ABSTRACT

Enterochromaffin cells were investigated in the jejunum of adult mice using electron microscope radioautography at various times during continuous infusion of ^{3}H-thymidine.

Enterochromaffin cells were classified into three types: biconcave granule cells or type I (60%), large spherical granule cells or type II (13%), and small spherical granule cells or type III (24%). Uncommon spherical granule cells accounted for about 3%.

Typical enterochromaffin cells do not divide, but undergo renewal by differentiation of unrecognized precursors in the lower half of intestinal crypts. Labeled enterochromaffin cells appear in crypts by $1\frac{1}{2}$ days, and in villi by 3 days after the infusion started. At 8 days, 80 percent cells were labeled both in crypts and villi. And 8 days after stopping a 4-day infusion most labeled cells had disappeared from crypts and villi.

Enterochromaffin cells migrate from crypt to villus, and are presumably extruded from villus tips. The turnover time would be less than eight days.

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RENEWAL OF ENTEROCHROMAFFIN CELLS IN THE MOUSE JEJUNUM

by

Mário N. Ferreira

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

Department of Anatomy
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November 1970.

To my Wife

Esmeralda

and children

Cristina

Pedro Manuel

and

Frederica

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INTRODUCTION

In the epithelium of the small intestine considerable work has been done on the renewal of the main cell type, the columnar cells, both in normal and experimental situations in animals of various species, as well as in pathological cases in human. Recently, a few authors have demonstrated that mucous (goblet) cells (Merzel and Leblond, '69; Cairnie, '70) and Paneth cells (Cheng, Merzel and Leblond, '69; Cairnie, '70) also undergo renewal.

On the other hand, little is known about the renewal of enterochromaffin cells. Are these cells permanent? Or are they being continually replaced by new ones as is the case with the other cell types of the small intestinal epithelium? The single study on this subject estimated that the replacement time of enterochromaffin cells in human rectal mucosa was 1-3 months (Deschner and Lipkin, '66).

The present work was undertaken to provide a more detailed understanding of the kinetic behavior of this cell population, and had two major objectives. The first was to describe, classify, and quantitate the enterochromaffin cells present in crypts and villi of the mouse jejunum. The second objective was to find out whether the various types of enterochromaffin cells undergo renewal. This has been done using ultrastructural radioautography after administration of tritiated-thymidine, either by a single injection or by continuous infusion into the peritoneal cavity of the mouse.

REVIEW OF LITERATURE

PART I — INTESTINAL KINETIC STUDIES

During embryologic development, the originally stratified epithelial layer of small intestinal mucosa transforms into a single layer of columnar cells (Johnson, '10; Kammeraad, '42; Coulombre and Coulombre, '58; Behnke, '63). The single layer then differentiates into a deep portion, the crypts, and a superficial portion, the villi which project into the lumen.

The epithelial cells lining the crypts and villi include the columnar cells, which vary in structural detail from the base of the crypts to the villi, the mucous (goblet) cells, the Paneth cells, and the enterochromaffin cells.

The base of the crypts contains cells which are constantly proliferating and differentiating. The villi are covered by cells which seem to be specialized in absorptive functions.

Cell migration along the columnar layer

It was known since the earliest references by Paneth, Heidenheim, and Bizzozero, at the end of the 19th century (cited by Stevens, '50), that mitoses were frequent in the crypts of the intestine; these divisions were thought to be part of a "regeneration process" in which cells damaged by mechanical action of food and bacterial activity in the intestinal lumen would be replaced (Stevens, '50; Leblond and

Messier, '58; Leblond, '65).

When these problems were reinvestigated by Leblond and Stevens ('48), they observed, using quantitative methods, that the mitotic index of duodenal and ileal epithelial cells was 3 percent and 3.5 percent, respectively, the mitotic figures being located in the crypts of Lieberkuhn; this proliferative activity occurred both in the presence of food in the lumen and in fasting animals. Furthermore, these experiments confirmed the fact that cells were extruded from the tip of the villus at what is today called the "extrusion zone" (Leblond and Stevens, '48; Stevens Hooper, '56; Creamer et al. '61b). The extrusion zone was recently observed with the scanning electron microscope (Asquith, Johnson and Cooke, '70).

The recognition of mitotic activity in cells at the base of the crypts, and the loss of cells at the very tip of the villi in normal conditions, led to the assumption that epithelial cells migrated from crypt to villus tips and that cell production balanced the cell loss.

The concepts of steady state and turnover time

The balance between cell production and cell loss results in a condition of dynamic equilibrium referred to as "steady state", a concept which implies that the number of cells present does not change significantly with time. To maintain the "steady state" half of the cells arising from mitosis must differentiate, and the other half must remain in the tissues and eventually divide again (Leblond, Greulich and Pereira, '64). The population having the ability both to renew

themselves and to differentiate are "stem-cells".

The time taken for the replacement of a number of cells equal to that of the entire population is "turnover time". While turnover time may be determined by methods using colchicine, X-ray, etc. (Leblond, '59), methods using radioactive markers for dividing and migrating cells give the most precise results.

Radioautographic studies of the kinetics of intestinal epithelial cells started with the work of Leblond, Stevens and Bogoroch ('48) using the inorganic phosphate (P^{32}); although the radioactive P^{32} is incorporated by cells synthesizing DNA, it is not specific since it may be incorporated in other substances; another disadvantage is that it emits strong beta-rays resulting in possible damage to cells and poor radioautographic resolution.

Later, Leblond and Walker ('56) and Walker and Leblond ('58) obtained more precise results in intestine of mice after the administration of adenine labeled with carbon-14 (interference from RNA synthesizing cells was eliminated by RNAase pretreatment).

Soon after, the use of another precursor, thymidine labeled with tritium, with higher specificity and lower beta energy than the isotopes just mentioned, gave sharper images and excellent resolution of the silver grains in photographic films (Leblond, Messier and Kopriwa, '59; Amano, Messier and Leblond, '59; Messier and Leblond, '60). Thymidine is a pyrimidine base incorporated into the DNA of cell nuclei about to divide. Provided that the dose of radioactivity

is kept low, the use of tritium, a radioisotope of hydrogen, to tag the thymidine does not interfere with the incorporation of thymidine into cell nuclei.

Since Howard and Pelc ('53) discovered the cell cycle, it became apparent that virtually all cells, either "in vivo" or "in vitro" duplicate their DNA prior to mitosis. The cell cycle in a particular cell type has a definite duration (the generation time) composed of four successive phases (post-mitotic, pre-synthetic phase or G1; DNA synthesis period or S; post-synthetic, pre-mitotic phase or G2; and mitosis, M). During the S phase it is possible to label the nuclei of cells with precursors of the DNA molecule, such as tritiated-thymidine (^{3}H-thymidine) and, then, to obtain various parameters for cell kinetic studies (rate of proliferation, labeling index, number of labeled mitosis) and to trace migratory patterns of labeled daughter cells.

In experiments with single injection or continuous infusion of radioactive tracers, if the number of labeled cells at different time intervals is linearly related to the administration of the radioisotope, the slope of the line obtained makes it possible to calculate the turn-over rate, and, from this, to estimate turnover time (Cheng, Merzel and Leblond, '69).

Renewal of columnar cells

The rapid process by which intestinal crypt cells divide, differentiate and migrate to the "extrusion zones" at the apex of the villi

provides a system in constant physiological renewal that has been extensively studied in the last two decades; particularly, the pattern of proliferative and migratory activity of the columnar cells is now very well known (Leblond and Stevens, '48; Leblond and Walker, '56; Leblond and Messier, '58; Leblond, '59; Quastler and Sherman, '59; Creamer et al., '61; Bertalanffy, '62; Lipkin et al., '63a and b; Shorter et al., '64; MacDonald et al., '64; Cairnie et al., '65; Lipkin, '65 a and b; Lesher et al., '66; Leblond et al., '67; Troughton and Trier, '68; etc.).

Before the use of radioisotopes, Stevens and Leblond, ('48) determined the turnover time of the columnar (chief) cells by using the colchicine-method; knowing the average duration of mitosis in the intestinal epithelium (1.3 hours) and the mitotic index (3 percent in duodenal crypt cells, and 3.5 percent in the ileal cells) they obtained a turnover time of 1.6 days and 1.4 days, respectively, for these cells in the duodenum and ileum.

Leblond, Stevens and Bogoroch ('48), by means of radioautography with P^{32}, and Walker ('57), using C^{14}-adenine, gave evidence for the migration of cells labeled in crypts upward to the base of the villi. In 1958, Leblond and Messier, who used ^{3}H-thymidine, observed labeled cells at the tips of the villi 72 hours after the administration of the radioisotope.

The sequence of the events after labeling of dividing cells in the crypts of the small intestine is now well established: (1) at 1 hour after the administration of a DNA radioactive precursor, cells syn-

thesizing DNA are seen at the base of the crypts; (2) at 12 hours, some labeled cells are at or immediately outside the mouth of the crypts, being now part of the villus epithelium; (3) at 24 hours, many are on the sides of the villi; (4) at 72 hours, radioactive cells have reached the tips of the villi and some of them are seen in the lumen of the intestine; (5) because only very labeled cells are seen at 3 days, it has been assumed that most radioactive cells have been extruded (Messier and Leblond, '60). These authors established that in rats the crypt epithelial cells migrated to the villi tips in about 2 days, whereas in the mouse it took about 3 days in jejunum and ileum, and about 2 days in duodenum.

Creamer et al. ('61a) found a turnover time of 1 - 2.5 days for the epithelial cells in the small intestine of the mouse.

Since the columnar cells make up about 80 percent of the intestinal epithelial population, investigations on the renewal of the other cell types were scarce until recent years.

Renewal of mucous (goblet) cells

The dynamic studies of these cells started with the observation of Friedman ('45) that goblet cells in the crypts, swollen by low doses of X-rays, with time migrated up the sides of the villus.

In the lower half of the crypts, 8 hours after ^{3}H-thymidine administration, Messier and Leblond ('58) and Leblond and Messier ('60) noticed the presence of labeled nuclei in cells containing purple-stained mucus, i.e., goblet cells, in radioautographs stained with

PA-Schiff hematoxylin. At 24 hours they saw nearly all the nuclei of goblet cells overlaid by a reaction. Judging by the time taken for goblet cells to reach the villus tip, these authors assumed that the renewal of these cells takes approximately 3 days in the small intestine of mice.

There is now evidence that "immature goblet cells" (Thrasher and Greulich, '66) or "oligomucous cells" (Merzel and Leblond, '69) or "pre-goblet cells" (Cairnie, '70) located in the crypts, incorporate ^{3}H-thymidine as soon as 1 hour after the injection. Merzel and Leblond ('69) and Troughton and Trier ('69) also referred to some mitotic figures which contained a small number of mucus globules, thus supporting the view that cells sufficiently differentiated to be recognizable as goblet cells undergo independent proliferation in the crypts.

The turnover time for goblet cells is 120.3 hours in the duodenum of mice, although some labeled cells reached the tip of the villus by 67 hours (Merzel and Leblond, '69). Cairnie ('70) calculated a turnover time of 40 hours for goblet cells in crypts and said that they migrate to the villus in about the same time as columnar cells. By extrapolation of the line of labeled goblet cells at different time intervals after injection of ^{3}H-thymidine he found 100 percent labeled at 86 hours.

Hence, goblet cells (like columnar cells) in the small intestine undergo renewal and migrate from crypts to villi; the turnover time

of 3-5 days found in the species studied indicates that the transit of these cells may be slightly slower than that of columnar cells.

Renewal of Paneth cells

Kinetic data on Paneth cells has been fragmentary until very recent years.

Although mitoses of Paneth cells have been claimed in the literature (for review: Cheng, '69; Cheng et al., '69), no labeling of these cells was found immediately after ^{3}H-thymidine injection (Leblond and Messier, '58). However, some authors did find labeled Paneth cells at 1 day (Hampton, '68) or even at 12 hours (Troughton and Trier, '69) after the administration of the radioisotope. Leblond et al. ('68) also found 2 percent labeled cells by 2 - 4 days after injection.

Immediately after injection of ^{3}H-thymidine, cells which are located among adult Paneth cells become labeled. These cells have been called "intercalated cells" by Cheng, Merzel and Leblond ('69) in the small intestine of mice. Using both single injection and continuous infusion of radioisotope, they found that with time the number of labeled intercalated cells decreased, while the number of labeled Paneth cells increased. They concluded that Paneth cells derive from the intercalated cells which proliferate in the bottom of the crypts. This was in agreement with Hampton ('68), who claimed that the Paneth cell population was distinct from other cell types.

From the slope of the line of percent labeled cells at different time intervals after continuous infusion, Cheng et al. ('69) went on to estimate a 3 week turnover time for Paneth cells in the small intestine of mice. Similarly, Cairnie ('70) observed labeled Paneth cells at the 3rd day after ^{3}H-thymidine administration. By the 14th day he found a labeling index of 40 percent, and he suggested a model analogous to that described for goblet cells, but with much longer turnover time.

PART II — THE ENTEROCHROMAFFIN CELLS

Histological and histochemical approaches

In the gastrointestinal epithelium of most Vertebrates (except Cyclostomes and Teleosts) and of many Invertebrates, Heidenheim, (1870), and later Nussbaum, Nicolas, Kultschtzky, Ciaccio, Masson, among others, described a population of cells characterized by cytoplasmic granules with specific staining properties (see: Cordier, '23; '26; Cordier and Lison, '30; Macklin and Macklin, '32; Erspamer, '54). These cells have been described as triangular or polygonal cells located in the epithelia derived from the embryonic gut, lying on the basement membrane, and rarely or never reaching the intestinal lumen. Their main characteristic is the existence of granules located throughout the cytoplasm, or concentrated in the infranuclear region.

It was soon observed that these granules have specific though not uniform staining reactions. Some of them have reducing properties

and stain directly by the silver nitrate method according to Masson, and modified by Fontana — cells with these granules have been called argentaffin cells. Other cells have granules which reduce the argentic substances only in the presence of extraneous reducers — these have been designated argyrophil cells, and could be demonstrated by the Bodian's protargol method or other argyrophile reactions. Argentaffin cells also have argyrophilic properties, but the argyrophile cells are not necessarily argentaffinic. Furthermore, the argentaffin cells show a chromaffin reaction, that is, their granules given a yellow-brownish staining after treatment with dichromate fixatives. This property is the basis of the designation enterochromaffin cells given by Erspamer ('37). It is assumed that chromaffinity is a staining property peculiar to the argentaffin cells.

These many observations, using different species and different staining methods, have been the basis for a variety of classifications of these cells at the light microscope level (Hamperl, '52; Helweg, '52; Erspamer, '54; Clara, '57; Monesi, '60b).

Recently, some authors (see Häkanson et al., '70) classified cells as enterochromaffin (in the stomach) if they exhibited a formaldehyde-induced yellow fluorescence after excitation with ultraviolet light. In addition, cells with a green fluorescence, after treatment with L-Dopa, have been named enterochromaffin-like cells, but non-argentaffin.

It was known from histochemical studies of the granules in some cells which showed a chromaffin reaction that they contained the melanic pigment, adrenaline, and melanine (for review: Lison, '31). The work of Gomori ('48) gave a new dimension to the search for the enterochromaffin-positive cell substance. Because several chemical products (pyrocathequine, resorcine, hydroquinone, and 5-hydroxytryptamine) were found to react with the enterochromaffin cell reagents, Gomori concluded that some of these substances (resorcine or 5-hydroxytryptamine) might be the cell substance responsible for the enterochromaffin reaction.

Biochemical approach

Already in 1937, Vialli and Erspamer (cited by Erspamer, '54) had obtained from acetonic extracts of the gastrointestinal mucosa of mammals a partially purified, di- or polyphenolic-active substance which they called enteramine. This substance was subsequently identified as 5-hydroxytryptamine (Erspamer and Asero, '52); its identity with serotonin, a vaso-constrictor discovered by Rapport, Geen and Page ('48) in bovine serum, was established.

5-hydroxytryptamine, (enteramine or serotonin), is not found in the content of the intestinal lumen of the animals in which the enterochromaffin cells are present, but is present in their blood stream and in extracts of the digestive mucosa (and in many other tissues as well). This substance is synthesized from tryptophan, an essential amino acid, and its production is dependent, to some extent, on tryptophan

uptake (Sokoloff, '68). Tryptophan may be metabolized by different pathways: (1) decarboxylation to tryptamine by an aromatic decarboxylase; (2) hydroxylation by tryptophan hydroxylase and conversion to 5-hydroxytryptophan, the substrate for 5-hydroxytryptophan decarboxylase, which transforms it into 5-hydroxytryptamine. However, the distribution of enzymes responsible for the metabolism of tryptophan does not coincide either with the tissue content of 5-hydroxytryptamine or the idea that most extracerebral 5-hydroxytryptamine synthesis occurs in the enterochromaffin cells of the gut (Page, '68). In fact, Falck, Hillarp et al. ('62) have demonstrated by fluorescence methods that 5-hydroxytryptamine is widespread in the body (enterochromaffin cells, platelets, nervous tissue, mast cells, etc.).

