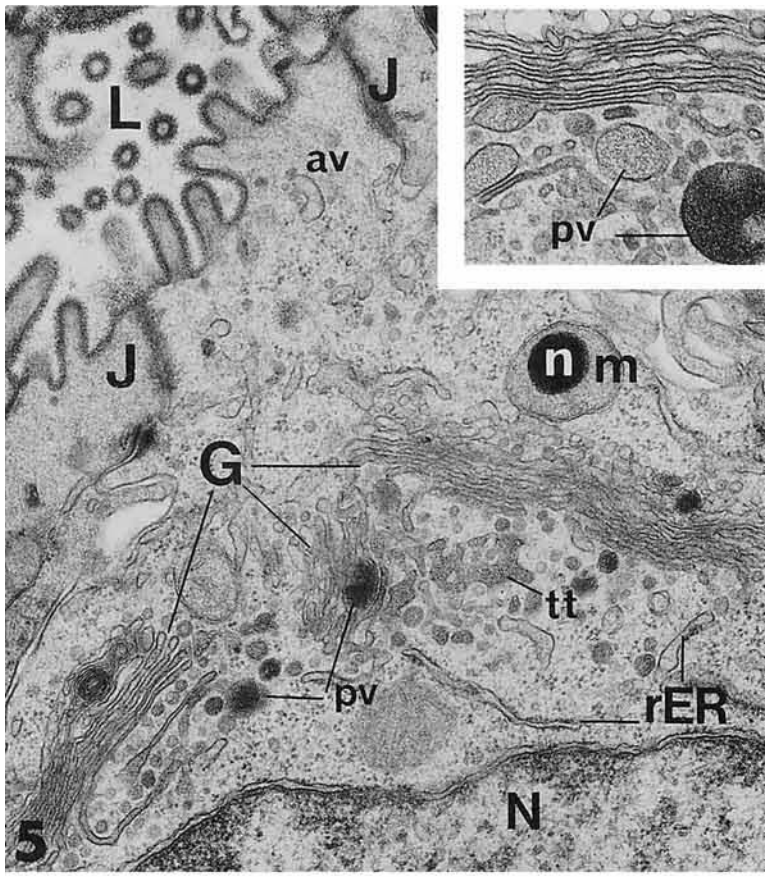
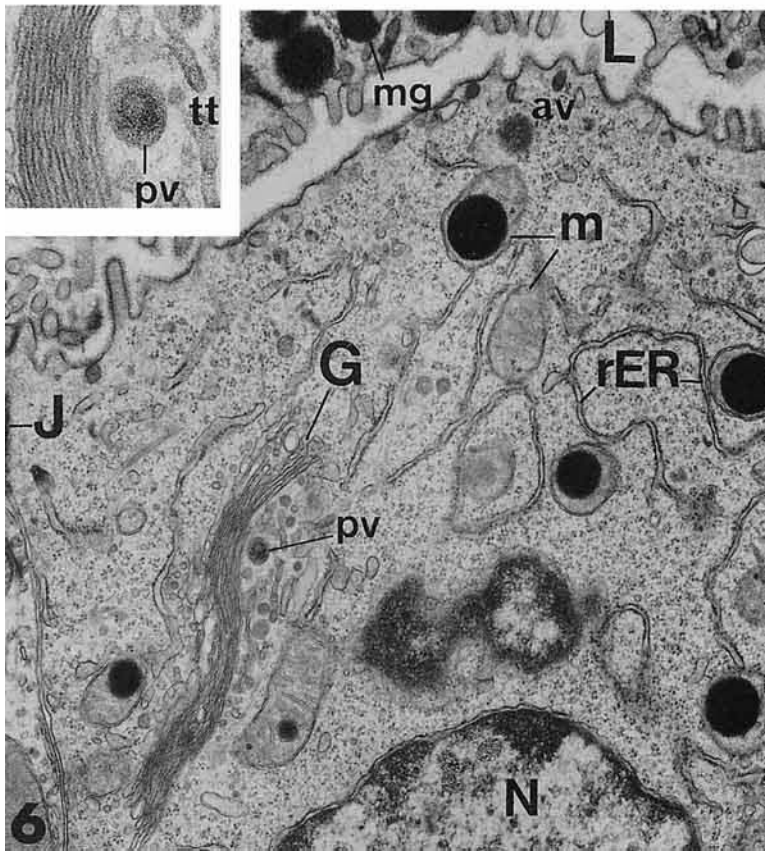


Fig. 4.





Figs. 5 and 6.

Fig. 7. Pre-pit cell, characterized by the presence of a small number of dense spherical secretory granules (sg). The cytoplasm contains a few scattered rER cisternae and mitochondria (m), while free ribosomes are abundant. Two Golgi stacks are in the supranuclear region. At their trans face, they exhibit prosecretory vesicles (pv) with fairly uniform, finely particulate content in different degrees of compactness as well as a spherical secretory granule (sg). Along the lumen (L), microvilli are coated with a prominent glycocalyx. av, apical vesicles including some C-shaped ones; J, junctional complex. ×39,000.

Fig. 5. Subtype II granule-free cell, referred to as pre-pit cell precursor. This cell is characterized by a Golgi apparatus (G) that includes prosecretory vesicles (pv) with fairly uniform content. Three Golgi stacks are visible, one of which is associated with a transtubular network (tt) containing finely granular material. The prosecretory vesicles contain material that varies in density, but is finely particulate throughout, as best seen in the inset. Because of the similarity between these features and those observed in the Golgi apparatus of pre-pit cells (Fig. 7), this cell is considered to be a pre-pit cell precursor. In the inset, the densest prosecretory vesicle shows two pale circular areas whose texture is different from that in the core of pre-neck cell granules; they are due to deep indentations of the vesicle membrane. The cytoplasm includes many free ribosomes, but few rER cisternae. The cell apex shows a few apical vesicles, one of which is C-shaped (av). Along the lumen (L), the microvilli show a prominent glycocalyx. J, junctional complex; m, mitochondrion with nodule (n); N, nucleus. ×30,400. **Inset:** ×38,000.

Fig. 6. Subtype III granule-free cell, referred to as pre-neck cell precursor. This cell is characterized by a Golgi apparatus (G) that includes prosecretory vesicles (pv) containing dense, irregular central material surrounded by a light gray periphery (inset)—that is, features similar to those of pre-neck cell prosecretory vesicles (Fig. 8). Hence the cell is designated pre-neck cell precursor. The nucleus (N), seen at bottom, is cut twice. Several of the mitochondria (m) contain nodules of various sizes. There are few rER cisternae and many free ribosomes. Across the isthmus lumen (L) the apical cytoplasm of a pre-neck cell includes cored mucous granules (mg). av, apical vesicles; J, junctional complex. Inset: tt, transtubular network. ×21,000. **Inset:** ×53,200.

TABLE 6. Secretory granule diameter in pre-pit and pre-neck cells (Experiment IV)

Cell type	Granule shape	Diameter ± s.e. (nm)[1]
Pre-pit	Spherical granule	210 ± 23
	Ovoid granules	205 ± 11
Pre-neck	Cored and uncored granules	398 ± 14

[1]Statistical analysis shows no significant difference between the spherical and ovoid granules of pre-pit cells, but both are significantly smaller than pre-neck cell granules.

taraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate, pH 7.4, for about 5 min. Small pieces from the upper left dorsal quarter of the corpus were cut out and immersed in the same fixative for 2 h. Remnants of the fixative were washed from the tissue in 0.1 M sodium cacodylate buffer, postfixed in 1% osmium tetroxide for 1 h, dehydrated in graded alcohols, and embedded in Epon in such a way that the zymogenic units could be cut along their length. To check whether such an orientation had been achieved, 0.5-μm thick sections were cut and stained by 0.3% toluidine blue in 0.3% sodium borate. If the orientation was not adequate, the blocks were trimmed and repositioned until some of the units were cut longitudinally and showed a lumen. Most of the work was done in serially cut longitudinal sections of units. In a few cases, serial cross sections cut through the whole length of the unit were used for cell counting. Every cell and nucleus was then drawn in every section as previously described (Karam and Leblond, 1992). In this manner every cell was counted and accurate results were obtained without stereological correction.

Experiment II: Cell Labeling After a Single ^{3}H-Thymidine Injection

Eighteen mice received, through the dorsal tail vein, an injection of 1 μCi per gm body weight of ^{3}H-thymidine (77.9 Ci/mmole; New England Nuclear). Four were sacrificed 30 min following the injection and the others in pairs after 3, 6, or 12 h or 1, 2, 4, or 8 days. Following sacrifice, stomach tissues were processed as in Experiment I.

The radioautographic study was carried out for each animal on a block from which 92 half-micrometer thick sections were cut serially. To avoid counting a cell more than once and to confirm the identify of each cell, sections 1, 11, 21, 31, 41, 51, 61, 71, 81, and 91 were placed on a first slide for radioautography and sections 2, 12, 22, 32, 42, 52, 62, 72, 82, and 92 were mounted on a second slide for toluidine blue staining. The intervening sections were discarded. Thus, each radioautographed section could be matched to the toluidine blue stained adjacent section. The sections destined for radioautography were exposed for 5 min to an iron alum solution and stained for 15 min in Regaud's iron hematoxylin on a hot plate at 80°C, then coated with Kodak NTB2 emulsion (Kopriwa and Leblond, 1962) and exposed for 45 days prior to development. Examination was carried out in two Reichert light microscopes placed side by side. In the first microscope, a radioautographed section was examined to find out whether any given cell was labeled, while in the other microscope the matching toluidine blue stained section helped in ascertaining the identity of the cell, using previously described criteria (Karam and Leblond, 1992). A cell was considered labeled if three or more silver grains were present over its nucleus. The results were expressed as labeling index, that is, the ratio of the number of labeled cells over the sum of labeled and unlabeled ones, times one hundred.

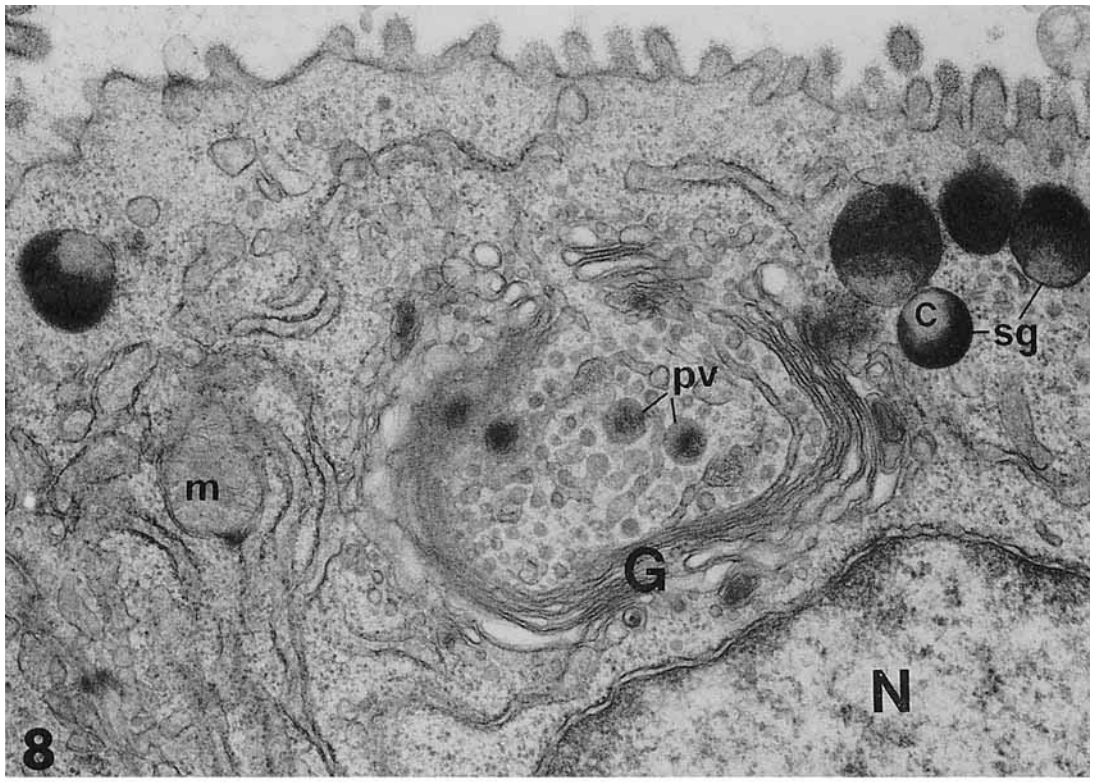
Experiment III: Cell Labeling After Continuous ^{3}H-Thymidine Infusion

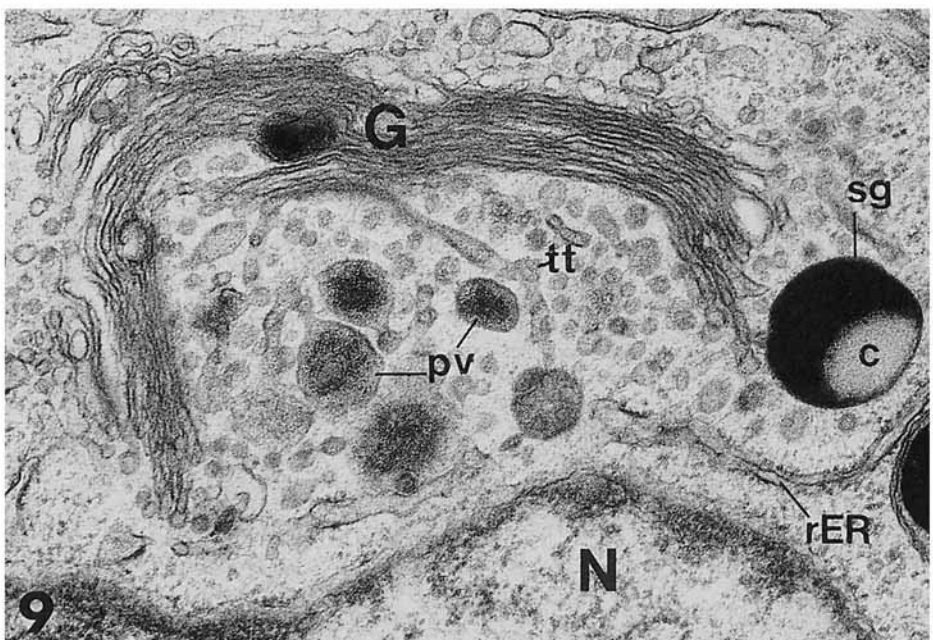
To make sure that all dividing cells and their progeny would be labeled during periods varying from 1–52 days, a very low dose of ^{3}H-thymidine, 0.1 μCi per gm body weight per day in 1 ml of Ringer's solution, was given by continuous infusion to each of 14 mice. A subcutaneously inserted PE20 polyethylene tubing (Clay Adams) was connected to a Sage (model 249-5) syringe pump, as described previously (Tsubouchi and Leblond, 1979). During the first day of infusion, the 1 ml syringe was filled with Ringer's solution to accustom the animal to the intubation. The next day, the syringe was filled with ^{3}H-thymidine solution to start the continuous infusion; however, the infusion was considered to begin 4.8 h later, that is, after the time required to fill up the tubing, so that the ^{3}H-thymidine could then reach the animal's tissues. The animals were sacrificed in groups of three or four by perfusion with glutaraldehyde-formaldehyde as in Experiment I. Tissues were postfixed in osmium tetroxide. As in Experiment II, paired slides were prepared from each animal—that is, toluidine blue stained slides and radioautographs which, because of the minute daily dose, had to be exposed as long as 110 days. The labeling index was then determined using paired sections as in Experiment II.