Much of the interest in 5-hydroxytryptamine arises from the possibility that this substance may be the hormone secreted by enterochromaffin cells. During the 'forties and 'fifties the opinion prevailed that enterochromaffin cells were a single cell type. The pure argyrophile (non-argentaffin) cells were regarded as cells depleted of their secretory product, 5-hydroxytryptamine. And a low frequency of argentaffin cells was believed to reflect a high rate of release of 5-hydroxytryptamine (Singh, '65). The striking cellular differences observed, either morphologic or histochemical, were thought to be due only to diverse functional stages of the cells.

Origin of enterochromaffin cells

Three main hypotheses involving the three germ layers of the embryo have been proposed for the origin of enterochromaffin cells (see Macklin and Macklin., '32):

(1) entodermal origin (the entoderm lining the gut);

(2) mesenchymal origin (the underlying mesenchyme);

(3) nervous origin (the neural crest).

The majority of investigators who have studied this problem agree that enterochromaffin cells differentiate "in situ", since they are first recognizable in the epithelial layer of the intestine (entodermal origin). However, as pointed out by Andrew ('62), the earlier stages prior to the appearance of the typical granules, cannot be identified by the available methods, so that the young cells could conceivably be found in locations other than the epithelium.

There is little evidence for a mesodermal origin of enterochromaffin cells (Macklin and Macklin., '32). The studies of Kull, quoted by the latter authors, and of Dias-Amado ('25a and b) were based on the staining of connective tissue cells underlying the gut epithelium, but the methods used were of low specificity and the cells they described probably correspond to leucocytes and globular-leucocytes.

The elegant experiments of Andrew ('62) indicate that enterochromaffin cells are not derived from the neural crest. She made

explants of blastoderms of chick embryos from definitive primitive streak- to 22-somite stages; definite and potential neural crest and neural plate material was included in control, but excluded from experimental explants. This material was cultured as chorio-allantoic grafts for 18-20 days to allow adequate differentiation of intestine. Enterochromaffin cells were found in both control and experimental grafts, and in number at least as great as in normal gut. These experimental results decisively eliminated the possibility of a nervous origin (neural crest hypothesis) for these cells.

There is, however, a good deal of indirect evidence for the entodermal origin of enterochromaffin cells. Firstly, they may be found in metastases from epithelial growths of the intestine (Friedman, '34; quoted by Singh, '63a). Moreover, islets of ectopic intestinal mucosa in the stomach show large numbers of enterochromaffin cells with morphological features of the intestinal ones (Singh, '63a). Similarly, in the ectopic intestinal mucosa of the stomach of patients with treated pernicious anemia, Rubin et al. ('66) observed that: "A full complement of intestinal type of epithelial cells was found (differentiated villus, undifferentiated crypts, goblet, Paneth and argentaffin cells). Undifferentiated crypt cells observed were identical to those of the normal small intestine and commonly in mitosis." It was assumed by these authors that the presence of enterochromaffin cells within these ectopic crypts "does not preclude a neural crest origin, but does suggest a possible epithelial origin."

Thus evidence to date favours an entodermal origin.

Differentiation during fetal life

The subsequent development and differentiation of enterochromaffin cells has received scant attention.

The work of Sharples ('45) on the histogenesis of argentaffin cells in the duodenum of the rat showed a relationship between the early appearance of these cells and the level of development of the intestinal epithelium. Argentaffin cells were first found in the still stratified duodenal epithelium, close to the pylorus, as early as the 15th day of gestation, i.e., two days prior to differentiation of crypts and villi. These cells were usually rounded, with a few uniformly distributed granules and were found at various levels in the epithelium. By the time of birth, the enterochromaffin cells "appear to be distributed widely and at random in the epithelium of villi and are rarely seen in the developing crypts of Lieberkühn. The argentaffin cells are rounded in crypts and at the bases of the villi, but have a tendency to become compressed into a more flasklike form further up on the villi" (Sharples, '45). Two aspects of these observations are relevant: when argentaffin cells first appeared in the embryonic intestine of the rat, they were already scattered in the epithelium; however, a morphologic difference was noted between the crypt cells (which were rounded with scanty granules) and the cells located in the villi (which tended to become compressed by adjacent columnar cells and to display a dense granulation).

In the duodenum of chick embryo (Monesi, '60 a and b) and of human embryo (Singh, '63 a and b), a distinct interval was described

between the appearance of pure argyrophile cells (called by them pre-argentaffin) and of argentaffin cells. According to Monesi ('60 a and b) the cells with smaller granules observed under the light microscope appeared first and were Bodian positive or argyrophile; and the cells with larger granules, which appeared later, were both Bodian positive and Hamperl positive or argentaffin. It was presumed that the interval between the appearance of the two cell types was related to the intercellular synthesis of 5-hydroxytryptamine in the argentaffin cell. These data suggested the possibility that argyrophile cells transformed into argentaffin cells. However, in organotypic cultures of the chick embryonic duodenum argyrophile and argentaffin cells appeared simultaneously (Monesi, '60 a). Furthermore, by simultaneously using the argyrophile and the argentaffin reactions in paraffin sections to study the granules of these cells in guinea-pig, rabbit and man small intestine, Singh ('66) concluded that, in addition to pure argyrophile cells and pure argentaffin cells (also argyrophile), there was an intermediary type containing a mixture of argentaffin and argyrophile granules. These findings pointed to the necessity of examining individual granules in the same cell.

Electron microscopic approach

The initial observations on enterochromaffin cells with the electron microscope furnished little detail about their structure, but did show that granules were not fixation artefacts. In a study of human colon and appendix, and guinea-pig duodenum, Christie ('55) mentioned that enterochromaffin cells contained osmiophilic granules 0.2 μ in

diameter. Similarly, Taylor and Hayes ('59), studying argentaffin cells in rat intestine, found osmiophilic granules ranging from 1,000 Å-2,500 Å in diameter, and indicated that the granules had varying degrees of osmiophilia and possibly an internal structure.

In 1960, Bullon-Ramirez, Langer and Shultze studied the argentaffin cells from the guinea-pig duodenum and a human carcinoid tumor. By applying Masson's argentaffin method to thin sections, they were able to see silver deposits at the periphery of the granules of these cells. In osmicated preparations, the granules of 2,000 Å in diameter were homogeneous or showed small microvesicules and were surrounded by a single membrane.

More detailed studies started with the work by Trier ('63) on the small intestinal crypt epithelium of fasting humans. Enterochromaffin cells were identified by their location between the basal portions of other cell types of the crypt and by their unique intracytoplasmic granules. This author compared the structure of these cells with those described previously by Helander ('61; '62) in the gastric mucosa. In Trier's observations, the granules varied considerably in density and shape. Some were completely black, and usually circular in shape, although elliptical or irregular forms were seen; others were spherical but contained a sparse amount of finely granular material within a lighter matrix; still others were more or less empty-appearing and seemed to be only partially surrounded by a fine membrane.

Toner ('64) described two main cell types in the gastrointestinal tract of the fowl: (1) cells with round, dense granules less than 0.2 μ in diameter, and enclosed within a single membrane (pale forms were also noted); this cell type was found mainly in the proventriculus and gizzard, as well as in the small intestine; (2) the other cell type had larger and denser granules, often up to 0.5 μ in diameter, with a variety of shapes from round to discoid, or even biconcave; these cells were found only in the small intestine, and outnumbered the cells with round granules. In Toner's observations these cells seldom reached the surface of the epithelium. Toner correlated the rounded granule cells with the argyrophile cells of light microscopists and the irregular granule cells with the argentaffin cells.

A similar description of the ultrastructure of argyrophile cells was made in the stomach of mammals (Kurosumi, '58; Helander, '61; '62); however, their conclusion that the cells with round granules were argyrophile was derived from the "a priori" view that argyrophile cells were the main cells present in the stomach.

Rubin et al. ('66), studying the fine structure of heterotopic islands of intestine in the gastric mucosa of patients suffering from pernicious anemia, described argentaffin cells as being located in the crypts, where they rested on the basement membrane without continuity with the free surface. These cells seemed to correspond to the cell type with irregular granules described by Toner ('64).

As a result of these studies, it appeared that argyrophile and argentaffin cells had differently shaped secretory granules. The idea of a single cell undergoing a functional or secretory cycle which would include a change in granule shape was strongly supported by Ratzenhofer and Leb ('65) and Ratzenhofer ('66) who stated that in the cells of the rabbit gastric mucosa "the gradual osmiophobic granules seem to contain either less 5-hydroxytryptamine or no 5-hydroxytryptamine at all"; they assumed that argentaffin cells (also argyrophile) were predominantly osmiophilic, and that the predominantly osmiophobic cells likely corresponded to the pure argyrophile cells. Ratzenhofer ('66) suggested the term endocrine cell (E-cell) for the cellular elements he saw in the gastric mucosa of the rabbit, with the idea of expressing their uniformity of functional performance.

The enterochromaffin cells were studied with the electron microscope during the prenatal development, in the chicken duodenum (Ferreira, '66; Penttilä, '68). Granular cells first appeared in the epithelial layer between $13\frac{1}{2}$ - 14 days of incubation. Ferreira ('66) presented evidence that between 15 and 21 days of incubation different ultrastructural cell types were present in this material: (1) cells with irregular and rounded large granules; (2) cells with large and rounded granules; (3) and cells with small rounded granules. Penttilä ('68) also mentioned cells with distinct types of granules (irregular shape and size for argentaffin cells, and round and small granules with little variance in size for argyrophile cells), claiming that only very

few cells of the latter type were found in the duodenum of the chicken embryo.

These findings of different cell types in the embryonic intestine agree with what is found in adults.

Intensive research on the enterochromaffin population is currently going on in the laboratories of Rouiller (Switzerland), Pearse (England), Cavallero (Italy) and Bencosme (Canada). Using the electron microscope, histochemistry and fluorescence methods, the classical concepts of these cells is being refuted, and the idea is emerging that cells with specific granulations and different staining properties form a distinct and separate cell type.

Forssmann et al. ('67) and Orci et al. ('67; '68 a and b) have pointed out the existence of distinct endocrine cells (the term they used for enterochromaffin cells) in comparative studies on the digestive mucosa of various mammals. Carvalheira, Welsch and Pearse ('68) distinguished two different cell types in the gastrointestinal epithelium of various mammals; they definitely correlated the cells with irregular granules with the true argentaffin cells, while the cells with round granules (argyrophile) were found to be cholinesterase rich cells.

Thus, these recent investigations, among others, have challenged the previous understanding of this cell population. However, the structure, kinetic behavior, and functional activity of enterochromaffin cells is far from being settled.

Pathological approach (enterochromaffin cells and "carcinoids")

The term carcinoid has been used to describe an epithelial tumor of the small intestine rich in argentaffin cells. This tumor is malignant, although less so than most carcinomas. During the 'fifties, the concept was of a tumor usually originating in the jejunum or ileum and with characteristic histologic appearance, metastasizing to the liver, while secreting 5-hydroxytryptamine (Peart et al. '63). In typical cases, a clinical syndrome was associated with a group of signs and symptoms, which could be divided into episodic (flushing, hyperperistalsis, diarrhea, asthmatic attacks, vasomotor collapse) or permanent (facial hyperemia, edema, valvular heart disease) (see Brown and associates, '67).

But in the later 'fifties, variants of this syndrome, in which the tumor secreted an excess of 5-hydroxytryptophan rather than 5-hydroxytryptamine were reported by a few authors (for review: Peart et al. '63; Brown et al. '67).

It appeared that tumors of foregut origin (bronchus, stomach, pancreas ducts and biliary passages) may often be 5-hydroxytryptophan secretors, and have histologic differences from typical carcinoids; the typical carcinoids appear in the midgut (small intestine from the mid-duodenum, caecum, and colon as far as the mid transverse colon) and secrete only 5-hydroxytryptamine; the carcinoids of the hindgut (descending colon and rectum) are usually inactive biochemically (Williams and Sandler, '63).

When a systematic study of the carcinoids was made with the electron microscope it became evident that the morphological appearance of the tumors' cells was not uniform. Black ('68) studied the ultrastructure of carcinoids from the human stomach, ileum, appendix and rectum, and found that cells of those tumors have the same structure as normal cells in the same segments of the gastrointestinal mucosa. He described the gastric carcinoids with uniform, round and small granules (argyrophilic); ileal carcinoids either with (1) pleomorphic, large granules (argentaffin) or (2) with round varied size granules (argentaffin); appendical carcinoids with pleomorphic, large granules, with central cores (argentaffin); and rectal carcinoids with rounded granules of variable density (negative to both argyrophile and argentaffin reactions).

It is evident that the "carcinoid syndrome" is complex, and that different cell types are involved in the broad spectrum of histologic, histochemical and ultrastructural patterns, as well as biochemical activity and clinical signs.

Aims of the present study

Several classifications of enterochromaffin cells have been recently proposed on the basis of electron microscopic observations. However, since no cell counts were made, it was not known whether the cell types described were common or exceptional. Furthermore, the location of these cells was not specified. The first objective of this study, then, was to accurately define, locate, and quantitate the

various types of enterochromaffin cells in mouse jejunum.

The dynamic behavior of intestinal epithelial cells, other than enterochromaffin cells, has been extensively investigated. However, little is known of the kinetic properties of enterochromaffin cells, which constitute a small percentage of the intestinal cell population. This lack of information probably exists because the morphologic identification of these cells with the techniques used for the other cell types, does not lend itself readily to quantitation. In the current study, the second objective was to use ultrastructural radioautography after ^{3}H-thymidine administration in the hope of solving this problem.

MATERIAL AND METHODS

Experimental Animals

Female Swiss albino mice (McIntyre Animal Centre, Faculty of Medicine, McGill University), 3 months old ($\pm$ 1 week), 30 gm average weight, were used in all the experiments described below. They were fed a Purina Chow diet and tap water "ad libitum" until sacrifice. Two animals were used in each experiment with routine techniques, as well as in each group or time interval with the radioisotope experiments.

Routine Methods

Anaesthesia

Each experimental animal was anaesthetized in a beaker jar in an atmosphere of fresh, cold-saturated ether, and maintained under slight anaesthesia until the moment the fixative perfusate acted (in perfusion procedures) or until exsanguination (in immersion procedures). For perfusion, the anaesthetized animal was placed on its back on a dissecting board and secured with rubber bands around its limbs.

Perfusion Procedures

Two methods for perfusion of the fixative solutions were followed:

(1) direct perfusion into the left ventricle — after opening the abdominal and thoracic cavities of the animal by a midline incision,

the anterior portions of the ribs and sternum were removed with fine scissors and the wall of the left ventricle was punctured with a bevelled catheter of polyethylene attached by an adaptor to a 50 ml syringe containing the fixative solutions at room temperature. The polyethylene tube was placed into the ventricle in the direction of or into the aorta. When the injection was started the wall of the right atrium was cut gently with fine scissors, to allow circulation of the fixative solution throughout the body. Animals were perfused for 5 minutes at a rate of about 20 ml per minute for a total of 100 ml. The animals became very stiff in the first minutes of perfusion, and organs (heart, liver and gastrointestinal tract) became pale with formaldehyde or yellowish with glutaraldehyde. These changes indicated good fixation. Animals which were not stiff when the perfusion was over were discarded, since this indicated poor circulation of the fixative solution, possibly as a result of vasoconstriction or loss of the fixative through a punctured aorta;

(2) use of a perfusion pump — in this case, the animals were first prepared for controlled anaesthesia with artificial respiration; the trachea (after dissection) was intubated with a polyethylene catheter attached to a glass tube with a small hole to allow respiration of air. This glass tube was connected with a rubber-Y-shaped tube which came from a bottle of ether; another rubber-Y-tube linked the bottle of ether to a bottle with a O_2 - CO_2 (95:5) mixture that could be regulated by a flowmeter. With this system, the appropriate amounts of

ether, O_2 - CO_2, and air for anaesthesia is achieved, and the animal is kept alive under controlled respiration rich in oxygen and slight anaesthesia until the moment the perfused fixative acts (for details see: Rau, '69).

The thoracic and abdominal cavities of the animal are opened and artificial respiration is begun immediately by rhythmically applying one finger to the hole of the glass tube.

The perfusion solution is kept in a bottle hung above the table and connected with the perfusion pump by a polyethylene tube; the flux is regulated by a drip chamber. The device must first be flushed with the fixative solution to avoid air bubbles and allow a continuous flow.

The wall of the left ventricle is punctured in the same way as described before using a bevelled catheter which is then attached to the rubber tube that comes from the perfusion pump. To start the perfusion, the pump is turned on; and the right atrial wall is opened immediately. The fixative is allowed to flow for 5 - 10 minutes, fast in the first 2 minutes, and slower thereafter. The signs of a well perfused animal are the same as described above.

Immersion procedures

In some experiments fresh tissues were fixed by immersion in the fixative solution, without perfusion. In these cases, lightly anaesthetized animals were opened by a midline incision of the abdominal wall, and the tissues were removed and placed in a drop

of cold fixative and thereafter processed as described below, using either formaldehyde - or glutaraldehyde - containing solutions.

Fixation procedures

After perfusion of the animals either with formaldehyde - or glutaraldehyde - containing solutions, or immersion of tissues directly, a small segment of jejunum was removed (10 - 15 cms away from the pyloric region), and sliced gently with a razor-blade into 1 mm segments perpendicular to the surface of the mucosa; these segments were then immersed in cold fixative solutions.

(1) Animals perfused with formaldehyde: after trimming the tissue the small pieces of the jejunum were immersed in the same fixative for 2 - 3 hours, and then post-osmicated for 2 hours;

(2) animals perfused with glutaraldehyde: the small pieces of jejunum, trimmed in the same fixative solution, were immediately post-osmicated in the following cold osmium tetroxide-containing solutions, for 2 hours:

2% OsO_4 in Sorensen's phosphate buffer, with 0.5% sucrose;

2% OsO_4 in Palade's veronal-acetate buffer;

(3) the same solutions were used in direct immersion fixation of the jejunum.

Washing

Post-osmicated tissues were rinsed for 1 hour in 2 changes of the same cold buffer systems used in each fixative solution.

Dehydration

Small pieces of the tissue from each animal were dehydrated either by ethyl alcohol (50%, 70%, 90%, 10 minutes each; 100% or absolute, 3 changes of 10 minutes each), or acetone (same increasing concentrations, during the same time) at room temperature; but in the case of glutaraldehyde-fixed material, dehydration with acetone was done in the cold up to the 90% step.

Embedding

Tissues dehydrated with ethyl alcohol were placed in propylene dioxide after the last bath of absolute alcohol. Tissues dehydrated in acetone were processed directly to the first embedding step. The following schedule was used:

(1) pieces of tissue dehydrated in alcohol:

propylene dioxide + Epon Mixture (1:1) — 2 hours

propylene dioxide + Epon Mixture (1:2) — overnight

Epon Mixture — 2-3 hours

final embedding in Epon Mixture using plastic capsules.

(2) pieces of tissue dehydrated in acetone:

pure acetone + Epon Mixture (1:1) — 2 hours

pure acetone + Epon Mixture (1:2) — overnight

Epon Mixture — 2-3 hours

final embedding in Epon Mixture using plastic capsules.

The final embedding was done in the Epon Mixture which had been well stirred with magnetic bars in plastic disposable cups; air

bubbles were avoided by using a vacuum-chamber. The pieces of tissue were placed on the flat cover of the plastic capsule by a wooden stick (1 piece per capsule), and oriented in such a way as to obtain longitudinal sections of crypts and villi. The capsule was placed over its cover and filled with the Epon Mixture, and placed in a 45°C oven for 12 hours, followed by a 60°C oven for 3 days to harden the resin mixture; finally, a flat surfaced block was obtained which allowed easy trimming of the tissue block with razor blades under the dissecting microscope.

Sectioning and staining

The sections were cut with the Porter-Blum ultramicrotome using glass knives. Semi-thin (0.5 - 1 μ) sections were made from selected blocks to test the fixation of the intestinal mucosa and orientation of the tissue. The sections were transferred to glass slides and stained with 1% toluidine blue in a warm saturated solution of sodium borate, and observed with the light microscope. Thin sections of crypts and villi were obtained from blocks containing the best fixed material, picked up with copper grids (300 mesh), and thereafter double stained with 4% uranyl acetate in 50% alcohol (10-15 minutes) and Reynold's lead citrate (10-15 minutes).

The electron microscopic study of enterochromaffin cells was done with the Siemens Elmiskope IA and Hitachi HS-TS electron microscopes, operated at 60 kv and 50 kv, respectively.

Preparation of the solutions:

Fixative solutions:

(1) Formaldehyde-containing solutions: 4% formaldehyde in phosphate buffer 0.1 M (pH 7.3) was used, with 0.005% calcium chloride, prepared as follows:

Stock solution (for 1.000 ml):

Formaldehyde 8% aqueous - 500 ml

Sorensen's buffer 0.2 M - 500 ml

Calcium chloride - 0.05 gm

To prepare the formaldehyde solution, paraformaldehyde was dissolved in distilled water by heating the solution to 60°C and adding 10-15 drops of 1N NaOH while stirring. After cooling, the solution was filtrated.

Sorensen's buffer:

Sol. A - 0.2 M $Na H_2 PO_4 H_2O$ - 6.88 gm in 250 ml dist. water;

Sol. B - 0.2 M $Na_2 H PO_4$ - 14.19 gm in 500 ml dist. water;

Stock buffer solution 0.2 M - 100 ml of Sol. A + 400 ml Sol. B.

(2) Glutaraldehyde-containing solutions:

Fresh and cold stock solutions of 25% glutaraldehyde were used; to obtain 1.000 ml of 2.5% glutaraldehyde in Sorensen's buffer 0.1 M with 0.1% sucrose, pH 7.2, the following materials were used:

Glutaraldehyde 25% aqueous (filtered) - 100 ml

Sorensen's buffer 0.2 M - 500 ml

Sucrose - 1 gm

Distilled water - 400 ml

(3) Osmium tetroxide-containing fixatives:

-2% OsO_4 in Sorensen's buffer 0.1 M, with 0.1% sucrose (pH 7.2):

Osmium tetroxide	- 0.5 gm
Sorensen's buffer 0.2 M	- 12.5 ml
Sucrose	- 0.025 gm
Distilled water	- 12.5 ml

-2% OsO_4 in Palade's veronal-acetate (pH 7.3):

Veronal stock solution: -	sodium veronal	- 1.47 gm
	sodium acetate	- 0.97 gm
	distilled water	- up to 50 ml
Stock salt solution: -	sodium chloride	- 4 gm
	potassium chloride	- 0.2 gm
	calcium chloride	- 0.1 gm
	distilled water	- up to 50 ml

Hydrochloric acid sol.: 0.86 ml concentrated HCl sol. to make 100 ml;

Final stock solution: - 10 ml of veronal stock sol.
2.8 ml of stock salt sol.
11 ml of hydrochloric acid sol.
31.2 ml distilled water;

Fixative solution:

2% OsO_4 in dist. water	- 25 ml
Final stock sol.	- 25 ml

Epon embedding:

Stock sol. A	- 62 ml of Epon 812
	- 100 ml of DDSA
Stock sol. B	- 100 ml of Epon 812
	- 89 ml of NMA
Final sol.	- 7 ml of sol. A
	- 3 ml of sol. B
DMP	- 0.2 ml per 10 ml of final sol. (A + B)
Epon Mixture	- Final sol. (A + B) + DMP

Labeling Methods

Animal care

Twenty three female Swiss albino mice were used in experiments for the "in vivo" incorporation of the radioisotope tritiated-thymidine (^{3}H-thymidine). Care was taken with these experimental animals in the following points: (1) the temperature of the room where the cages were kept was constant during the time of the experiments (72-76^{o}F); (2) during the experiments each animal was kept in a separate cage under the same conditions; (3) the experiments all started at the same hour of the day (11 a.m.); the sacrifice was also done at 11 a.m. (except for the group sacrificed at the $1\frac{1}{2}$ days time interval).

Labeling by single intraperitoneal injection

Three animals were each injected with a single 100 μCi intraperitoneal dose of ^{3}H-thymidine (New England Nuclear Corp., specific activity 16.1 c/mM). They were sacrificed one hour later, by perfusion of 4% formaldehyde in Sorensen's buffer 0.1 M; the pieces of jejunum of each animal were then double - fixed by immersion in the same fixative for 2 hours and post-osmicated in 2% OsO_4 in the same buffer, and embedded in Epon. Then, after checking the labeling reaction with light microscope radioautographs, thin sections were prepared for electron microscope radioautography.

Continuous labeling by infusion of the radioisotope

In order to obtain material continuously labeled for varying periods of time, the animals were intubated and thereafter, the continuous infusion was performed, according to a method based upon the technique of intravenous infusion of rats by Evans ('68) and modified for this purpose and improved by Leblond and associates (Cheng, '69; Cheng, Merzel and Leblond, '69; Chang, '70).

Intubation

All animals were operated on in order to introduce a permanent plastic tube into the peritoneal cavity for continuous infusion of the radioisotope. Under ether anaesthesia, an incision was made through the dorsal skin, from the midline region near the ears and continuing along the left side down to the abdominal region. A Clay-

Adams Intramedic polyethylene tube (PE 20) was then inserted 1-2 cms into the peritoneal cavity, and the other end of the tube was kept long enough to reach the syringe placed in the plastic holder of a Sage syringe pump (for details see: Cheng, '69; Cheng, Merzel and Leblond, '69). The tube has to be firmly attached by thread to the abdominal wall where it enters the abdominal cavity, as well as at the occipital region; it is also attached to the subcutaneous tissue, and overlying skin. To avoid damage to the tube, a shielded cable enclosed it between the animal and the cage. After the operation each animal was placed in a separate cage and immediately received an infusion of 0.9 percent sterilized saline until the moment the continuous infusion of the radioisotope started (1 day interval).

Continuous labeling procedure

After the animals recovered from the stress operation (1 day), the ^{3}H-thymidine was placed in a tuberculine syringe of 1 ml that was held by the plastic holder adapted to the Sage syringe pump; the free extremity of the implanted tube was connected to the syringe. The plastic holder was mobile, pushing the piston of the syringe in a continuous movement, that allowed an infusion of 1 ml per day. The ^{3}H-thymidine had to be replaced every day; six animals could be infused simultaneously, by using six syringes.

Each animal received an infusion of 100 μCi / per ml of sterilized saline/per day of ^{3}H-thymidine (New England Nuclear Corp., specific activity 18.9 c/mM).

The animals were sacrificed in pairs at intervals of 1 day, $1\frac{1}{2}$ days, 2 days, 3 days, 4 days, 6 days, and 8 days after the beginning of infusion; after a 4-day infusion, two animals were sacrificed 4 days later, and the other two were sacrificed 8 days later.

The tissue (jejunum) was fixed by immersion in formaldehyde and glutaraldehyde containing solutions following the methods described in routine procedures. Then, the best fixed blocks were selected, and semi-thin sections were prepared for light microscope radioautography (to control the labeling reaction), and after the tissue was processed systematically for electron microscope radioautography.

Radioautographic methods

Light microscope radioautography

All materials were processed according to the method of Kopriwa and Leblond ('62), using Kodack NTB2 coating emulsion. A time exposure of 2 weeks was used to check the labeling reaction.

Electron microscope radioautography

According to the method of Kopriwa ('66), pre-cleaned microscope slides were dipped vertically into freshly diluted 0.8 percent celloidin in isoamilacetate — and dried vertically in a dust free cabinet overnight.

Pale gold to silver ultrathin sections of jejunum were cut with the Porter-Blum microtome and transferred to the celloidin coated slides with a platinum wire loop.

Coating method

The sections then were coated with Ilford L4 liquid emulsion diluted with double distilled water (1:4), and exposed for 2-3 months.

The exposed sections were developed for 1 minute in diluted D-19b developer in distilled water (1:4).

Transfer to E.M. grids

After drying the developed slides, the celloidin-emulsion complex was cut around the place the sections were located, and floated in distilled water. E.M. copper grids (300 or 200 mesh) were placed with forceps on the coated sections. Then with an aspirating device, the celloidin-emulsion layer with the sections covered by the grids was picked up on filter paper, and dried.

Post-staining

The grids containing sections were double stained with 4% uranyl acetate in 50% ethyl alcohol, 10-15 minutes and washed with the same alcohol solution; and with Reynold's lead citrate for another 10-15 minutes and washed in distilled water; grids were dried at room temperature.

Enterochromaffin cells, whether labeled or not, were then carefully identified in the electron microscope; all cells in crypts and villi were photographed.

Photographic methods

Electron-exposed plates, from routine or radioautographic material were developed in Kodak HRP developer for 3.5 minutes, rinsed in running tap water, fixed in Edwald Quick Fix for 10 minutes, and allowed to wash for one-half hour in running tap water; thereafter, they were dipped in Kodak Photo-Flo-200 solution a few seconds and dried for 30 minutes at 50°C in a dust free ventilator.

For analysis, enlarged prints were made of these plates.

RESULTS

Intestinal Epithelial Cells (other than Enterochromaffin)

Crypt Cells

Besides the columnar cells, the cells of the crypt include the Paneth cells, mucous (goblet) cells, and granulomucous cells, as well as the enterochromaffin cells which will be described below in detail. All epithelial cell types are separated from the connective tissue of the lamina propria by a basement membrane.

Located at the very bottom of the crypt among the Paneth cells are elongated columnar cells. Because these columnar cells appear to be squeezed between the adult Paneth cells, they have been called recently "intercalated cells" (Cheng, Merzel and Leblond, '69). These cells are structurally similar to those on the lateral walls of the crypt up to its middle third. They have small microvilli at the apical border, a cytoplasm rich in polysomes and free ribosomes, and a small number of mitochondria. Their nucleus is slender, with clumps of dense chromatin attached to the inner aspect of the nuclear membrane and interspersed in the nuclear sap. The nucleus, however, becomes more regular in shape and the complexity of the cytoplasmic organelles (endoplasmic reticulum, Golgi stacks) increases in cells at successively higher positions. The columnar cells are closely apposed to each other by interdigitating plasma membranes. These columnar cells frequently

show mitotic figures, which can be seen either between adult Paneth cells or in the two lower thirds of the crypt. Generally, the dividing cells have a less dense cytoplasm with the chromosomes in the centre. The mitochondria and small cisternae of rough-endoplasmic reticulum are at the periphery of the cytoplasm. Small dense granules can be observed in some mitotic figures, in the peripheral cytoplasm (Figure 16) or near the apical portion when this is seen in the plane of the sections.

A few cells in mitosis which contained a small number of membrane-bound typical mucous granules were identified as proliferating "oligomucous cells". This term has been used by Merzel and Leblond ('68) to describe a few cells seen in the lower third of the crypt above the Paneth cell zone, which had a small number of pale, homogeneous mucous granules; generally, in interphase, these cells have a dense cytoplasm with an organized rough-endoplasmic reticulum that surrounds the nucleus and extends to the apical cytoplasm, where the granules are located. Oligomucous cells are considered a stage in the differentiation of typical goblet cells which are first seen in the middle and upper thirds of the crypt. Typical goblet cells have a peculiar goblet shape, caused by distention of the supranuclear and apical portions (theca) with mucous granules. The apex has microvilli, most of which disappear when the cell starts secreting mucous substance. Typical goblet cells have a more developed rough-endoplasmic reticulum than oligomucous cells, which occupies the basal

cytoplasm, the paranuclear region and the cytoplasm around the Golgi apparatus.

Another cell type seen in the same areas as oligomucous and goblet cells, are the so-called "granulomucous cells" (Merzel and Leblond, '68). (These cells have been termed "intermediate cells" by Troughton and Trier ('69)). While they have the same general structure as goblet cells, the mucous granules may contain a small dense core which is centrally or excentrically located. Their granules resemble those of the Paneth cells but they are smaller and the mucous-like material in which they are enclosed is less dense than the substance of the capsules of the Paneth cells' secretory granules. The rough-endoplasmic reticulum of these cells, formed by a system of parallel cisternae, is generally less developed than in typical goblet cells and free ribosomes are more abundant in the cytoplasm.

Paneth cells are located at the very bottom of the crypt, lining the base of the gland. The supranuclear cytoplasm of Paneth cells is filled with characteristically large, rounded secretory granules, up to 3 μ in diameter. The granules have dense, homogeneous cores, and are enclosed by a capsule of less dense material (glutaraldehyde fixation) or may be surrounded by a clear halo (formaldehyde fixation) between the bounding membrane (always present) and the dense core. The well developed Golgi complex is located above the nucleus, and secretory granules are seen in close association with its components. Typical lysosomes can be observed in the basal portion of the Paneth

cells or near the Golgi apparatus.