Experiment IV: Ultrastructural Analysis

Eight mice were perfused for about 15 seconds with lactate Ringer's solution, followed by a mixture of glutaraldehyde, paraformaldehyde, and tannic acid in concentrations of 2.5, 2.0, and 0.2%, respectively, in 0.1 M cacodylate buffer, pH 7.4, for 10–15 min. Pieces were taken from the upper left dorsal quarter of the stomach corpus, postfixed in osmium tetroxide partially reduced in ferrocyanide (Karnovsky, 1971), and embedded in Epon for EM study. Blocks were trimmed and, if necessary, reoriented until the units were cut longitudinally. Thin, silver colored sections were stained with uranyl acetate and lead citrate.

Since the large majority of the 30-min labeled cells was in the isthmus, the EM study was focused on the four poorly differentiated cell types present in this region. The size of their nucleoli, mitochondria, and secretory granules, when present, was estimated in micrographs, usually at ×30,000. The six largest nucleoli observed for each type, presumed to be cut through their central region, were selected for measurement, outlined on the micrographs, and their surface area determined in a Zeiss MOP (Manual Optical Planimeter). The average width of clearly outlined mitochondria was also determined in six or more micrographs from different cells for each cell type. In addition, the mean diameter of the dense intramitochondrial spherical inclusions referred to as nodules (see Figs. 5 and 6) was also estimated on these micrographs. Fi-





Figs. 8, 9. Pre-neck cells, characterized by dark secretory granules (sg) containing a pale spherical or elliptical core (c). G, Golgi stack; N, nucleus.

Fig. 8. The cell is rich in free ribosomes. The Golgi stack surrounds the trans face, which includes several prosecretory vesicles (pv) characterized by a dark irregular center and a grayish periphery. m, mitochondria. ×30,400.

Fig. 9. Trans face of a Golgi stack at higher magnification, illustrating a structure that may be part of the transtubular network (tt) and several prosecretory vesicles (pv) composed of a grayish peripheral area and dense central material within which irregular tubule-like material may be discerned. ×39,000.

Fig. 10. Variant I pre-parietal cell, devoid of secretory granules. This cell is identified as pre-parietal by the presence at the apex of long microvilli (MV) without a distinct glycocalyx. The narrow cytoplasm is packed with free ribosomes and includes small mitochondria (m), one of which shows a nodule (n), and few short rER cisternae. N, nucleus. ×11,700.

nally, the diameter of secretory granules, if present, was measured whenever their outline was clearcut. The longest and shortest axes were averaged to obtain the mean diameter of the granules. When the two diameters of the granules were equal or did not differ by more than 10%, they were said to be spherical. Otherwise, whether ellipsoidal or piriform, they were designated ovoid. The mean diameter was measured in 10 spherical and 10 ovoid granules in each of six micrographs.

RESULTS

LM Identification of Epithelial Cells in the Four Regions of the Zymogenic Unit

The pit is lined by pit cells, among which parietal cells are scattered (Fig. 1). In toluidine blue stained semithin sections, pit cells are characterized by the presence of a group of closely packed secretory granules at their apex. This group is thin in the pit cells located close to the isthmus border (Fig. 2) and usually increases in size in the cells along the pit up to its opening, but decreases at the free surface (Fig. 1). The parietal cells stand out by their large size, secretory canaliculus, and numerous mitochondria.

The isthmus is characterized by the presence of four small cell types crowded between parietal cells, namely pre-pit cells, which include a few hazy specks identified as secretory granules (Fig. 2); granule-free cells characterized by the absence of secretory granules; pre-neck cells containing a few light secretory granules (Fig. 2), which may include a minute dark particle corresponding to the core seen in the EM (see Fig. 8); pre-parietal cells, characterized by a pale area at the apex corresponding to crowded microvilli (Fig. 1) and/or by a light region in the cytoplasm produced by an incipient canaliculus (Fig. 2).

The neck is composed of neck cells that seem to be squeezed between parietal cells and are characterized by numerous pale granules (Fig. 2) within which a minute dark particle is occasionally distinguished.

The base is characterized by zymogenic cells with intensely stained secretory granules and, close to the neck-base border, pre-zymogenic cells with granules intermediate in density between those of neck and zymogenic cells. Parietal cells are less packed in the base than in isthmus or neck (Fig. 1), while entero-endocrine cells are mainly scattered in the base.

Radioautography

Cell labeling after a single ^{3}H-thymidine injection (Experiment II)

The 30-min radioautographs show labeled cells within the isthmus and close to it (Fig. 3). Cell counts in the LM (Table 1) indicate that cells may be classified into four groups: 1) unlabeled cells, including parietal, pre-parietal and caveolated cells; 2) occasionally labeled cells—that is, zymogenic (four labeled cells, all in the same animal), pre-zymogenic (one labeled) and entero-endocrine cells (unlabeled in this series); 3) regularly but weakly labeled cells—that is, pit cells (0.8%) and neck cells (1.8%) with the label restricted to those in the neighborhood of the isthmus (Fig. 3); and 4) highly labeled cells—that is, granule-free cells (32.4%), pre-pit cells (24.6%), and pre-neck cells (11.3%), all in the isthmus.

The labeling index of the four small isthmal cell types has also been examined at various time intervals after a single ^{3}H-thymidine injection (Table 2). The labeling of granule-free, pre-pit, and pre-neck cells reaches a peak toward 6 h, declines by 1 day, and keeps on decreasing thereafter to disappear by 8 days. In pre-parietal cells the label does not appear until 1 day after injection, reaches a peak at 2 days, decreases thereafter, and is absent by 8 days (Table 2).

Continuous ^{3}H-thymidine infusion (Experiment III)

In granule-free and pre-pit cells (Table 3), the labeling index rises at 1 day to approach 100% by 2 days. The rise occurs later in pre-neck cells and even more so in pre-parietal cells, but in both the index approximates 100% by 4 days.

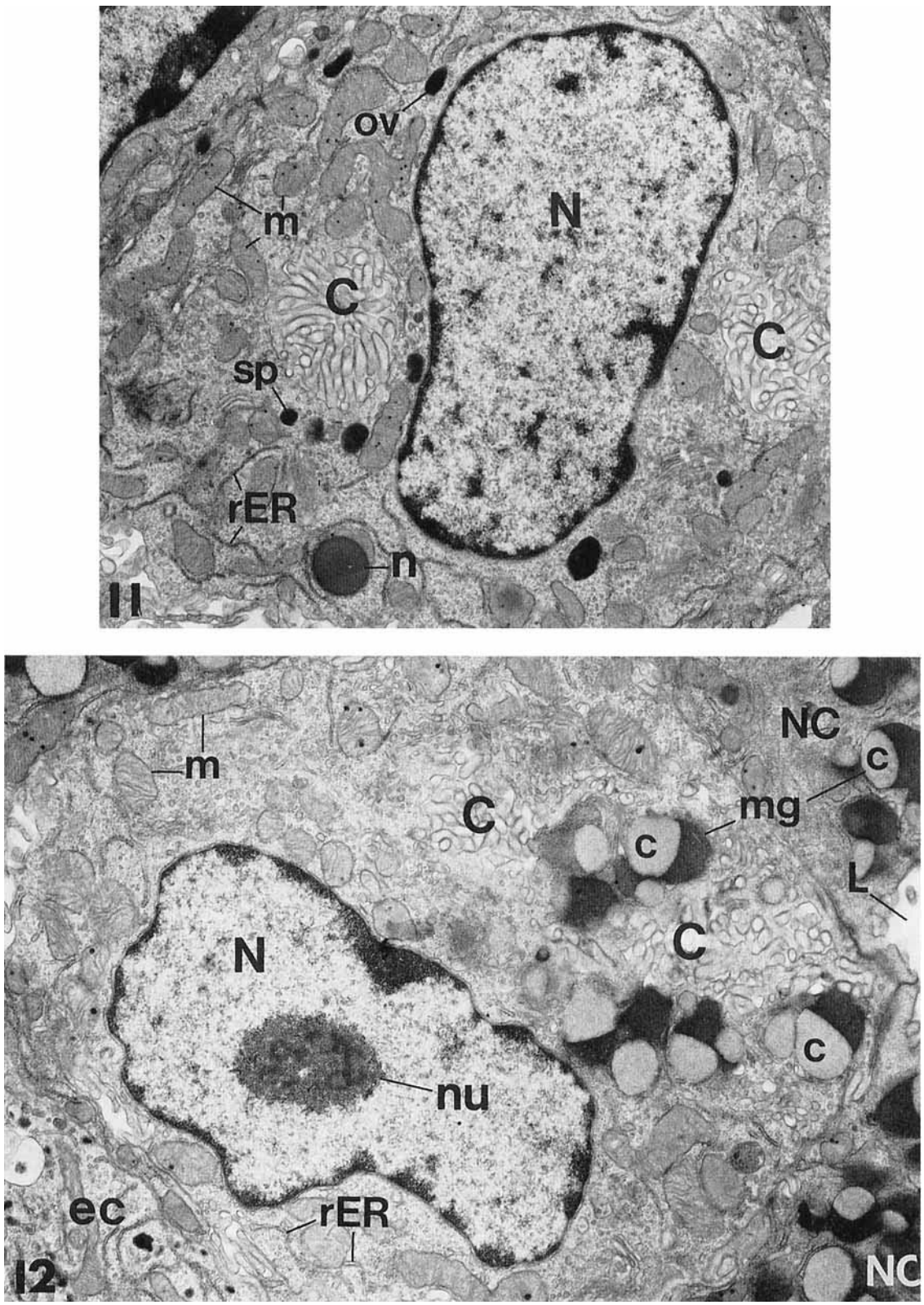
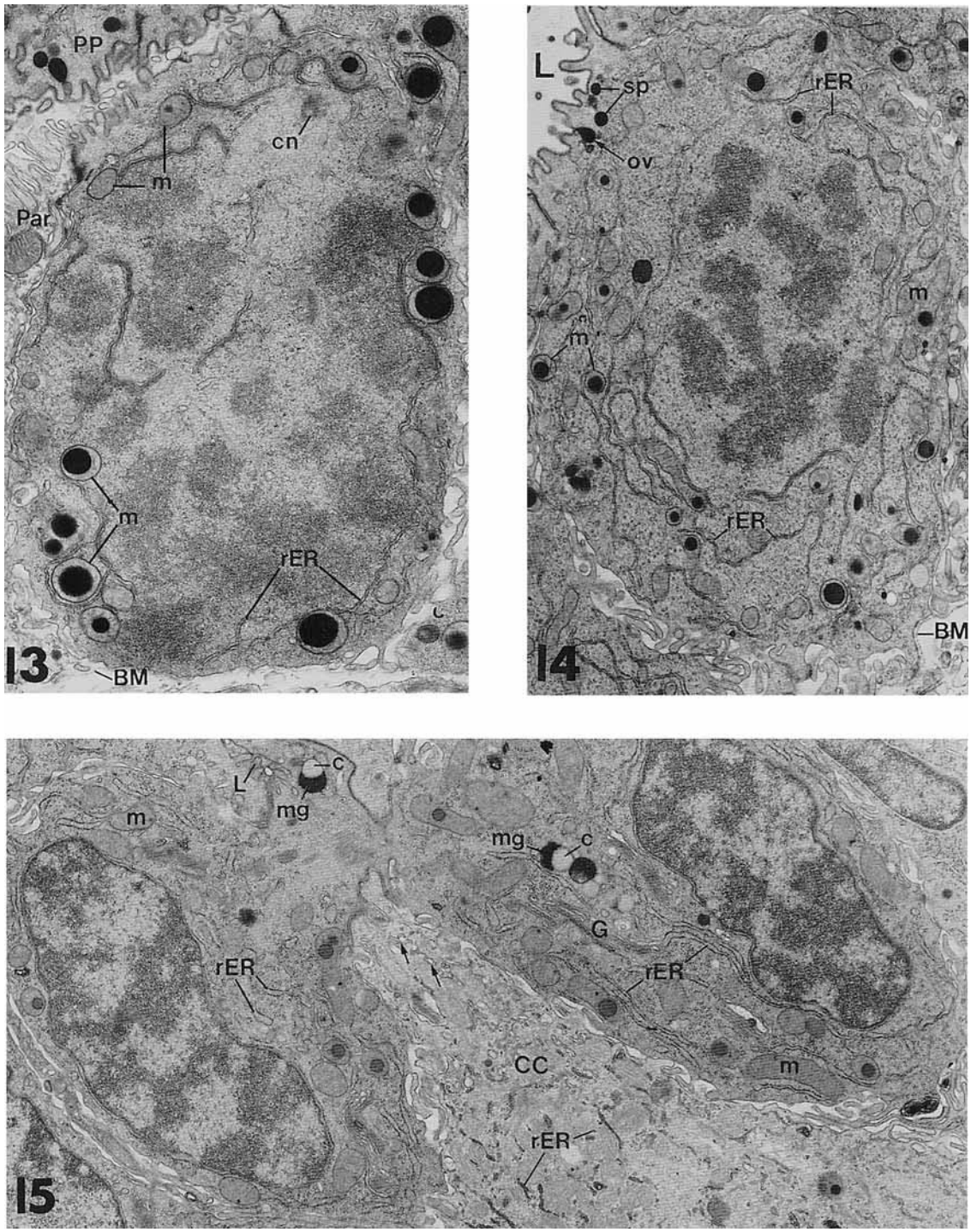


Fig. 11. Variant II pre-parietal cell, characterized by the presence of a few ovoid (ov) and spherical (sp) dense granules similar to those in pre-pit cells. This cell is identified as pre-parietal by the presence of two small intracellular canaliculi (C) packed with long microvilli and by relatively numerous mitochondria (m) which may contain a nodule (n). There are many free ribosomes and few rER cisternae. N, nucleus. ×11,700.

Fig. 12. Variant III pre-parietal cell, characterized by a few mucous granules (mg) with light core (c) similar to those in two adjacent neck cells (NC). The two canaliculi (C) filled with microvilli identify the cell as pre-parietal. The nucleus (N) contains a moderately large nucleolus (nu) and is surrounded by cytoplasm rich in mitochondria (m) and free ribosomes. Cisternae of rER are few. ec, entero-endocrine cell; L, lumen. ×13,490.



Figs. 13–15. Mitotic figures of three immature cell types in the isthmus region. ×11,000.

Fig. 13. Granule-free cell mitosis characterized by the lack of secretory granules. Metaphase chromosomes and free ribosomes occupy most of the cytoplasm, while rER cisternae and nodule-containing mitochondria (m) are scattered at the periphery. The cell apex projects a few small microvilli into the lumen at upper center. The long microvilli at upper left indicate the presence of a parietal cell (Par). BM, basement membrane; cn, centriole; PP, pre-pit cell.

Fig. 14. Pre-pit cell mitosis characterized by the presence of few dense spherical (sp) and oval granules (ov) near the apical membrane. Metaphase chromosomes occupy the central cytoplasm, whereas rER cisternae and nodule-containing mitochondria (m) are scattered at the periphery. BM, basement membrane; L, lumen.

Fig. 15. Pre-neck cell in telophase, with the two daughter cells about to separate. Two mucous granules (mg) exhibit a core (c), one in the left daughter cell close to the narrow lumen (L) and the other in the right daughter cell next to the Golgi apparatus (G). The cytoplasm of a caveolated cell (CC) at lower right contains small tubular elements (arrows) and dense rER cisternae. m, mitochondria.