Villus Cells

In the epithelial layer covering the villus, four epithelial cell types can be identified with the electron microscope: the columnar cells, which predominate; the goblet cells, with a conspicuous theca; a few granulomucous cells with a well developed theca; and the enterochromaffin cells. Lymphocytes, globule-leucocytes and eosinophils are frequent, between the epithelial cells, and more abundant than in the crypts; many of them are near the basement membrane.

As columnar cells of the base of the crypt migrate up to the villi, they undergo significant ultrastructural changes. From the mouth of the crypt to the villi they undergo a sudden change.

The columnar cells of the villus have an apical surface covered with long microvilli with a "fuzzy" coat and core filaments which are well preserved in glutaraldehyde fixed and acetone (cold) dehydrated material. The filaments in the cores of microvilli penetrate the apical cytoplasm merging with the terminal web. Laterally, in the cell membrane, there are terminal bars between adjacent cells.

The lateral membranes of the columnar cells in villi are increasingly interdigitated, and desmosomes may be found elsewhere along the lateral membranes of adjacent cells. Intercellular spaces are normally filled with a pale granular material.

The apical cytoplasm between the nucleus and the terminal web contains smooth-surfaced vesicles, coated vesicles, and multivesicular bodies, as well as profiles of the rough-endoplasmic reticulum and free ribosomes. On the apical side of the nucleus, stacks of the Golgi apparatus, with sacules and vesicles, are present; small secretory-like granules, enclosed by a membrane, may be seen in some cells that are structurally not different from the columnar cells, except for a lighter cytoplasm. Mitochondria of variable size are larger and more abundant than in crypt cells. They almost fill up the basal portion of the cells, which is apposed to the basement membrane.

Goblet cells of the villus are typical. They are interspersed between columnar cells. The Golgi complex forms a cuplike structure, made up of several stacks of saccules, some of which are swollen with material; mucus-filled globules occupy almost the entire apical cytoplasm (theca) and are seen to discharge from the cell (see Neutra and Leblond, '69).

Granulomucous cells are rare in villi, but here they also present a theca containing mucous-globules with central dense cores of different sizes.

Enterochromaffin Cells in Mouse Jejunum

The typical enterochromaffin cells in crypts and villi of the jejunum, as well as cells that are believed to be related to them, will now be described.

Enterochromaffin cells were defined, under the electron microscope, as cells containing a sizable group of dense cytoplasmic granules, mostly in the basal portion. In sections, these cells may appear to be away from the lumen, or they may reach it. In the latter case, like columnar, mucous, and Paneth cells, they present an apical border with microvilli and lateral terminal bars.

In this investigation, two main groups of enterochromaffin cells were found:

Biconcave granule cells (Type I)

These cells contain two kinds of granules. Most of them are biconcave, as will be demonstrated below, but some are spherical. These cells have been referred to in the literature as true argentaffin cells (Carvalheira, Welsch and Pearse, '68), as enterochromaffin cells type I (Forssmann et al. '69), or E C cells (Vassallo et al. '69).

Spherical granule cells (Types II and III)

These cells contain only spherical granules, which may be either large or small: cells with large granules were referred to as type II (Forssmann et al. '69), L cells (Vassallo et al. '69) or D cells (Kobayashi et al. '70); cells with small granules were called type III (Forssmann et al. '69), S cells (Vassallo et al. '69), or small rounded granule cells (Kobayashi et al. '70).

Other spherical granule cell types with a somewhat different appearance were rare in this material, but will also be described.

(I) Biconcave granule cells (Type I)

These cells were themselves subdivided into three classes, according to the frequency of the biconcave granules.

Subgroup Ia is characterized by the fact that nearly all granules are biconcave (95-100%) and few or none are spherical (0-5%) (see Figures 1 and 2). Examination of the shape of the granules in these cells (crescent-, comma-, or dumbbell-like) reveals a striking similarity to sections of red blood cells in which the biconcave shape is well known. Moreover, when sections of a biconcave-plasticine model (red blood-like) are cut and photographed, it can be seen that both sections of red blood cells (Figure 5) and of the plasticine model (Figure 4) are very similar to sections of the granules of enterochromaffin cells which have been described in the literature as irregular granules (Figure 3). Therefore, the term 'irregular granules" really applies to irregular sections of the biconcave granules which characterize this class of cells. These granules have an average diameter of 165 mμ (105-240 mμ).

In the present classification a few granules with a different appearance have been called "particulate granules". These are spherical, less dense, and have occasional opaque cores. "Particulate granules" are somewhat larger than biconcave granules with a diameter between 250-450 mμ.

This class of cells is found mostly in crypts (Figure 6).

Subgroup Ib is characterized by a predominance of biconcave granules (85%) plus a larger number of "particulate granules" (15%), in which the opaque cores are more evident than in subgroup Ia (Figures 2 and 7). These cells are found mostly in crypts, although a fair number are found in villi (Figure 6).

Subgroup Ic usually has about equal numbers of biconcave granules and "particulate granules", although the "particulate granules" may predominate (Figures 2 and 8). The "particulate granules" may have many dense cores, as was also found in subgroup Ib. A few biconcave granules may also contain intermediate density and opaque cores. This ultrastructural aspect of the granulations is seen in both formaldehyde-, or glutaraldehyde-fixed material.

Although Ic cells may be present in the crypts, they are mostly found in villi (Figure 6).

(II) Spherical granule cells (Type II and III)

As the name indicates, these cells are characterized by the spherical shape of all granules, which appear round in sections. They are usually somewhat smaller than biconcave granule cells. Although the overall appearance is the same, their Golgi apparatus appears larger and displays the forming granules more often than do biconcave granule cells. The spherical granules are usually dense and homogeneous with a narrow halo, but in many cells may consist of more or less empty-appearing vesicles; forms transitional between these two extremes are common. The spherical granule cells are divisible into two main cell

types according to the size of the granules:

Large spherical granule cells or type II have granules with a diameter between 300-420 mμ (360 mμ, average) (Figure 9); the halo of the granules is narrow and may be absent.

Small spherical granule cells or type III have granules with a diameter between 200-300 mμ (250 mμ, average) (Figure 10); the halo is very narrow (50 mμ) so that the diameter of the core averages 200 mμ.

(III) Other spherical granule cell types are observed in a small number of cases. In decreasing numbers, they are:

Cells with both spherical and oval granules (Figure 11): the spherical granules are smaller than the oval ones, but both types are very dense and, in most of them, there is no halo. While some granules may be less dense than the average, empty-appearing vesicles are not observed in these cells.

Cells with small spherical granules and large halos (Figure 12): most of the granules display a small dense excentrically located core which is close to the bounding-membrane. These cells are similar to the type IV cells of Forssmann et al. ('69).

Cells with spherical granules of different sizes and densities (Figure 13): the variability of these granules in both size (diameter from 300-600 mμ) and density (from pale to dense) is a constant feature. In the less dense granules, the bounding-membrane seems to incompletely surround the secretory material. These cells are similar to the type V cells of Forssmann et al. ('69).

(IV) Enterochromaffin cells of types I, II and III with few granules

In the lower half of the crypts, a small number of cells with a prominent round or slightly irregular nucleus contain only few biconcave (Figure 14, right) or spherical granules (Figure 15). These cells generally have a light cytoplasm which is rich in free ribosomes, but contains only a few cisternae of rough-endoplasmic reticulum.

Occasionally, some mitotic figures may display small dense spherical granules, although it is difficult to know if they are of the same kind as in spherical granule cells (Figure 16).

(V) Cells with light cytoplasm and no secretory granules

Also located in the lower crypt are a few cells with light cytoplasm, globular nucleus, and no granules at all. These cells always have a scanty rough-endoplasmic reticulum, but are rich in polysomes and free ribosomes. Desmosomes may be observed in these so-called light cells of the crypts (Figure 14, left).

Quantitative Study of Enterochromaffin Cells in Mouse Jejunum

In these investigations, a total of 1,300 typical enterochromaffin cells were recorded, both in crypts and villi, from routine preparations and radioautographic material. It was noticed that the most frequent cell type in mouse jejunum was the biconcave granule cells or type I (60 %) followed by the spherical granule cells (37%). The relative percentage of the type I cell subgroups and of the various spherical granule cell types is presented in the following text Table:

Quantitation of Enterochromaffin Cells
(Mouse Jejunum)

Type I or biconcave granule cells	60%	
-with rare "particulate" (Ia)		19%
-with about 1/4 "particulate" (Ib)		30%
-with about 1/2 "particulate" (Ic)		11%
Spherical granule cells	37%	
-type II, all spherical, large		13%
-type III, all spherical, small		24%
Other cell types	about 3%	
-cells with spherical and oval granules		2%
-cells with small granules and large halos		< 0.3%
-cells with granules of different sizes and densities		< 0.7%

Labeling of Enterochromaffin Cells with ^{3}H-Thymidine

Electron microscope radioautographs were prepared from thin sections of the crypts and villi of the mouse jejunum after ^{3}H-thymidine administration; and each enterochromaffin cell encountered was photographed.

The numbers of cells recorded, either labeled or not labeled, as well as the percentage of labeled cells, are presented in Tables 1 and 2.

The percentages of labeled cells at every time interval were also plotted against days after the beginning of the continuous infusion of ^{3}H-thymidine. The graphs obtained (Figures 17-25) show the behavior of the total enterochromaffin cell population in the columnar layer (Figure 17) and in crypts and villi (Figure 18); and also show the dynamics of the different cell types: biconcave granule cells (Figures 19 and 20) and their subgroups (Figure 21); spherical granule cells, as a total (Figures 22 and 23) and, separately as large spherical (Figure 24) and small spherical (Figure 25) granule cells.

Single injection

At 1 hour following a single intraperitoneal injection of ^{3}H-thymidine, none of the 61 typical enterochromaffin cells recorded in crypts and villi were labeled (Table 1). At this time interval a search for labeling of cells with light cytoplasm — light cells — was similarly negative.

Labeled mitoses never showed the typical dense granules of enterochromaffin cells; however, some mitotic figures, labeled or not,

exhibited a very few round dense granules as shown in Figure 16.

Continuous Infusion

After 1 day of continuous infusion, no typical enterochromaffin cells were labeled. In the crypts 74 cells, and in the villi 57 cells from two animals were photographed (Table 1). At the bottom of the crypts, however, a few cells with light cytoplasm and without dense granules were labeled.

By $1\frac{1}{2}$ days the situation had changed and a few labeled typical enterochromaffin cells appeared in the crypts, although not in villi. At this time interval 11 out of 124 enterochromaffin crypt cells, that is, 8.8 percent, were labeled (Table 1). The labeled cells were found at different levels in the lower crypt. Biconcave granule cells (Figure 26) and spherical granule cells (cells with large granules) (Figure 27) and cells with small granules (Figure 28) were both found labeled.

These initially labeled cells were called young enterochromaffin cells, since their main characteristic was the existence in their cytoplasm of a small number of typical secretory granules. Their nuclei were larger than those of adjacent columnar cells, somewhat irregular in shape, and showed the same electron-density as in the latter. The rough-endoplasmic reticulum was present in the form of a few long cisternae with attached ribosomes. Free ribosomes were numerous, but less than in adjacent columnar cells. Therefore, the cytoplasm of young enterochromaffin cells was lighter than in the other cell types.

At 2 days, typical labeled enterochromaffin cells were again seen only in the crypt where 10 out of 57, that is, 17.5 percent were recorded (Tables 1 and 2 and Graphs).

The labeled cells either resemble the young enterochromaffin cells or may be more granulated. Biconcave granule cells have a few "particulate granules" among the biconcave ones, as described for Ib cells. And the spherical granule cells may present a few empty-appearing vesicles among the dense granules.

At 3 days, for the first time labeled cells were found in villi (30.0 percent), and the percentage of crypt labeled cells increased (48.6 percent).

At this time interval some labeled cells, particularly those located in villi, were well granulated. In the case of biconcave granule cells they resembled the cells described as Ib. Labeled spherical granule cells were also more granulated than at the previous time intervals, and in some cells the number of empty-appearing vesicles increased.

At 4 days, about equal numbers of enterochromaffin cells in crypts (68 percent) and villi (59 percent) were labeled; at 6 days the labeling percentage increased and about 76 percent of enterochromaffin cells in both crypts and villi were labeled; and at 8 days, the percentage of labeled cells increased again to 84.3 percent in crypts, and 77.7 percent in villi.

The structure of the labeled cells at these time intervals either had the general features of young labeled cells described previously, or

resembled mature cells. Many crypt labeled biconcave granule cells had, at 4 days, the structure of Ia cells (Figure 29) or Ib cells (Figure 30), the latter with "particulate granules". At 8 days, the number of cells with many "particulate granules" increased (Ic cells) (Figure 34).

Labeled spherical granule cells at 4 days also contained an increasing number of dense granules, either in crypts (Figures 31 and 32) or villi, where granules with different densities were seen (Figure 33). At 6 and 8 days, spherical granule cells presented a more variable density in the granulations (Figure 35) or even a great number of empty-appearing vesicles (Figure 36).

A number of animals were sacrificed 4 days after cessation of a 4-day continuous infusion. Two of them were sacrificed 4 days later, i.e., 8 days after the beginning of the infusion; and the other two were sacrificed 8 days later, that is, 12 days after the beginning of the infusion.

In the first group (sacrificed 4 days after stopping the 4-day infusion) more than half of the cells were labeled. A total of 89 enterochromaffin cells were recorded, 49 in crypts (with 53.0 percent labeled), and 40 in villi (with 53.0 percent labeled). Labeled cells were evenly distributed from the bottom of the crypt to the upper villi.

Labeled crypt biconcave granule cells at this time interval all had a considerable number of granules; many had a structure of Ib and Ic cells. In villi, most had features of Ic cells, with a predominance of "particulate granules" (Figure 37), thus indicating that this

kind of granule was related to ageing of the cell.

Labeled spherical granule cells at this time interval were also well granulated and had a variable number of empty-appearing vesicles. One of these cells, presenting dense granules, empty-appearing vesicles, and forms transitional between both dense granules and vesicles, was found near the Paneth cells' zone (Figure 38).

Once again, labeled cells located at the bottom of the crypts may present a structure of mature cells. Such observations indicate that at least some enterochromaffin cells failed to migrate with other members of the same generation.

In the animals sacrificed 8 days after the end of the 4-day infusion, a total of 183 enterochromaffin cells were photographed (82 in crypts, and 101 in villi), and only 12 cells were labeled; three of the labeled cells were located in crypts (3.6 percent), and nine were found in villi (8.9 percent). Most of the cells, then, have disappeared from the crypts, as well as from the villi.

The labeled cells at this later time interval represented old enterochromaffin cells. In crypts, they all were biconcave granule cells. In villi, 6 out of 9 had biconcave granules, and the other 3 had spherical granules. Old biconcave granule cells had an irregular nucleus, and the granules were biconcave and "particulate" in texture (Figure 39); old spherical granule cells had dense granules and empty-appearing vesicles (Figure 40).

One of the biconcave granule cells found at the bottom of the crypt had a markedly irregular nucleus, and lysosome-like structures in the cytoplasm. These cells may eventually migrate upward or degenerate "in situ". Strong evidence was found for the latter, since some "Karyolitic bodies" labeled at 6 days (Figure 42) contained dense granules compatible with the identification of a "degenerating" enterochromaffin cell. Besides, a typical enterochromaffin cell was found at the lower crypt which seemed to be phagocytosed by a columnar cell (Figure 41). These findings suggested that a few enterochromaffin cells, at least in this material, were being removed by this way.

DISCUSSION

For many years, before the electron microscope period, the main problem that morphologists have investigated with regard to enterochromaffin cells was related to their ability to react with silver salts, either after treatment by an extraneous reducer (argyrophilia) or by direct reduction of a solution of a silver diamine complex to metallic silver, with blackening of the cytoplasmic content (argent-affinity) (Lison, '60).

The interest in these peculiar cells of the gastrointestinal tract was enhanced by the identification of 5-hydroxytryptamine or serotonin as the secretory product of enterochromaffin cells. (This has also been identified in many other cells and tissues) (for review: Page, '68.)

The differences between argyrophilic and argentaffin cells was attributed in the past to cyclic changes of a single cell type. The argyrophilic cells were regarded as being depleted of their secretory product, 5-hydroxytryptamine; whereas argentaffin cells were believed to contain this substance (Singh, '65; Ratzenhofer, '66).