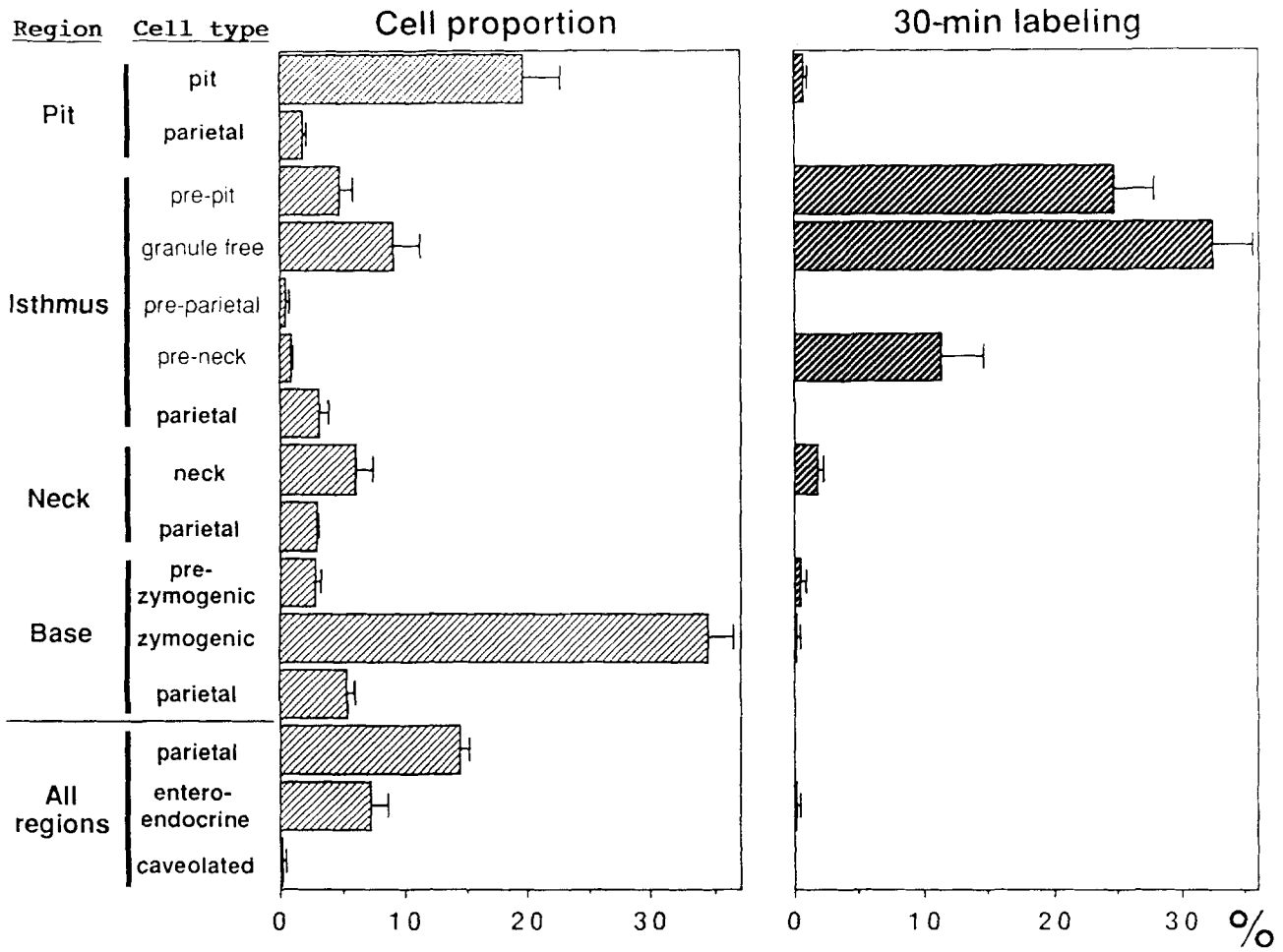


Fig. 16. On the left, the frequency of the cells of each type is expressed as a percent (taken from Karam and Leblond, 1992). On the right, the labeling index of the cells of each type 30 min after a ^{3}H-thymidine injection is the ratio of the number of labeled over total cells. Comparison between the two sides of the diagram reveals that the most abundant cells—zymogenic, pit, and parietal cells—are little or not labeled, while only three isthmal cell types are highly labeled, namely granule-free, pre-pit, and pre-neck cells.

EM Studies (Experiment IV)

Ultrastructure of granule-free, pre-pit, pre-neck, and pre-parietal cells

The previous description of these cells (Karam and Leblond, 1992) will be summarized and completed by examination of the Golgi apparatus and by measuring the size of some organelles. First, the granule-free cells (Figs. 4–6) are small, poorly differentiated, and devoid of secretory granules. In the nucleus, diffuse chromatin predominates. Nucleoli are large (Fig. 4) with a 2.1-μm^2 mean surface area (Table 4). The scanty cytoplasm is rich in free ribosomes and includes mitochondria (Fig. 4) whose width averages 333 nm (Table 5). Some mitochondria display dense spherical inclusions, previously named "nodules" (Karam and Leblond, 1992); their diameter averages 288 nm (Table 5). Next to the apical membrane there are small irregular, spherical, oval, or C-shaped structures referred to as apical vesicles (Figs. 5, 6). The Golgi apparatus shows marked variation, but can be categorized into one of three patterns, which have been used to classify granule-free cells into three subtypes. In subtype I (Fig. 4), Golgi stacks are small and their trans face exhibits no distinct prosecretory vesicles (inset of Fig. 4). In subtype II (Fig. 5), Golgi stacks are moderately well developed and include prosecretory vesicles which have a uniformly fine particulate content but highly variable density (Fig. 5, inset). In subtype III (Fig. 6), Golgi stacks are also moderately well developed but the prosecretory vesicles are characterized by irregular dense material in the center and a light gray periphery (Fig. 6, inset). Out of 19 granule-free cells randomly selected and photographed, four have been classified as subtype I, eleven as subtype II, and four as subtype III cells.

Pre-pit cells are also small and poorly differentiated but include a few dense spherical and oval secretory granules (Fig. 7) whose respective diameters average 210 and 205 nm (Table 6). The apical membrane exhibits microvilli with prominent glycocalyx. Below this membrane, it is often possible to distinguish a narrow zone which is devoid of rER cisternae and mitochondria, but may contain secretory granules and apical vesicles (Fig. 7). Because of the absence of major

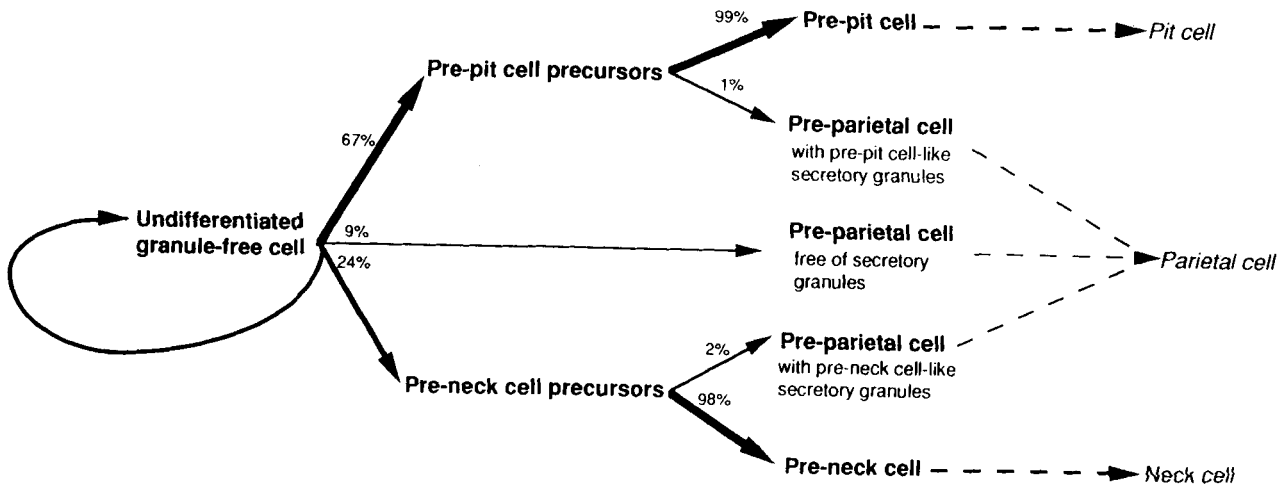


Fig. 17. Model for the origin of cells in the isthmus of the mouse corpus epithelium. The undifferentiated granule-free cell (subtype I) is the pluripotential stem cell, which not only reproduces itself, but gives rise to the pre-pit cell precursor (granule-free cell subtype II), pre-neck cell precursor (granule-free cell subtype III), and the pre-parietal cell free of secretory granules (variant I). The pre-pit cell precursor is a partially committed progenitor from which arise the pre-pit cell and the pre-parietal cell with pre-pit cell-like secretory granules (variant II). Similarly, the pre-neck cell precursor is another partially committed progenitor yielding the pre-neck cell and the pre-parietal cell with pre-neck cell-like secretory granules (variant III). The proportion of cells produced by each granule-free subtype is given on its right along the arrows, as calculated in Appendix A. Finally, as indicated by dashed arrows, the fate of pre-pit cell, pre-neck cell, and pre-parietal cell is tentatively the differentiation into pit, neck, and parietal cell, respectively.

organelles in this zone and its peripheral position, it has been previously referred to as ectoplasm in pre-pit as well as pit cells (Karam and Leblond, 1992). In the nucleus, most of the chromatin is diffuse. Nucleoli are large, with a mean 2.45 μm^2 surface area (Table 4). The cytoplasm contains an abundance of free ribosomes. Both mitochondrial width (299 nm, Table 5) and nodule diameter (244 nm) are smaller than in granule-free cells. Golgi stacks comprise prosecretory vesicles exhibiting a uniformly fine particulate content in various degrees of condensation (Fig. 7), a pattern similar to that observed in subtype II granule-free cells (Fig. 5).

Pre-neck cells (Fig. 8) are poorly differentiated and display a few secretory granules which usually have a light core. The granule diameter is about twice as large as in pre-pit cells (Table 6). The nucleus has mainly diffuse chromatin, while the surface area of nucleoli averages 1.9 μm^2 (Table 4). In the cytoplasm, free ribosomes are numerous. Mitochondrial width (374 nm, Table 5) does not differ from that in granule-free cells, but nodules are less common and smaller (232 nm) than in these cells. Golgi stacks are fairly well developed and include prosecretory vesicles which contain dark irregular material with a light gray periphery (Figs. 8, 9), as in subtype III granule-free cells (Fig. 6).

Finally, pre-parietal cells are in contact with the unit's lumen by a narrow surface showing long microvilli without distinct glycocalyx (Fig. 10). They vary in size and degree of differentiation, and may include small canaliculi lined with long, bent microvilli (Figs. 11, 12). Three variants of these cells have been observed. Variant I, the most frequent and least differentiated, shows no structure resembling secretory granules (Fig. 10). Variant II displays a few spherical or oval dense secretory granules (Fig. 11) similar to those in pre-pit cells. Variant III exhibits a few secretory granules with a pale core (Fig. 12) as observed in pre-neck cells. Examination of 33 randomly photographed pre-parietal cells has revealed that 22, 7, and 4, respectively, belong to the first, second, and third variant.

Search for mitotic figures in the EM

Mitotic figures are often observed in the isthmus. Some of them are devoid of secretory granules and accordingly identified as granule-free cells (Fig. 13). Others include the dense spherical and oval secretory granules characteristic of pre-pit cells (Fig. 14). Others still display cored granules typical of pre-neck cells (Fig. 15).

DISCUSSION

Using the 30-min labeling index as a measure of mitotic activity, the eleven cell types were classified into four groups according to whether mitotic activity was absent, rare, low, or high. Absence of mitotic activity was observed in parietal cells in accord with Willems et al. (1972), MacDonald et al. (1964), and Creamer et al. (1961), as well as in pre-parietal and caveolated cells. Rare mitotic activity occurred in zymogenic cells, as previously reported by Kataoka (1970), Chen and Withers (1975), Tamura and Fujita (1983), and Willems et al. (1972) and also in pre-zymogenic cells and entero-endocrine cells (Karam and Leblond, 1993c). Low mitotic activity was regularly observed in pit and neck cells. High mitotic activity was present in three isthmal cell types: granule-free, pre-pit, and pre-neck cells, which together accounted for 90% of the label taken up in the oxyntic epithelium. Comparison of cell frequency (left of Fig. 16) and 30-min labeling index (right) emphasized that the cells which are little or not mitosable—namely zymogenic, parietal, and pit

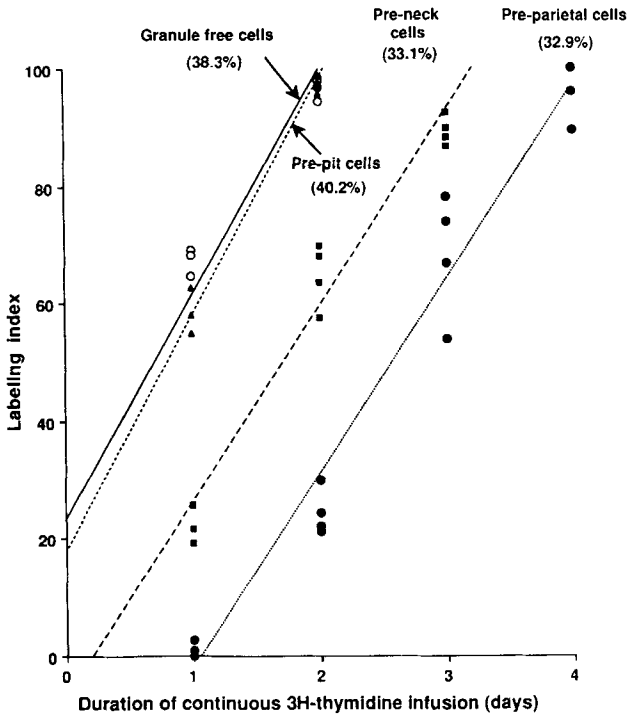


Fig. 18. Labeling index of the four immature isthmal cell types plotted against the duration of continuous ^{3}H-thymidine infusion. The slope of the regression lines provides an estimate of the daily turnover rate (that is, the number below the name of each cell type).

cells—were the most numerous, whereas the highly mitosable granule-free, pre-pit and pre-neck cells were in relatively small numbers.

The ability of some gastric epithelial cells to divide was attributed to mitogenic factors, such as TGFα (transforming growth factor alpha) which was shown to be produced by cultured cells isolated from the oxyntic mucosa (Chen et al., 1990a,b).

Sequence of Events in the Isthmus

Stem cells are generally characterized in two ways. Functionally, they have the ability to proliferate so as to ensure their own renewal while producing committed cells (Hall, 1989). Morphologically, they display embryonic-like features such as a scanty cytoplasm rich in free ribosomes and a nucleus containing diffuse chromatin and large nucleoli (Leblond, 1981). Three of the four small isthmal cell types exhibited the proliferative ability and morphological features of stem cells: granule-free, pre-pit, and pre-neck cells. The presence of three potential stem cells raised the possibility of a hierarchy between them, as in the bone marrow, where a "pluripotential stem cell" was known to give rise to stem cells with a limited potential, known as "committed progenitors" (Hall, 1989). In fact, two of the potential stem cells, the pre-pit and pre-neck cells, produced a few small but distinct secretory granules and, therefore, were partly differentiated; they could be committed progenitors. In contrast, the third potential stem cell, the granule-free cells, were poorly differentiated and could play the role of pluripotential stem cells.