Initially, it was thought that enterochromaffin granules require a formaldehyde-containing fixative to be preserved and, thereafter, stained. However, the first studies with the electron microscope used osmic acid as a fixative, and the blackening of the secretory granules and their fixation without formaldehyde was the main comment in those earlier papers (Christie, '55; Taylor and Hayes, '59). Thereafter,

either glutaraldehyde- or formaldehyde-containing solutions were used, and both showed the same general structure of these cells and their secretory granules. The usefulness of those fixatives was confirmed in the present work on the mouse jejunum, where the best methods were with perfusion of 4% formaldehyde or 2.5% glutaraldehyde, both in 0.1M Sorensen's phosphate buffer (pH 7.2), followed by post-osmication in 2% osmic acid in the same buffer system; also, when 2% osmic acid in Palade's veronal-acetate buffer was applied after the perfusion of 2.5% glutaraldehyde, a good fixation was achieved for all cell types of the jejunum.

I. ULTRASTRUCTURE OF ENTEROCHROMAFFIN CELLS

A few investigators, working with the electron microscope during the 'sixties, showed morphologic evidence for the presence of different types of enterochromaffin cells in the gastrointestinal mucosa of some mammals, including man (Helander, '62; Toner, '64; Rubin et al., '66; Ferreira, '66; Orci et al., '67; '68a and b; Forssmann et al., '68; Carvalheira, Welsch and Pearse, '68).

Forssmann et al. ('69) identified five different cell types of what were called endocrine cells of the gastrointestinal mucosa: (1) type I, or enterochromaffin cells (EC), which would be the source of 5-hydroxytryptamine; (2) type II, or intestinal A cells with spherical granules of 500-700 mμ in diameter, which would secrete glucagon; (3) type III, or intestinal D cells, with spherical granules of 150-250 mμ in diameter, related to the production of secretin; (4) type IV, or

catecholamine-like, with spherical granules of 150 mμ and others of 300-800 mμ in diameter, only found in the gastric mucosa; (5) and type V, with spherical granules of different densities and a diameter of 300-500 mμ, observed only in the pyloric region and postulated to be the source of gastrin.

At about the same time, groups of workers from Italy (Solcia et al., '67; '68; '69; Capella et al., '69; Vassallo et al., '69) identified at least six types of endocrine cells in the gastrointestinal mucosa of several mammals (cat, guinea-pig, rabbit, etc.). Of these, five types were found in the gastric epithelium, and three in the intestine. In the gastric mucosa, they described three types in the pyloric region (enterochromaffin cells, G cells secreting gastrin, and X cells of unknown function); four types were seen in the fundic region (enterochromaffin cells, enterochromaffin-like cells, A-like cells (i.e., compared to pancreatic), and X cells). In the intestinal mucosa, they recognized three types in the duodenum (enterochromaffin cells, and two polypeptide-producing cells — some with small granules or S cells, and others with large granules or L cells); in the ileum, only enterochromaffin cells and L cells were observed.

These publications neither indicated the position of these various types of cells in crypts or villi (in the case of the small intestine), nor did they provide quantitative data on their frequency.

In the present investigation on mouse jejunum, at least three well characterized enterochromaffin cell types were observed; other

cells, extremely rare in the material under study, will also be discussed.

(1) Type I or biconcave granule cells

These were the most frequently seen and were characterized by an abundance of granules with highly irregular shape. Because reconstructions showed that entire granules were similar in shape to red blood cells (Figures 3-5), the term "biconcave granules" was introduced. A survey of these type I cells in crypts and villi revealed that the majority of crypt cells have only biconcave granules (Ia cells), whereas the majority of villus cells have two kinds of granules in about the same ratio: biconcave granules and spherical granules, which are less dense and contain opaque cores ("particulate granules") (Ic cells). Forms transitional (Ib cells) have biconcave granules similar to those in Ia cells, and "particulate granules" are more abundant than in the latter, but less than in Ic cells (Figures 2 and 6).

When these findings were compared with the labeling data at different time intervals, it was observed that labeled Ia cells, with biconcave granules, were more abundant at earlier time intervals, and labeled Ic cells were more abundant at later time intervals. Therefore, the appearance of "particulate granules" appeared to be related to the maturation of the enterochromaffin cell type I.

It has been discussed in the literature that the secretory granules of these cells have two main components: a protein which displays basophilia following HCl treatment, and 5-hydroxytryptamine (Solcia et al., '68; Vassallo et al., '69); sites of maximal concentration of silver

deposits in sections treated with ammoniacal silver corresponded to the dense material of the biconcave granules or to the opaque cores of the "particulate granules" ("internal bodies" in observation of Vassallo et al., '69). As the Italian group claimed, the less dense material of the granules seemed to be less reactive.

It is opportune to refer to the work of S. Ito et al. ('69) who studied the pattern of incorporation and depletion of 5-hydroxytryptamine and 5-hydroxytryptophan, using ^{3}H-hydroxytryptamine creatinine sulphate in incubating minced gastric mucosa: in radioautographs from this material, they observed a rapid and preferential localization of label in enterochromaffin cells with large granules which had a low density matrix and a small electron-opaque core. These cells probably corresponded to cell types Ib and Ic of the present work.

Forssmann et al. ('69b), by means of radioautography after ^{3}H-hydroxytryptophan injection, found that the radioactivity was incorporated "chiefly in enteroserotonin cells" (same as type I cells). These authors also found that "other endocrine cells are capable of incorporating the serotonin precursor, at a different rate from endocrine cells type I."

(2) Spherical granule cells

In the mouse jejunum two main cell types containing spherical granules, were classified as large spherical granule cells, and small spherical granule cells. Both cell types were found either in crypts or villi. Also, a few cells with different types of spherical granules

were observed, but these were quite rare (see Table on p. 49).

a) Large spherical granule cells or Type II — In this material about one third of the spherical granule cells were of this type. Comparing the granule size of these cells with the ones described by previous authors, they may be similar to cells described by Forssmann et al. ('69) as "intestinal A cells", and thought to be concerned with glucagon secretion; or to cells described by Vassallo et al. ('69) as "L cells", and thought to be implicated in the cholecystokinin-pancreozymin production; or to cells described as "intestinal D cells" by Kobayashi et al. ('70), and considered to be involved in the production of secretin.

b) Small spherical granule cells of Type III — In mouse jejunum these were the most frequent of the spherical granule cells. Cells with granules of the same size and shape have been described by Toner ('64) in the fowl gastrointestinal mucosa, and were referred to by Forssmann et al. ('69) as "intestinal D cells" in the rat; by Vassallo et al. ('69) as "S cells" (150-250 mμ) in some mammals; and by Kobayashi et al. ('70) in human duodenum as "small-rounded granule cells" (150-250 mμ). While Forssmann et al. ('69) and Vassallo et al. ('69) proposed that cells with this kind of granule were responsible for secretin production, Kobayashi et al. ('70) compared them with the pancreatic A cells, thus assuming that they secreted glucagon. So, a discrepancy exists in the recent literature as to the designation of cells with small spherical granules and their physiological activity.

(3) Other spherical granule cells

In this material only about 3 percent of the enterochromaffin cells were different from types II and III:

a) Spherical and oval granule cells were found in crypts and villi. The granules were distinctive; in some cells, a few granules had a dense core surrounded by a less dense material, suggesting their similarity to the "intestinal D cells" of Kobayashi et al. ('70). These authors also referred to cells with both "large sized rounded granules and irregular shaped granules", in human duodenum, and showed a picture of this cell type in a crypt. In the present work, although cells with both spherical and oral granules were found in crypts, they were mainly found in villi — and it was almost impossible to know if they represented a different cell type or a variation of the large spherical granule or type II cells.

b) Small spherical granule and large halo cells were very rare in mouse jejunum (7 out of 1,300), and are characterized by the appearance of the granules with a small dense core located excentrically near the bounding-membrane. In the literature, cells having a similar structure were referred to by Forssmann et al. ('69) as Type IV cells or "catecholamine-like"; similar cells were described by Vassallo et al. ('69) as "EC-like". Both groups of authors found them only in the gastric mucosa. Vassallo and co-workers saw them in the lower two thirds of the fundic glands of the cat, lying on the basement membrane;

whereas Forssmann and associates found them particularly near the pylorus in the rat. Vassallo et al. ('69) claimed that in the cat both enterochromaffin or EC cells and EC-like cells "contribute to the positive reactions with the 5-hydroxytryptamine methods; the distribution between these two types of cells in light microscope is achieved only by means of the HCl-basic dye methods, which stains EC but not EC-like cells." In addition, they claimed that "EC-like cells may account in part" for EC cells storing only small amounts of 5-hydroxytryptamine.

The significance, here, of this rare cell type is not known. However, there was no evidence to correlate these cells morphologically with the changes which we described in the granulations of the biconcave granule cells (enterochromaffin cells type I or EC cells).

c) Cells with spherical granules of different sizes and densities were occasionally observed in crypts. Structurally similar cells have been mentioned by Forssmann et al. ('69), who described them in rat pylorus as "type V, the gastrin secreting cells"; Vassallo et al. ('69) described "G cells" in the fundus and pyloric region of the stomach and correlated such cells with the type V cells of Forssmann and associates. Further investigation has to be done to know if every group of workers is referring to the same cell. In this material, only 5 out of 1,300 were classified as this cell type.

II. KINETIC PROPERTIES OF ENTEROCHROMAFFIN CELLS

a) Origin and Maturation of Enterochromaffin Cells

It has been mentioned that 1 hour after a single injection of ^{3}H-thymidine no typical enterochromaffin cells were labeled. Therefore, DNA-synthesis does not take place in typical enterochromaffin cells; the lack of label at 1 hour and 1 day of continuous infusion, and the absence of mitosis in these cells (in a total of 1,300 cells recorded) leads to the conclusion that typical enterochromaffin cells do not divide.

Since a few labeled crypt cells have a small number of granules at $1\frac{1}{2}$ days, this means that unrecognized precursor of enterochromaffin cell had divided, giving rise to daughter cells which acquired a few granules, and thus could be recognized. Both biconcave granule cells and spherical (small and large) granule cells were found labeled at this early time interval: this indicates that they evolve independently, that is, they are different cell types. No support was found for the old theories of enterochromaffin cells being transformed into another cell type (see Popoff, '38; Schofield, '51-'53).

The young enterochromaffin cells labeled at $1\frac{1}{2}$ days of continuous infusion are similar to others described in routine preparations (Figures 14-15), as characterized by a small number of secretory granules, a lighter cytoplasm and a large nucleus. Therefore, it is now possible to identify these young cells on the electron microscope level in routine methods.

A few other cells, with light cytoplasm and no secretory granules at all, had become labeled by 1 day of continuous infusion. These cells have been called here "light cells". When semi-thin sections (0.5μ) were observed in the light microscope radioautographs, it was found that cells with round shape, light cytoplasm and large nucleus were present in the lower crypt and middle crypt, and a few of them were labeled at 1 day. We assume that these cells are the same as those which have been referred to in the literature as "light cells", and which have been related to the pre-enterochromaffin cells. The labeling of these cells 12 hours before the appearance of typical granules in cells recognized as typical enterochromaffin leads to the possibility of these cells being related to the immediate precursors of enterochromaffin cells. As light cells are not labeled at 1 hour after a single injection, they apparently do not self-proliferate.

Therefore, between 1 hour and 24 hours, proliferative cells located in the lower two thirds of the crypts evolve into cells with light cytoplasm; within 12 hours more, a few cells acquire typical secretory granules (young enterochromaffin cells); thereafter, these cells become well granulated, with specific granulation according to cell type. By 3 days they have characteristics of mature cells, and these features become better defined at 4, 6 and 8 days.

b) The "Cycle" of Enterochromaffin Cells

For many years, histologists have accepted the view that enterochromaffin cells constitute a single cell type, with a cycle which involved

the transformation of argyrophile cells into argentaffin cells, which in turn would become argyrophile again after the release of the secretory product, the 5-hydroxytryptamine. Using the electron microscope, the idea of a cycle in each cell type was also considered in some papers, but no sequence of the events was presented.

Here, it was found, knowing the structure of the cells labeled at different time intervals, that some morphological aspects of the granules are related to time:

(1) Initially, young biconcave granule cells have typical biconcave granules which are dense and homogeneous (Ia cells); later, these cells acquire an increasing number of spherical granules, less dense and with opaque cores ("particulate granules"), as found in cells Ib and specifically in Ic cells. In radioautography, Ic cells are found among cells labeled at later time intervals and are more frequent in villi.

(2) Spherical granule cells have a small number of granules, at early time intervals. With time the number of the granules increases, and many cells contain not only dense granules but also empty-appearing vesicles, and forms transitional between both.

Therefore, it is proposed that these morphological changes reflect a cycle within biconcave granule cells and spherical granule cells.

c) Migration and Renewal of Enterochromaffin Cells

Since enterochromaffin cells first appear labeled in crypts (at $1\frac{1}{2}$ days), and later in villi (at 3 days), this means that cells labeled in crypts have migrated to positions in the villi between $1\frac{1}{2}$ days and

3 days. At 4 days of continuous infusion of ^{3}H-thymidine, more than half of the enterochromaffin cell population has been replaced, since the labeling at this time interval was about 64 percent. And at 8 days of continuous infusion, about 80 percent of the cells were labeled, either in crypts or villi. In a different experiment, in which the infusion was stopped at the 4th day, and animals were sacrificed 4 and 8 days later, a decrease in the percentage of labeled cells was observed in the first group (4 days after the end of the infusion); and in the second group (8 days after the end of the infusion), almost all labeled cells had disappeared both from crypts and villi.

This means that enterochromaffin cells have migrated from the crypts to the villi, and are extruded. Cells which evolve from the lower levels in crypts may account for the few cells still present at the upper villi 8 days after termination of the 4-day infusion (old enterochromaffin cells); cells evolving from higher positions may be extruded sooner.

Therefore, it is proposed that the turnover time of the enterochromaffin cells in mouse jejunum is less than 8 days.

It was observed that some "Karyolitic bodies" in the base of the crypts may present a structure compatible with the identification of degenerating enterochromaffin granules. Also a typical enterochromaffin cell was found to be phagocytosed by a columnar cell (4 days after a 4-day infusion) in the lower crypt. These findings suggested that the rare labeled cells which still remain at the bottom

of the crypt 8 days after the availability of the radioisotope may be unable to move upward, become exhausted and, therefore, are attacked by phagocytic columnar cells (Hugon and Borgers, '66) in the base of the crypt.

Thus, like columnar, goblet and Paneth cells, enterochromaffin cells in mouse jejunum are renewed. The majority of enterochromaffin cells are extruded from the villus tip, by migrating from crypt to villus. A few may degenerate "in situ". But they all will be replaced in less than 8 days.

SUMMARY AND CONCLUSIONS

The enterochromaffin cells of mouse jejunum were investigated by electron microscopic radioautography at various time intervals after continuous infusion of ^{3}H-thymidine.

Three main cell types were identified in crypts and villi: a) type I or biconcave granule cells in which the predominant biconcave granules are associated with spherical granules having a "particulate" texture; b) type II or large spherical granule cells containing round-appearing granules with an average diameter of 350 mμ: c) type III or small spherical granule cells containing round-appearing granules with an average diameter of 250 mμ.

The biconcave granule cells are the most numerous (60%) whereas large spherical granule cells represent 13%, and small spherical granule cells 24% of the enterochromaffin cell population. Cells with different spherical granules are also present, but they account for about only 3 percent of the population.

Labeling of typical enterochromaffin cells was not observed 1 hour after a single injection of ^{3}H-thymidine, nor after 24 hours of continuous infusion of the substance. Even though 1,300 enterochromaffin cells with typical granules were photographed, mitosis was not observed in any of them. Hence, typical enterochromaffin cells do not divide in the jejunum of mice.

However, labeling of typical enterochromaffin cells was observed after $1\frac{1}{2}$ days of continuous infusion, but exclusively in cells located in

crypts; cells of the three main types were labeled although they presented only a small number of dense secretory granules. It was concluded that the three cell types evolve independently and are, therefore, different from one another. The crypt is the site of the transformation of unrecognized precursors into the various enterochromaffin cells.

The percentage of labeled enterochromaffin cells in crypts rose at 2 days of continuous infusion, although no labeled cells were found in villi at this time interval. At 3 days, with a further increase in labeled cells, some of them made their appearance in villi. Enterochromaffin cells of the three types had migrated from crypt to villus by the third day. After 4, 6, and 8 days of continuous infusion, the percentage of labeled cells of the three types increased with about 80 percent labeling at the eighth day.

When the continuous infusion was stopped at the 4th day and the animals sacrificed 4 days later, the percentage of labeled cells was decreased; if the animals were sacrificed 8 days after the end of the 4-day infusion, most of the labeled cells had disappeared from crypts and villi.

It was concluded that enterochromaffin cells turn over in the jejunum of mice, and that the turnover time is less than eight days.