Granule-free cells

Corpron (1966) and Kataoka (1970) had previously mentioned the existence of cells devoid of granules in the oxyntic epithelium and suggested that they were stem cells. In the epithelium of the mouse antrum, granule-free cells were described in the isthmus and considered to be stem cells, from which arose "dense granule cells" (similar to pre-pit cells) and "core granule cells" (similar to pre-neck cells) (Lee and Leblond, 1985a). In the present work, the granule-free cells were subdivided into three subtypes according to the Golgi pattern. The subtype I cell, which lacked prosecretory vesicles in the Golgi stacks (Fig. 4), was primitive, showed no sign of differentiation, and will be referred to as undifferentiated granule-free cell. The subtype II cell, which had prosecretory vesicles with a finely particulate content (Fig. 5) as in those of pre-pit cells (Fig. 7), was presumably committed to become a pre-pit cell and will be referred to as pre-pit cell precursor. The subtype III cell, which was characterized by prosecretory vesicles containing dense irregular material with a light periphery (Fig. 6) as in pre-neck cells (Figs. 8, 9) was presumably committed to become a pre-neck cell and will be referred to as pre-neck cell precursor. Of the three subtypes, the undifferentiated granule-free cell alone had all the immature features expected from a pluripotential stem cell (Leblond, 1981). It will accordingly be taken to be the stem cell of the oxyntic epithelium. As such, it will give rise to the other two subtypes—that is, the pre-pit cell precursor and the pre-neck cell precursor. In addition, evidence will be presented below that the three subtypes are involved in the production of pre-parietal cells.

In general stem cells were known to be restricted to a limited region such as the crypt base in mouse small intestine (Bjerknes and Cheng, 1981a,b). In the mouse corpus, granule-free cells, including the undifferentiated ones, were known to occupy the central part of the isthmus (Karam and Leblond, 1992). Such restriction implied that they were more or less stationary, while the pre-pit, pre-neck, and pre-parietal cells differentiating from them migrated away.

Pre-pit cells

Pre-pit cells could arise either from the differentiation of pre-pit cell precursors (subtype II) or from their own mitoses. The proportion of cells arising from each one of these two sources was calculated on the assumption that the labeling of each one of the three granule-free subtypes was proportional to its frequency (Appendix B). Under these conditions, 57% of the pre-pit cells arose from differentiation while 43% came from their own mitoses. Moreover since, in the isthmus, these cells were generally located on the distal side of the centrally located granule-free cells (Karam and Leblond, 1992), the pre-pit cells arising from differentiation had to migrate away from the center in a distal direction toward the gastric lumen.

The fate of pre-pit cells was suggested by their behavior after a single ^{3}H-thymidine injection (Table 2). The labeling was high initially but soon decreased and,

by 8 days, had vanished. The loss of label was not due to degeneration or death of pre-pit cells, of which no sign was observed, and was, therefore, attributed to their transformation into other cell types, such as pit cells. This possibility will be critically examined in the next article of this series.

Pre-neck cells

Pre-neck cells could be derived either from the differentiation of pre-neck cell precursors (subtype III) or from their own mitoses. Assuming that the labeling of the three granule-free subtypes was proportional to their frequency, it was estimated that 85% of the pre-neck cells arose from differentiation and 15% from mitosis (Appendix B). Since pre-neck cells predominated on the proximal side of the isthmus (Karam and Leblond, 1992), their differentiation from the centrally located granule-free cells implied migration away from the center in a proximal direction away from the gastric lumen.

The pre-neck cells labeled after a ^{3}H-thymidine injection decreased in number until they vanished by 8 days (Table 2), even though they showed no sign of damage. They likely transformed into other cells, such as neck cells, a possibility that will be critically examined in a subsequent article in this series.

Pre-parietal cells

Pre-parietal cells were not labeled 30 min after a ^{3}H-thymidine injection (Table 1), nor were their nuclei seen in any of the mitotic phases previously described in the EM (El-Alfy and Leblond, 1989). Yet by 1 day after injection (Table 2) or continuous infusion (Table 3), they became labeled. Hence the label must have been acquired from the transformation of one or more of the initially labeled isthmal cell types. Only granule-free cells reached a higher labeling index peak than pre-parietal cells after a single ^{3}H-thymidine injection (Table 2) and, therefore, they alone were likely to be their predecessors. In fact, each one of the three pre-parietal variants had features that could be related to one of the granule-free subtypes. Thus, the secretory granules in variant II pre-parietal cells were identical to those in pre-pit cells, the main progeny of pre-pit cell precursors (subtype II). It was accordingly postulated that variant II cells came from pre-pit cell precursors. Similarly, most secretory granules in variant III pre-parietal cells had a light core as did most of those in pre-neck cells, the main progeny of pre-neck cell precursors (subtype III). It was then postulated that variant III cells came from pre-neck cell precursors. Finally, variant I pre-parietal cells were devoid of secretory granules and, therefore, could come directly from undifferentiated granule-free cells (subtype I). Pre-parietal cells were no longer labeled by 8 days after injection (Table 2). Since they were not seen to degenerate, their likely fate was to transform into parietal cells, a possibility that will be examined in the fourth article in this series (Karam, 1993).

To sum up our proposals on the interrelations between the four immature cell types, a model of cell interactions in the isthmus was designed in which the undifferentiated granule-free cell was presented as the self-replicating pluripotential stem cell, from which arose pre-pit, pre-neck, and pre-parietal cells (Fig. 17). The approximate numbers of cells produced by the three granule-free subtypes are given in this model as percentages calculated in Appendix A.

Cell Turnover

The loss of label from granule-free, pre-pit, pre-neck, and pre-parietal cells by 8 days after a single ^{3}H-thymidine injection indicated that they turned over. The fraction of each cell population replaced per day, or turnover rate, was estimated from the continuous infusion data on the assumption of a steady state of the epithelial cells (Fig. 18). However, only a small number of points were available to construct regression lines and the reliability of values close to 100% was low, so that the results should be taken as approximations. This was particularly the case for granule-free and pre-pit cells, so that the turnover rates given in brackets under the names of the two cell types in Figure 18 might be underestimates and, if so, would exceed 38.3% per day for granule-free cells and 40.2% per day for pre-pit cells. The inverse of the turnover rate—that is, the turnover time, (the time interval required to replace a number of cells equal to the total number in the population)—was, therefore, 2.6 days for granule-free cells and 2.5 days for pre-pit cells; both figures might be overestimates. On the other hand, the data for pre-neck and pre-parietal cells appeared to be reliable. The calculated turnover rate was, respectively, 33.1% and 32.9% per day for pre-neck and pre-parietal cells, while the turnover time was 3 days for both. In conclusion, all four immature cell types were renewed fairly rapidly with turnover times of 3 days or less.

CONCLUSIONS

Within the units making up the corpus epithelium, cell proliferation essentially occurs in the isthmus, which includes three rapidly dividing cell types (granule-free, pre-pit, and pre-neck cells) and two non-dividing ones (pre-parietal and parietal cells).

A model based on the available evidence considers the undifferentiated granule-free cells as the pluripotential stem cells of the epithelium; these cells are located in the center of the isthmus and are generally stationary; they divide to maintain themselves as well as to give rise to three committed cell types: pre-pit cells, pre-neck cells, and pre-parietal cells (Fig. 17) which migrate away from them. Since other cells show little or no mitotic activity, these four cell types are likely to be the source of the other cells in the epithelium. Evidence presented in the following four articles indicates that pit, neck, parietal, and entero-endocrine cells directly come from isthmal cells, whereas zymogenic cells are derived from neck cells through a pre-zymogenic stage.

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APPENDIX A

The proposed turnover times and the data available on the numbers of granule-free cells (4, 11, and 4, respectively, for subtypes I, II, and III) and pre-parietal cells (22, 7, and 4, respectively, for variants I, II, and III) were used to obtain approximate estimates of cell production on the assumption that the cell filiation outlined in the Figure 17 model was correct.

Cell Production

The daily turnover rate of a given cell type times its number per unit gave the daily cell production in the unit, as shown for granule-free and pre-parietal cells in Table 7.

The daily production of granule-free cells was then

TABLE 7. Calculation of the number of cells produced per day

Cell type	Turnover rate (% per day)	Number of cells per unit[1]	Daily cell production[2]
Granule-free	38.3	17.2	6.6
Pre-parietal	32.9	0.6	0.2

[1]From Karam and Leblond (1992).
[2]Turnover rate multiplied by the number of cells per unit, divided by 100.

parceled out between the three subtypes in proportion to their numbers (Table 8, third column) and so was the daily production of pre-parietal cells parceled out between the three variants (Table 8, sixth column). The last column in this table indicated that, for 11 subtype I granule-free cells produced per day, only one became a variant I pre-parietal cell. In the other two cases, the difference was much greater: 95 subtype II cells for one variant II pre-parietal cell, and 58 subtype III cells for one variant III pre-parietal cell. Thus, only a low proportion of each granule-free cell subtype gave rise to the corresponding variant pre-parietal cell.

The subtype I granule-free cell, presumed to function as stem cell, had a progeny consisting of three offspring: subtype II, subtype III, and the variant I pre-parietal cell. The daily cell production rate of subtype I cells was parceled out among the three offspring (Table 9, top), yielding 9% variant I pre-parietal, 67% subtype II, and 24% subtype III cells.

Similarly, the subtype II cell was found to produce 1% variant II pre-parietal cells and 99% pre-pit cells (Table 9, middle).

Finally, the granule-free subtype III cell was found to give rise to 2% variant III pre-parietal cells and 98% pre-neck cells (Table 9, lowermost part).

The percentages obtained from Table 9 provided estimates of cell production which were recorded in the Figure 17 diagram.

TABLE 9. Progeny of granule-free cells

Progeny of subtype I (undifferentiated cell)	Daily cell production	Proportion of cells produced by subtype I cells (%)
Variant I pre-parietal cell	0.13[1]	9
Subtype II	0.93[2]	67
Subtype III	0.34[3]	24
Progeny of subtype II (pre-pit cell precursor)	**Daily cell production**	**Proportion of cells produced by subtype II cells (%)**
Variant II pre-parietal cell	0.04[4]	1
Pre-pit cell	3.76[5]	99
Progeny of subtype III (pre-neck cell precursor)	**Daily cell production**	**Proportion of cells produced by subtype III cells (%)**
Variant III pre-parietal cell	0.024[6]	2
Pre-neck cell	1.38[7]	98

[1]From Table 8.
[2]Obtained from the daily production of subtype I, that is, 1.4 cells per unit (Table 8) minus the daily production of variant I pre-parietal cells, that is, 0.13 cells per unit (Table 8), then multiplied by the number of subtype II cells, 11 (Table 8), and finally divided by the sum of subtype II and III cells, 15 (from Table 8).
[3]Obtained from the daily cell production of subtype I cells, minus that of variant I pre-parietal and subtype II cells.
[4]From Table 8.
[5]Obtained from the daily production of subtype II, that is, 3.8 cells per unit (Table 8) minus the daily production of variant II pre-parietal cells, that is, 0.4 cells per unit (Table 8).
[6]From Table 8.
[7]Obtained from the daily production of subtype III, that is, 1.4 cells per unit (Table 8) minus the daily production of pre-parietal variant III, that is, 0.024 cells per unit (Table 8).

APPENDIX B

Since pre-pit cells arise either from differentiation of pre-pit cell precursors (subtype II of granule-free cells) or from their own mitoses, an attempt was made to estimate the proportion coming from each one of these two sources. 1) The number of pre-pit cells formed by differentiation was obtained in several steps. First, the number of granule-free cells labeled 30 min after a ^{3}H-thymidine injection (32.9%: Table 1) was expressed per unit (in which 17.2 of these cells are present [Karam and Leblond, 1992]), as follows (17.2 × 32.9)/100 = 5.66 (Table 10). Secondly, the proportion of the labeled granule-free cells consisting of labeled pre-pit cell precursors was obtained on the assumption that the labeling of the latter was proportional to their frequency (which was 11 pre-pit cell precursors out of 19 granule-free cells studied). Accordingly 5.66 × (11/19) = 3.28 pre-pit cell precursors were labeled per unit. Thirdly, 99% of their progeny consisted of pre-pit cells (Appendix A)—that is, 3.25 labeled pre-pit cells arose from them per unit. 2) To estimate how many pre-pit

TABLE 8. Comparison between the production of granule-free subtypes and pre-parietal variants in the unit

Granule-free cells			Pre-parietal cells			Ratio of daily cell production (subtype over variant)
Subtype	Relative number	Daily cell production[1]	Variant	Relative number	Daily cell production[2]	
I	4	1.4	I	22	0.13	11:1
II	11	3.8	II	7	0.04	95:1
III	4	1.4	III	4	0.024	58:1

[1]Obtained by multiplying the daily cell production of all granule-free cells, that is, 6.6 cells per unit (Table 7) by the relative number for each subtype and dividing by the sum of the relative numbers—that is, 19.
[2]Obtained by multiplying the daily cell production of all pre-parietal cells, that is, 0.20 cells per unit (Table 7) by the relative number for each variant and dividing by the sum of the relative numbers—that is, 33.

TABLE 10. Isthmal cell production from mitosis expressed per unit

Cell type	Cell number per unit[1]	Labeled cells 30 min after ^{3}H-thymidine (%)[2]	Number of labeled cells per unit 30 min after ^{3}H-thymidine[3]
Granule-free	17.2	32.9	5.66
Pre-pit	10.0	24.0	2.46
Pre-neck	1.8	11.3	0.203

[1]From Karam and Leblond (1992).
[2]From Table 1.
[3]Obtained by multiplying the unit cell number by the percent labeled 30 min after ^{3}H-thymidine injection.

cells came from their own mitoses, the number of these cells labeled at 30 min, 24.5%, was multiplied by their number per unit, 10.0 (Karam and Leblond, 1992), yielding 2.46 pre-pit cells labeled per unit (Table 10). Thus per unit the number of pre-pit cells labeled by differentiation was 3.25, while 2.46 were labeled directly. These estimates translated into percentages indicated that, for the 57% of pre-pit cells produced by differentiation, 43% came from mitosis.

Similarly, an attempt was made to estimate the proportion of pre-neck cells coming from differentiation of pre-neck cell precursors (subtype III) and from their own mitoses. 1) It was known from the above that 5.66 granule-free cells per unit were labeled 30 min after a ^{3}H-thymidine injection. It was assumed that the proportion of these labeled cells consisting of pre-neck cell precursors was the same as the frequency of the latter (4 pre-neck cell precursors out of 19 granule-free cells). Accordingly, $5.66 \times (4/19) = 1.19$ pre-neck cell precursors were labeled per unit; and 98% of their progeny consisted of pre-neck cells (Appendix A)—that is, 1.17 labeled pre-neck cells arose from them per unit. 2) To estimate the number of pre-neck cells arising from their own mitoses, the number of these cells labeled 30 min after ^{3}H-thymidine injection, 11.3%, was multiplied by their number per unit, 1.8, yielding 0.203 pre-neck cells labeled per unit (Table 10). Thus per unit, the number labeled by differentiation was 1.17, whereas 0.203 were directly labeled. These estimates indicated that 85% of the pre-neck cells were produced by differentiation, whereas 15% arose from mitosis.