BIBLIOGRAPHY

Amano, M., B. Messier and C. P. Leblond - Specificity of labeled thymidine as a DNA precursor in radioautography. J. Histochem. Cytochem., 1959, 7:153-155.

Andrew, A. - A study of the development relationship between enterochromaffin cells and the neural crest. J. Embryol. exp. Morph., 1963, 11:307-324.

Asquith, P., A.G. Johnson and W.T. Cooke - Scanning electron microscopy of normal and celiac jejunal mucosa. Am. J. Dig. Dis., 1970, 15:511-521.

Behnke, O. - Demonstration of acid phosphatase-containing granules and cytoplasmic bodies in the epithelium of fetal rat duodenum during certain stages of differentiation. J. Cell. Biol., 1963, 18:251-265.

Bertalanffy, F.D. - Cell renewal in the gastrointestinal tract of man. Gastroenterology, 1962, 43:472-475.

Black, W.C. - Enterochromaffin cell types and corresponding carcinoid tumors. Lab. Invest., 1968, 19:473-486.

Brown, H., N.E. Kahil and C.H. Gregory - Functioning carcinoid tumors. In: Cancer of the Gastrointestinal Tract (Year Book Medical Publishers, Inc.), Chicago, 1967, pp. 155-174.

Bullon-Ramirez, A., E. Langer and H. Shultze - La visualizacion de los granulos de la celulas cromoenteroargentafines mediante el microscopio electronico. Rev. Clin. Esp., 1960, 79:321-328.

Cairnie, A.B., Lamerton, L.F. and G.G. Steel - Cell proliferation studies in the intestinal epithelium of the rat. I. Determination of the kinetic parameters. Exp. Cell Res., 1965, 39:528-538.

Cairnie, A.B. - Renewal of goblet and Paneth cells in the small intestine. Cell Tissue Kinet., 1970, 3:35-45.

Cameron, I.L. and R.C. Greulich - Evidence for an essentially constant duration of DNA synthesis in renewing epithelia of the adult mouse. J. Cell Biol., 1963, 18:31-40.

Capella, C., E. Solcia and G. Vassallo - Identification of six types of endocrine cells in the gastrointestinal mucosa of the rabbit. Arch. histol. jap., 1969, 30:479-495.

Carvalheira, A.F., U. Welsch and A.G.E. Pearse - Cytochemical and ultrastructural observations on the argentaffin and argyrophil cells of the gastrointestinal tract in mammals, and their place in the Apud series of polypeptide-secreting cells. Histochemie, 1968, 14:33-46.

Chang, W. W.-L. - Renewal of the epithelium of the descending colon of the mouse. Ph.D. Thesis, McGill University, Montreal, Canada, 1970.

Cheng, H.P.L. - Renewal of Paneth Cells. M.Sc. Thesis, McGill University, Montreal, Canada, 1969.

Cheng, H.P.L., J. Merzel and C.P. Leblond - Renewal of Paneth cells in the small intestine of the mouse. Am. J. Anat., 1969, 126:507-526.

Christie, A.C. - A study of the Kultschitzky (argentaffin) cells with the electron microscope, after fixation by osmium tetroxide. Quart. J. Microscop. Sci., 1955, 96:295-299.

Clara, M. - Über die Morphologie und Histochemie der basalgekörnten Zellen. Acta Neuroveg. (Wien), 1957, 16:294-312.

Cordier, R. - Contribution à l'étude de la cellule de Ciaccio-Masson et de la cellule de Paneth. C.R. Soc. Biol. (Paris), 1923, 88:1227-1230.

Cordier, R. - Recherches morphologiques experimentales sur la cellule chromo-argentaffine de l'épithelium intestinal des Vertébrés. Arch. Biol. (Liège), 1926, 36:427-463.

Cordier, R. and L. Lison - Étude histochimique de la substance chromo-argentaffine de la cellule de Kultschitzky. Bull. Hist. Appl., 1930, 7:140-148.

Coulombre, A.J. and J.C. Coulombre - Intestinal development. I. Morphogenesis of the villi and musculature. J. Embryol. exp. Morphol., 1958, 6:403-411.

Creamer, B., R.G. Shorter and J. Bamforth - Turnover and shedding of epithelial cells. I. The turnover in the gastrointestinal tract. Gut, 1961a, 2:110-115.

Creamer, B., R.G. Shorter and J. Bamforth - Turnover and shedding of epithelial cells. II. The shedding in the small intestine. Gut, 1961b, 2:115-118.

Deschner, E.E. - Observations on the Paneth cell in human ileum. Exp. Cell Res., 1967, 49:624-628.

Deschner, E.E. and M. Lipkin - An autoradiographic study of the renewal of argentaffin cells in human rectal mucosa. Exp. Cell Res., 1966, 43:661-665.

Dias-Amado, L. - Sur l'existence de cellules argentaffines dans le tissue conjunctif des villosités intestinales. C.R. Soc. Biol. (Paris), 1925a, 93:1544-1549.

Dias-Amado, L. - Sur la signification des cellules de Nicolas. C.R. Soc. Biol. (Paris), 1925b, 93:1550-1551.

Erspamer, V. - Ricerche sperimentali sul significato biologico delle cellule enterochromaffini. Arch. Fisiol., 1937, 37:156-169.

Erspamer, V. - Il sistema cellulare enterochromaffine e l'enteramina (5-idrossitriptamina). Rend. Sci. Farmitalia, 1954, 1:1-193.

Erspamer, V. and B. Asero - Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature (London), 1952, 169:800-801.

Evans, J.S. - A technique for chronic vascular intubation for continuous infusion in unanesthetized and relatively unrestrained rats. Anat. Rec., 1968, 160:510.

Falck, B., N.A. Hillarp, G. Thieme and A. Torp - Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytochem., 1962, 10:348-354.

Ferreira, M.N. - On the existence of two ultrastructural types of enterochromaffin cells in the duodenum of the chick embryo. Arch. Port. Sc. Biol. (Lisbon), 1966, 15:75-86.

Forssmann, W.G., L. Orci, R. Pictet and C. Rouiller - Zur Ultrastruktur der endokrinen Zellen im Epithel des Magendarmtraktes der Ratte. Acta Anat., 1967, 68:605-606.

Forssmann, W.G., L. Orci and C. Rouiller - The problem of gastrin-producing cells. J. Cell Biol., 1968, 39:167a.

Forssmann, W.G., L. Orci, R. Pictet, A.E. Renold and C. Rouiller - The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. J. Cell Biol., 1969, 40:692-715.

Forssmann, W.G., A. Perrelet, D. Tesi and C. Rouiller - Experimental evaluation of the function of the enterochromaffin cell (Proc.) (Ninth Annual Meeting, Am. Soc. for Cell Biology, Detroit), 1969, paper no. 88.

Friedman, N.B. - Cellular dynamics in the intestinal mucosa : the effect of irradiation on epithelial maturation and migration. J. exp. Med., 1945, 81:553-558.

Gomori, G. - Chemical character of the enterochromaffin cells. Arch. Path., 1948, 45:48-55.

Häkanson, R., Ch. Owman, N.-O. Sjöberg and B. Sporrong - Amine mechanisms in enterochromaffin and enterochromaffin-like cells of gastric mucosa in various mammals. Histochemie, 1970, 21:189-220.

Hamperl, H. - Über argyrophile Zellen. Arch. path. Anat., 1952, 321:482-507.

Hampton, J.C. - Effects of ionizing radiation on the fine structure of Paneth cells of the mouse. Rad. Res., 1966, 28:71-83.

Hampton, J.C. - Further evidence for the presence of a Paneth cell progenitor in mouse intestine. Cell Tissue Kinet., 1968, 1:309-317.

Heidenheim, R. - Untersuchungen über den Bau der Labdrüsen. Arch. mikr. Anat., 1870, 6:368-406 (cit. by Erspamer, 1954).

Helander, H.F. - A preliminary note on the ultrastructure of the argyrophile cells of the mouse gastric mucosa. J. Ultrastruct. Res., 1961, 5:257-262.

Helander, H.F. - Ultrastructure of fundus glands of the mouse gastric mucosa. J. Ultrastruct. Res., 1962, Suppl. 4.

Hellweg, G. - Über Vorkommen und gegenseitiges Verhalten der argentaffinen und argyrophilen Zellen im menschlichen Magen-Darmtrakt. Z. Zellforschung, 1952, 36:546-551.

Howard, A. and S.R. Pelc - Synthesis of desoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. Heredity (Suppl.), 1953, 6:261-273.

Hugon, J.S. and M. Borgers - Ultrastructural differentiation and enzymatic localization of phosphatases in the developing duodenal epithelium of the mouse. I. The foetal mouse. Histochemie, 1969, 19:13-30.

Ito, S., R.E. Coggeshall, J.S. Mueller and B. Yaksta - Radioautography of enterochromaffin cells (Proc.) (Ninth Annual Meeting, Am. Soc. for Cell Biology, Detroit), 1969, paper no. 136.

Johnson, F.P. - The development of the mucous membrane of the oesophagus, stomach, and small intestine in the human embryo. Am. J. Anat., 1910, 10:521-561.

Kammeraad, A. - The development of the gastro-intestinal tract of the rat. I. Histogenesis of the epithelium of the stomach, small intestine and pancreas. J. Morph., 1942, 70:323-342.

Kobayashi, S., T. Fujita and T. Sasagawa - The endocrine cells of human duodenal mucosa. An electron microscope study. Arch. hist. jap., 1970, 31:477-494.

Kopriwa, B.M. and C.P. Leblond - Improvement in the coating technique of radioautography. J. Histochem. Cytochem., 1962, 10:269-284.

Kopriwa, B.M. - A semiautomatic instrument for the radioautographic coating technique. J. Histochem. Cytochem., 1966, 14:925-928.

Kurosumi, K., S. Shibasaki, G. Uchida, and Y. Tanaka - Electron microscope studies on the gastric mucosa of normal rats. Arch. hist. jap., 1958, 15:587-624.

Leblond, C.P. - Classical techniques for the study of the kinetics of cellular proliferation. In: Stohlman, F. Jr. - The kinetics of cellular proliferation. (Grune and Stratton Inc.), New-York, 1959, pp. 31-49.

Leblond, C.P. - Classification of cell populations on the basis of their proliferative behavior. Nat. Cancer Inst. Monogr., 1964, 14:119-150.

Leblond, C.P. - The time dimension in Histology. Am. J. Anat., 1965, 116:1-18.

Leblond, C.P. and C.E. Stevens - The constant renewal of the intestinal epithelium in the albino rat. Anat. Rec., 1948, 100:357-377.

Leblond, C.P., C.E. Stevens, and R. Bogoroch - Histological localization of newly-formed desoxyribonucleic acid. Science, 1948, 108:531-533.

Leblond, C.P. and B.E. Walker - Renewal of Cell populations. Physiol. Rev., 1956, 36:255-275.

Leblond, C.P. and B. Messier - Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of ^{3}H-thymidine into mice. Anat. Rec., 1958, 132:247-259.

Leblond, C.P., B. Messier and B. Kopriwa - Thymidine-H^3 as a tool for the investigation of the renewal of cell populations. Lab. Invest., 1959, 8:296-308.

Leblond, C.P., R.C. Greulich and J.P.M. Pereira - Relationship of cell formation and cell migration in the renewal of stratified squamous epithelia. Adv. on Biology of Skin, 1964, 5:39-67.

Leblond, C.P., Y. Clermont, and N.J. Nadler - The pattern of stem cell renewal in three epithelia (oesophagus, intestine and testis), Proc. Can. Cancer Res. Conf., 1967, 7:3-30.

Leblond, C.P., J. Merzel and H. Cheng - Cell renewal in the crypts of Lieberkühn of the small intestine in mice. IIIrd Int. Congr. Histochem. Cytochem., Springer-Verlag, New-York, 1968, pp. 138-139.

Lesher, S., L.F. Lamerton, G.A. Sacher, R.J.M. Fry, G.G. Steel and P.J. Roylance - Effect of continuous gamma irradiation of the generation cycle of the duodenal crypt cells of the mouse and rat. Rad. Res., 1966, 29:57-70.

Lipkin, M., B. Bell and P. Sherlock - Cell proliferation in the gastrointestinal tract of man. I. Cell renewal in colon and rectum. J. Clin. Invest., 1963a, 42:767-776.

Lipkin, M., P. Sherlock and B. Bell - Cell proliferation in the gastrointestinal tract of man. II. Cell renewal in stomach, ileum, colon, and rectum. Gastroenterology, 1963b, 45:721-729.

Lipkin, M. - Cell replication in the gastrointestinal tract of man. Gastroenterology, 1965a, 48:616-624.

Lipkin, M. - Cell proliferation in the gastrointestinal tract of man. Fed. Proc., 1965b, 24(1), Part I:10-15.

Lison, L. - Recherches histochimiques sur les phenols et leur dérivés. Arch. Biol., 1931, 41:343-436.

Lison, L. - Histochimie et Cytochimie Animales. Principes et Méthodes. (Ed. Gauthier Villards), Paris, 1960, Vols. I and II.

MacDonald, W.C., J.S. Trier and N.B. Everett - Cell proliferation and migration in the stomach, duodenum, and rectum of man: radioautographic studies. Gastroenterology, 1964, 46:405-417.

Macklin, C.C. and M.T. Macklin - The intestinal epithelium. In: Special Cytology (Ed. E.V. Cowdry), 1932, Vol. I, pp. 233-331.

Merzel, J. and C.P. Leblond - Origin and renewal of goblet cells in the epithelium of the mouse small intestine. Am. J. Anat., 1969, 124:281-306.

Messier, B. and C.P. Leblond - Cell proliferation and migration as revealed by radioautography after injection of thymidine-H^3 into male rats and mice. Am. J. Anat., 1960, 106:247-285.

Monesi, V. - The appearance of enterochromaffin cells in the intestine of the chick embryo. Acta Anat. (Basel), 1960a, 41:97-114.

Monesi, V. - Differentiation of argyrophil and argentaffin cells in organotypic cultures of embryonic chick intestine. J. Embryol. exp. Morph., 1960b, 2:302-313.

Neutra, M. and C.P. Leblond - The Golgi Apparatus. Sci. Amer., 1969, 220:100-107.

Orci, L., W.G. Forssmann and R. Pictet - Mise en évidence des types de cellules à granulations denses dans le système digestif du rat. J. Microscopie, 1967, 6:74a.

Orci, L., R. Pictet, W.G. Forssmann, A.E. Renold and C. Rouiller - Structural evidence for glucagon producing cells in the intestinal mucosa of the rat. Diabetologia, 1968a, 4:56-67.

Orci, L., W.G. Forssmann, W. Forssmann and C. Rouiller - Electron microscopy of the intestinal endocrine cells. Comparative study. Fourth European Conference on Electron Microscopy, 1968b, 2:369-370.

Page, I.H. - Serotonin. (Year Book Medical Publishers, Inc.), Chicago, 1968.

Peart, W.S. et al. - Carcinoid syndrome due to pancreatic duct neoplasm secreting 5-hydroxytryptophan and 5-hydroxytryptamine. Lancet, 1963, i:239-242.

Penttilä, A. - Enterochromaffin cells in the chicken duodenum during prenatal development. Z. Zellforschung, 1968, 91:380-390.

Popoff, N.W. - Epithelial functional rejuvenation observed in the mucous cells of gastrointestinal tract and the Paneth cells of stomach. Arch. Path. 1939, 27:841-887.

Quastler, H. and F.G. Sherman - Cell population kinetics in the intestinal epithelium of the mouse. Exp. Cell Res., 1959, 17:420-438.

Rapport, M.M., A.A. Green and I.H. Page - Serum vasoconstrictor (serotonin). IV. Isolation and characterization. J. Biol. Chem., 1948, 176:1243.

Ratzenhofer, M. and D. Leb - Über die Feinstruktur der argentaffinen und der anderen Erscheinungsformen der "Hellen Zellen" Feyrter's im Kaninchenmagen. Z. Zellforschung, 1965, 67:113-150.

Ratzenhofer, M. - Zur Biologie der endokrinen Zellen (des Helle-Zellen - Organs, Feyrter) im Verdauungstrakt. (Nach Untersuchungen am Kaninchenmagen). Klin. Wschr., 1966, 44:109-115.

Rau, E. - The effects of varying the composition of aldehyde fixatives for perfusion of young rats. M.Sc. Thesis, McGill University, Montreal, Canada, 1969.

Rubin, W., L.L. Ross, G.H. Jeffries and M.H. Sleisenger - Intestinal heterotopia. A fine structural study. Lab. Invest., 1966, 15:1024-1049.

Sandler, M. and P.J.D. Snow - An atypical carcinoid tumour secreting 5-hydroxytryptophan. Lancet, 1958, i:137-139.

Schofield, G. - The argentaffin cells of the small intestine of the guinea-pig. Acta Anat., 1951, 11:414-430.

Schofield, G. - The argentaffin and mucous cells of the small and large intestines of the mouse. Acta Anat., 1952, 16:1-15.

Schofield, G. - The argentaffin and mucous cells of the human intestine. Acta Anat., 1953, 18:256-272.

Sharples, W. - The histogenesis of the argentaffin cells in the stomach and duodenum of the rat. Anat. Rec. 1945, 91:107-117.

Shorter, R.G., C.G. Moertel, J.L. Titus and R.J. Reitemeier - Cell kinetics in the jejunum and rectum of man. Amer. J. Dig. Dis., 1964, 9:760-763.

Singh, I. - The prenatal development of enterochromaffin cells in the human gastro-intestinal tract. J. Anat. (London), 1963a, 97:377-387.

Singh, I. - On the alleged presence of non-argyrophile argentaffin cells in the human gastro-intestinal tract. Z. Zellforschung, 1963b, 59:615-624.

Singh, I. - Further observations on the alleged presence of non-argyrophile argentaffin cells in the human gastro-intestinal tract. Z. Zellforschung, 1964, 62:121-124.

Singh, I. - The relative proportion of pre-enterochromaffin (argyrophile) and enterochromaffin (argentaffin) cells in the gastro-intestinal tract of the human foetuses. Z. Zellforschung, 1965, 67:338-342.

Singh, I. - The distribution of cells of the enterochromaffin system in the gastro-intestinal tract of human foetuses. Acta Anat. (Basel), 1966, 64:544-588.

Solcia, E., Vassallo, G., and R. Sampietro - Endocrine cells in the antro-pyloric mucosa of the stomach. Z. Zellforschung, 1967, 81:474-486.

Solcia, E., G. Vassallo and C. Capella - Studies on the G cells of the pyloric mucosa, the probable site of gastrin secretion. Gut, 1969, 10:379-388.

Sokoloff, B. - Carcinoid and Serotonin. In: Recent Results in Cancer Research, 1958, 15:1-109.

Stevens, C.E. - Cell turnover of the intestinal epithelium as shown by mitotic counts and the incorporation of P^{32} into desoxyribonucleic acid. Ph.D. Thesis, McGill University, Montreal, Canada, 1950.

Stevens Hooper, C.E. - Cell turnover in epithelial populations. J. Histochem. Cytochem., 1956, 4:531-540.

Taylor, J.J. and E.R. Hayes - An electron microscopic study of the ultrastructure of the argentaffin cell. J. Applied Physics, 1959, 30:3033 (Proc.).

Thrasher, J.D. and R.C. Greulich - The duodenal progenitor population. III. The progenitor cell cycle of principal, goblet and Paneth cells. J. Exp. Zool., 1966, 161:9-20.

Toner, P. - Fine structure of argyrophile and argentaffin cells in the gastro-intestinal tract of the fowl. Z. Zellforschung, 1964, 63:830-839.

Trier, J.S. - Studies on small intestinal crypt epithelium. I. The fine structure of the crypt epithelium of the proximal small intestine of fasting humans. J. Cell Biol., 1963, 18:599-620.

Troughton, W.D. and J.S. Trier - Cell proliferation in intestinal crypts. Clin. Res., 1968, 16:119.

Troughton, W.D. and J.S. Trier - Paneth and goblet cell renewal in mouse duodenal crypts. J. Cell Biol., 1969, 41:251-268.

Vassallo, G., E. Solcia and C. Capella - Light and electron microscopic identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. Z. Zellforschung, 1969, 98-333-356.

Walker, B.E. - Sites of cellular renewal visualized by radioautography after C^{14}-adenine injection. Anat. Rec., 1957, 127:383 (Proc.).

Walker, B.E., and C.P. Leblond - Sites of nucleic acid synthesis in the mouse visualized by radioautography after administration of C^{14} labeled adenine and thymidine. Exp. Cell Res., 1958, 14:510-531.

Williams, E.D. and M. Sandler - The classification of carcinoid tumors. Lancet, 1963, i:238-239.

TABLE 1

^{3}H-Thymidine Labeling of Enterochromaffin Cells in Mouse Jejunum*

		ALL CELL TYPES					
		CRYPTS			VILLI		
	Days After Beginning	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label
Single Injection	0.04	24	0		5	0	
		18	0		1	0	
		12	0	0	1	0	0
Continuous Infusion	1	60	0		48	0	
		14	0	0	9	0	0
	1.5	51	6		65	0	
		24	2		27	0	
		22	2		11	0	
		16	1	8.8	11	0	0
	2	30	7		8	0	
		17	3	17.5	4	0	0
	3	25	23		13	5	
		14	14	48.6	8	4	30.0
	4	14	28		12	21	
		4	11	68.4	8	8	59.1
	6	13	45		3	13	
		5	17	77.5	4	8	75.0
	8	4	18		1	9	
		4	25	84.3	5	12	77.7
Four-Day Infusion	8	16	19		8	7	
		7	7	53.0	9	16	57.5
	12	17	3		42	6	
		62	0	3.6	50	3	8.9

* Each line corresponds to one animal.

TABLE 2

^{3}H-THYMIDINE LABELING OF ENTEROCHROMAFFIN CELLS IN MOUSE JEJUNUM*

		BICONCAVE GRANULES						LARGE SPHERICAL GRANULES						SMALL SPHERICAL GRANULES					
		Crypt cells			Villus cells			Crypt cells			Villus cells			Crypt cells			Villus cells		
	Days after start	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label	Non Label	Label	Mean % Label
Single injection	0.04	19	0		2	0		3	0		2	0		2	0		-	-	
		14	0		1	0		2	0		1	0		2	0		-	-	
		10	0	0	1	0	0	1	0	0	-	-	0	1	0		-	-	-
Continuous infusion	1	36	0		32	0		7	0		4	0		17	0		12	0	
		12	0	0	7	0	0	-	-	0	1	0	0	2	0	0	1	0	0
	1.5	32	3		42	0		8	1		10	0		11	2		13	0	
		15	1		24	0		3	0		1	0		4	1		2	0	
		11	1		10	0		3	0		-	-		6	1		1	0	
		8	1	8.3	5	0	0	3	0	5.5	2	0	0	4	0	13.7	4	0	0
	2	15	3		5	0		8	2		1	0		7	2		2	0	
		6	1	16.0	3	0	0	6	1	17.6	-	-	0	5	1	20.0	1	0	0
	3	17	15		8	3		4	4		1	1		4	4		4	1	
		9	6	44.6	5	3	31.6	2	4	57.1	1	0	33.3	3	4	53.3	2	1	25.0
	4	10	21		7	15		2	3		3	4		2	4		2	3	
		2	6	69.2	4	4	63.3	1	2	62.5	2	1	50.0	1	3	70.0	2	2	55.5
	6	7	34		1	6		1	4		2	5		2	7		2	2	
		5	11	78.9	2	4	76.9	1	2	75.0	-	3	80.0	2	4	73.3	-	1	60.0
	8	3	13		1	6		-	-		0	2		1	5		2	3	
		3	20	85.0	3	7	76.0	0	2	100.0	0	2	100.0	1	3	80.0	-	1	66.6
Four-day infusion	8	14	16		6	5		-	-		-	-		2	3		2	2	
		6	5	51.2	6	12	57.5	1	1	50.0	2	2	50.0	-	1	66.6	1	2	57.1
	12	8	3		17	3		2	0		9	0		7	0		16	3	
		29	0	7.5	36	1	7.0	6	0	0	5	0	0	27	0	0	9	2	16.6

* Each line corresponds to one animal

Fig. 1. Biconcave granule cell (Type I), subgroup Ia, characterized by the presence of granules in the basal cytoplasm, which borders on the basement membrane (BM). The granules that appear irregular are biconcave, as demonstrated in the next figure. The cells of subgroup Ia may have an occasional less dense, spherical granule, here called "particulate granule" (arrow) but the proportion of these granules is quite small. 30,000 X. (Glutaraldehyde.)

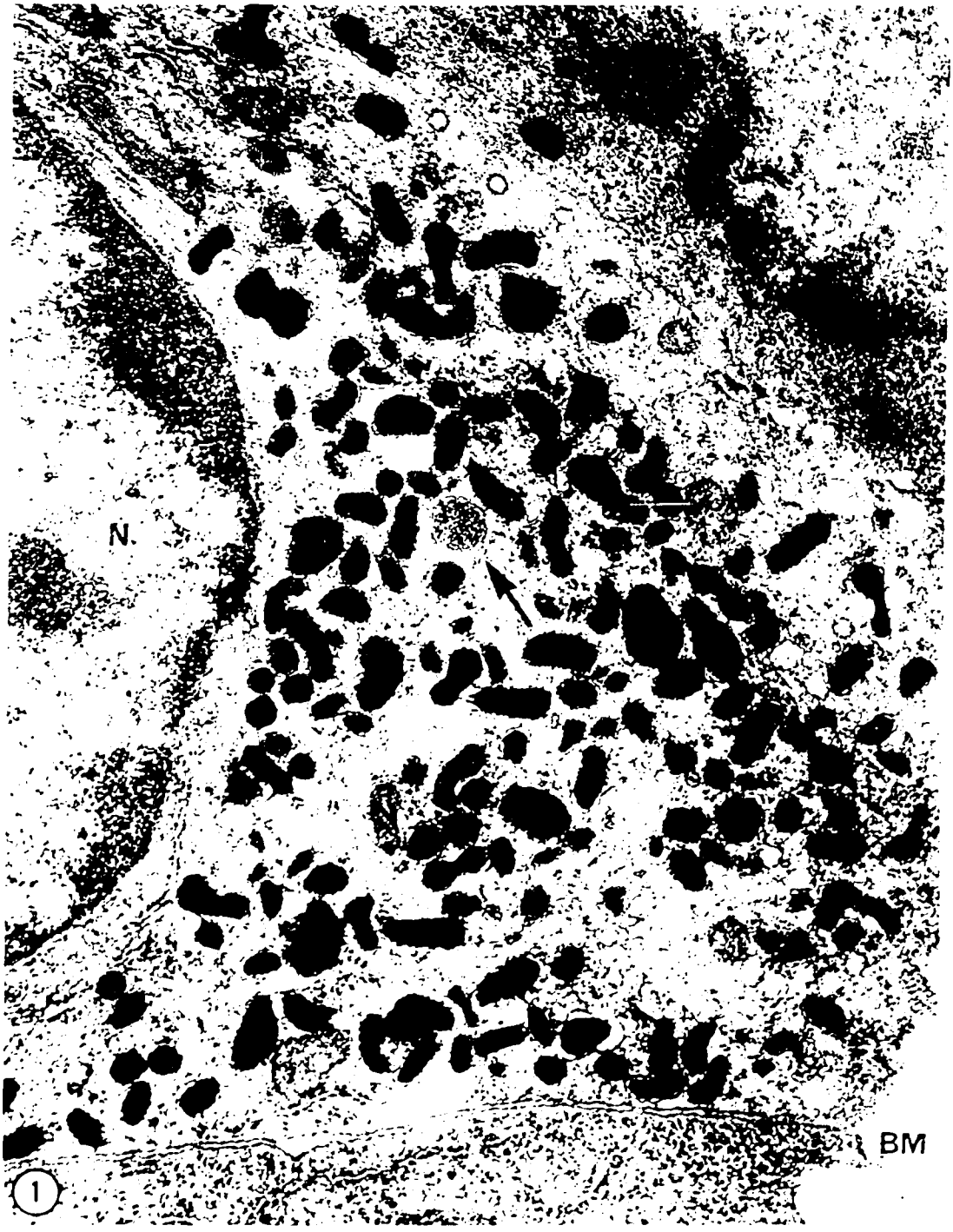
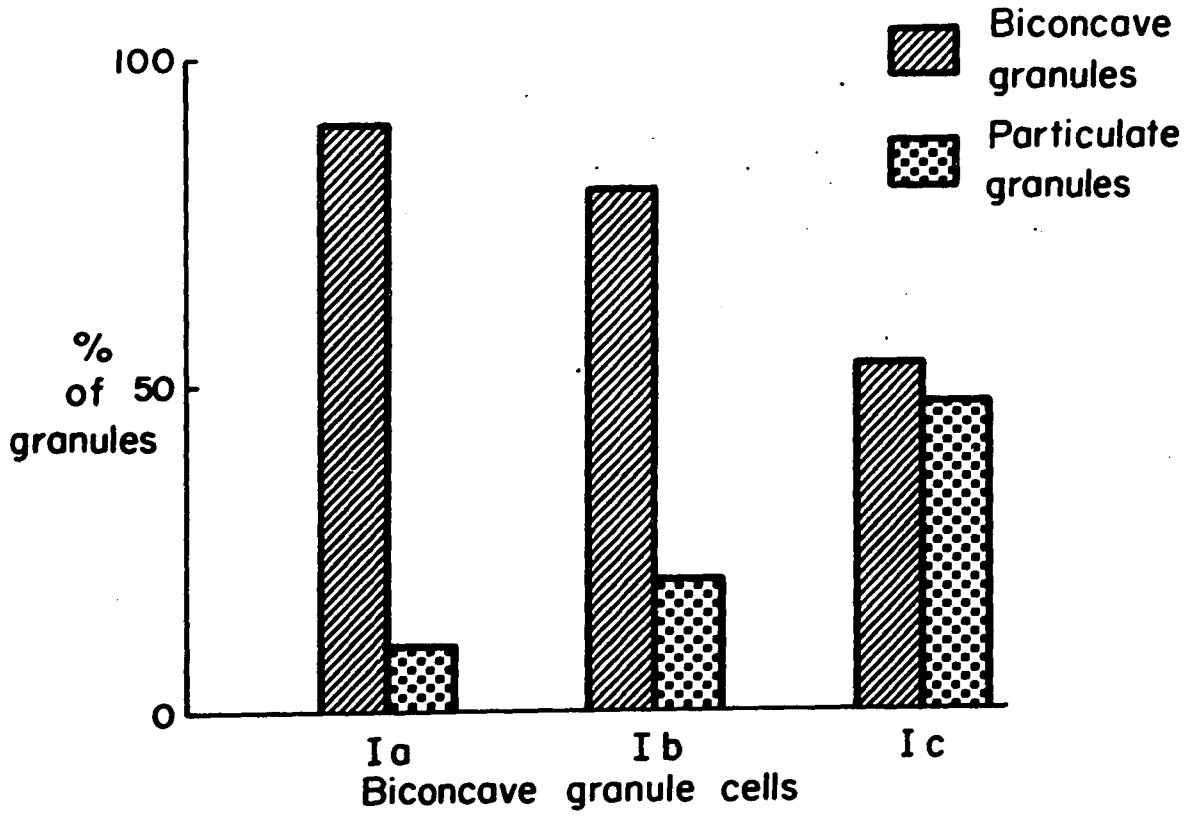

N
BM
1

Fig. 2. Biconcave granule cells (Type I).
Histogram demonstrating the percentage of the two types of granules (biconcave and particulate) present in the three subgroups of biconcave granule cells.
The frequency of particulate granules increases from subgroup Ia to Ic.


Biconcave granules
Particulate granules
100
% of granules
50
0
Ia
Ib
Ic
Biconcave granule cells

Figs. 3 - 5. Sections of "irregular-looking granules" were compared with sections of a biconcave model of plasticine and with E.M. sections of red-blood cells:

Fig. 3. Sections of irregular-looking granules found in cells of subgroup Ia. A variety of shapes is observed (comma-, dumbbell-like, etc.).

Fig. 4. Sections of a biconcave model of plasticine. The sections are similar to those of the granules shown above.

Fig. 5. E.M. sections of red-blood cells found in small vessels of the intestinal mucosa. The similarity with either the sections of the irregular granules of enterochromaffin cells or with the sections of the biconcave model is evident.

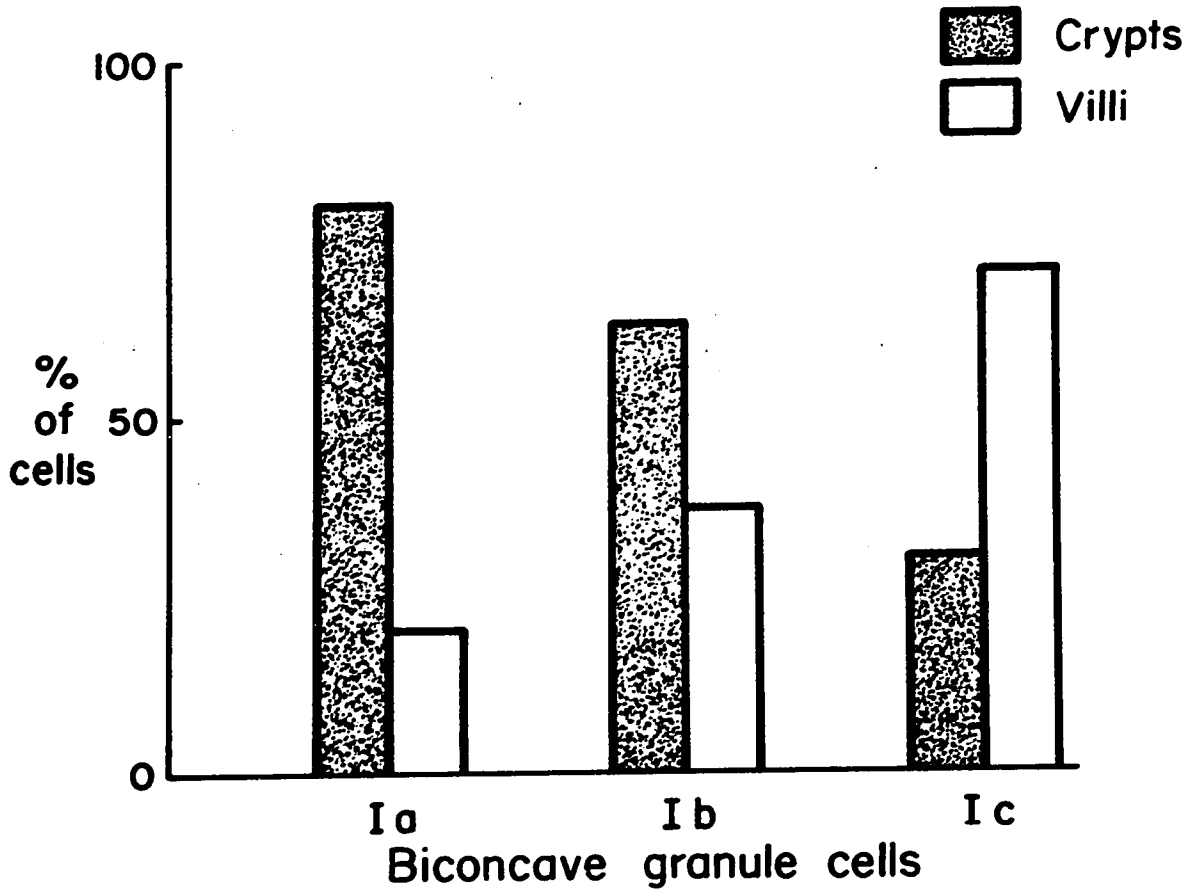
Therefore, "irregular granules" are, in fact, sections of <u>biconcave granules</u>.


3


4


5

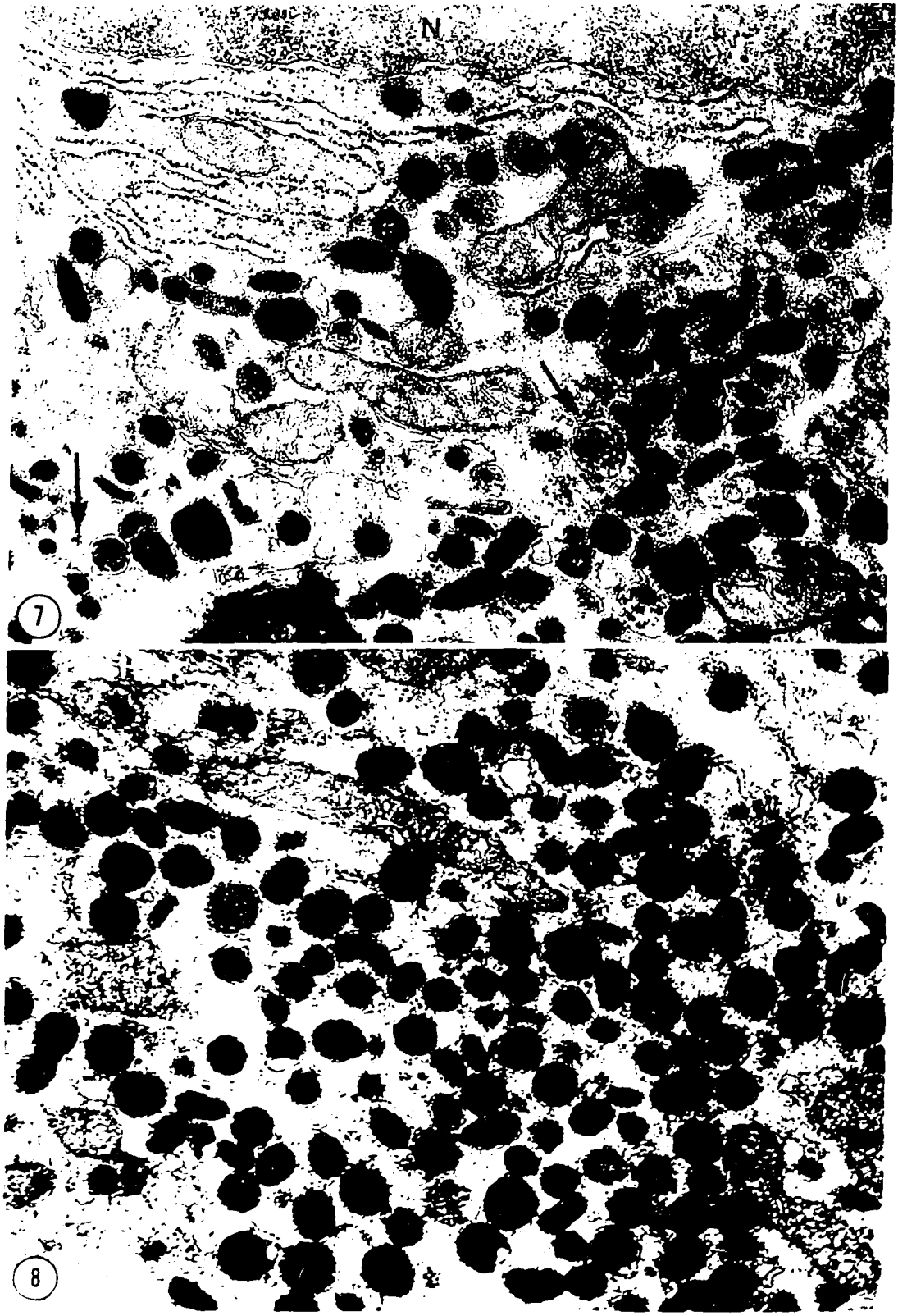
Fig. 6. Biconcave granule cells (Type I).
Histogram showing the frequency of occurrence of the three subgroups in crypts and villi. Subgroup Ia cells predominate in crypts; Subgroup Ib cells are less numerous in crypts but more numerous in villi than Ia cells; Subgroup Ic cells predominate in villi, but may be found in crypts.


Crypts
Villi
100
% of cells
50
0
Ia
Ib
Ic
Biconcave granule cells

Figs. 7 - 8.

Fig. 7. Biconcave granule cell (Type I), subgroup Ib, characterized by the presence of biconcave granules and various kinds of "particulate granules" (arrows). Biconcave granules similar to those in Ia cells (Fig. 1) predominate. "Particulate granules" are more abundant and display less density and more opaque cores than in Ia cells. 30,000 X. (Formaldehyde.)

Fig. 8. Biconcave granule cells (Type I), subgroup Ic, characterized by the presence of many "particulate granules" in which there may be more than one dense core. The biconcave granules are fewer than in cells Ia and Ib, and are occasionally less dense than in cells of the other two subgroups. 30,000 X. (Glutaraldehyde.)

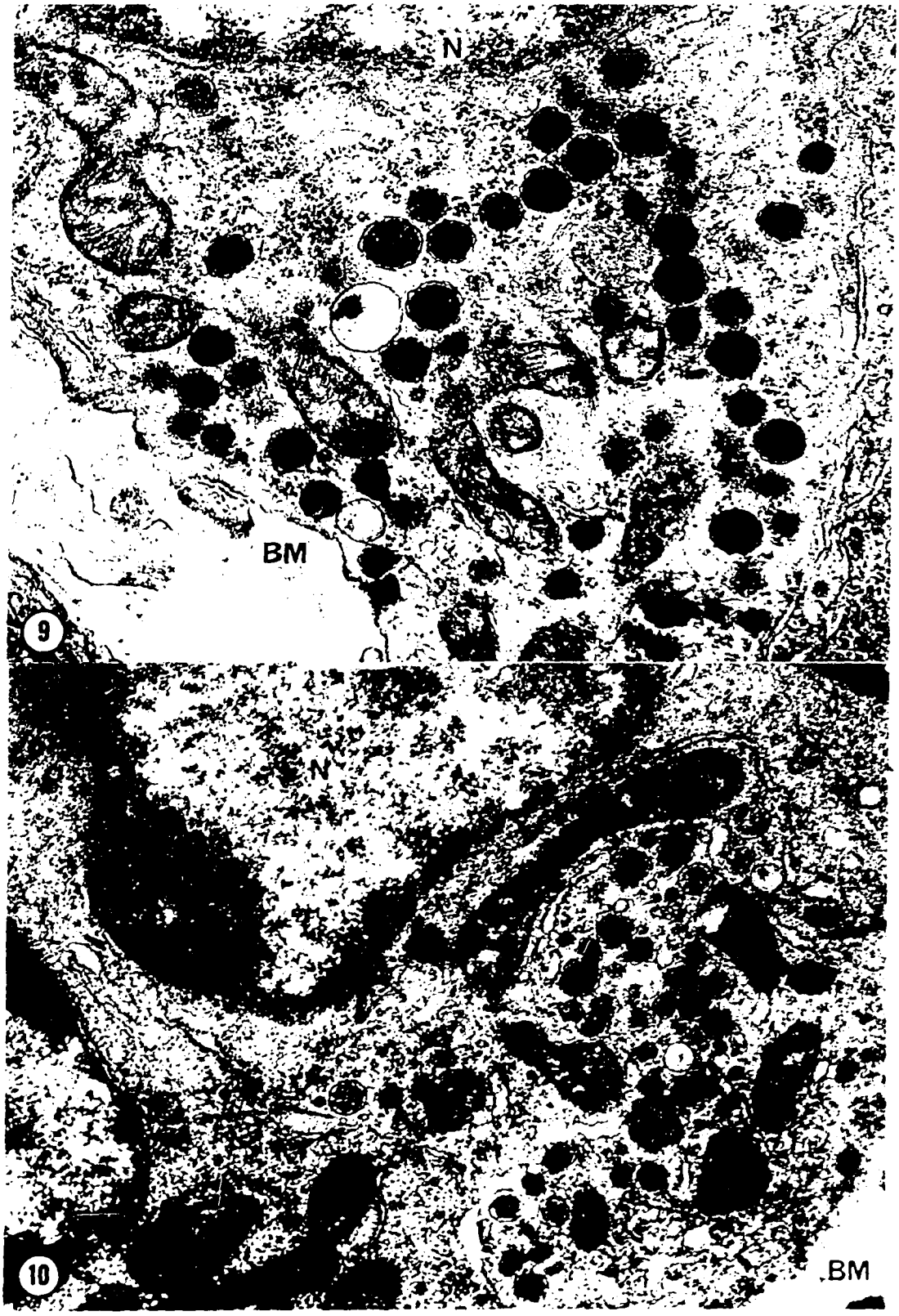

N
7
8

Figs. 9 - 10.

Spherical granule cells (Types II and III).

Fig. 9. Large spherical granule cell or Type II, with granules of an average diameter of 350 mμ. The granules are usually dense, but forms transitional from dense to empty-appearing vesicles may be observed. Mitochondria and coated vesicles are present. Polysomes are frequent, but less so than in columnar cells. 30,000 X. (Glutaraldehyde.)

Fig. 10. Small spherical granule cell or Type III, with granules having an average diameter of 250 mμ. The granules may be dense, pale or empty-appearing vesicles. Microfilaments, microtubules, coated vesicles, and mitochondria are seen. Polysomes are less numerous than in adjacent columnar cells. 30,000 X. (Glutaraldehyde.)


N
BM
9
N
10
BM

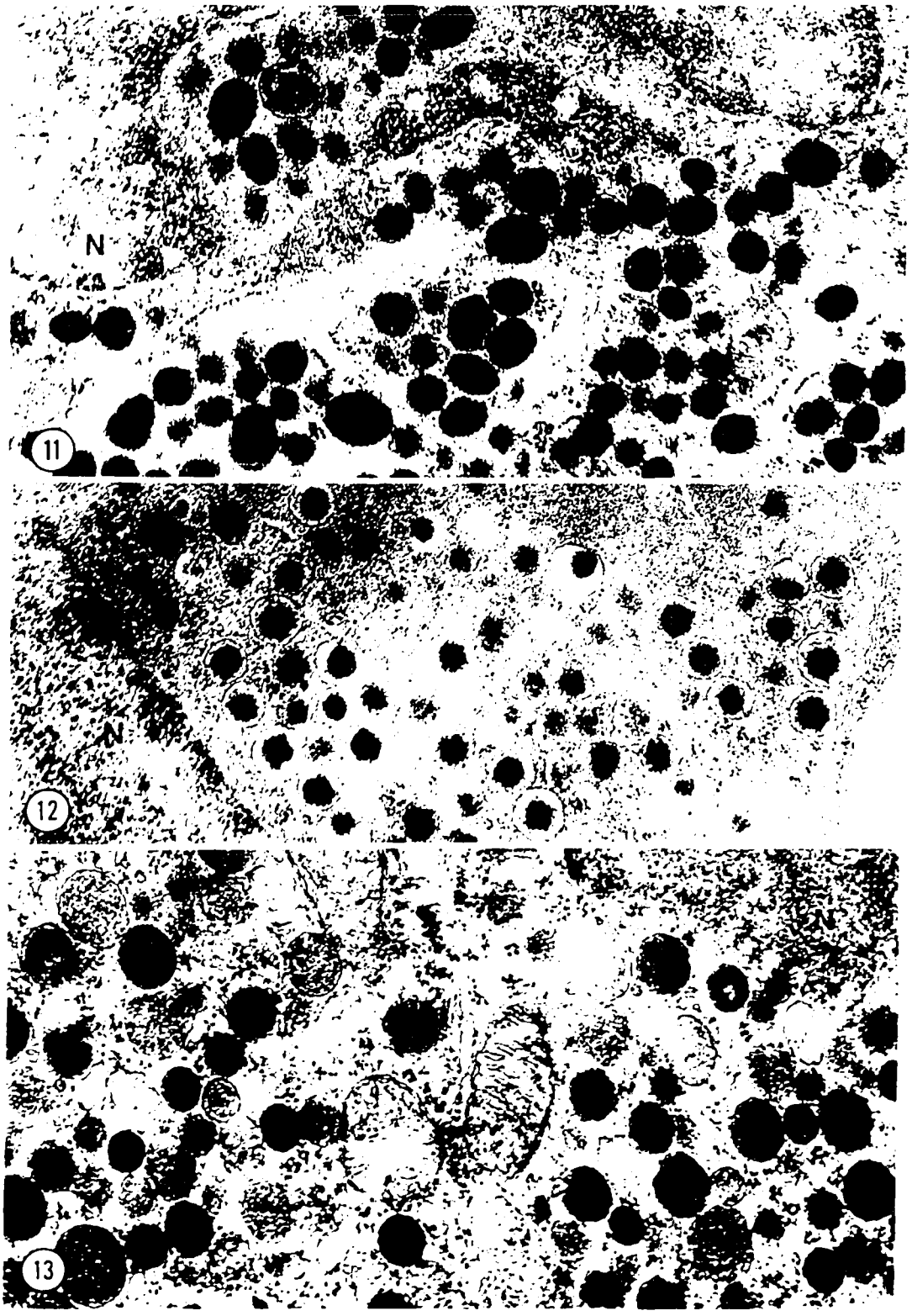
Figs. 11 - 13.

Uncommon types of enterochromaffin cells in mouse jejunum

Fig. 11. Spherical and oval granule cell: many dense granules fill the cytoplasm but empty-appearing vesicles are not observed. 30,000 X. (Glutaraldehyde.)

Fig. 12. Cell with small spherical granules presenting a large halo; many of the granules have a dense small core close to the bounding-membrane. 30,000 X. (Formaldehyde.)

Fig. 13. Cell with spherical granules of different sizes and densities; the granules are even larger than in cells described as large spherical granule cells or type II. The different density, from dense to pale, is a constant feature. In the less dense granules the bounding-membrane is incomplete. 30,000 X. (Glutaraldehyde.)

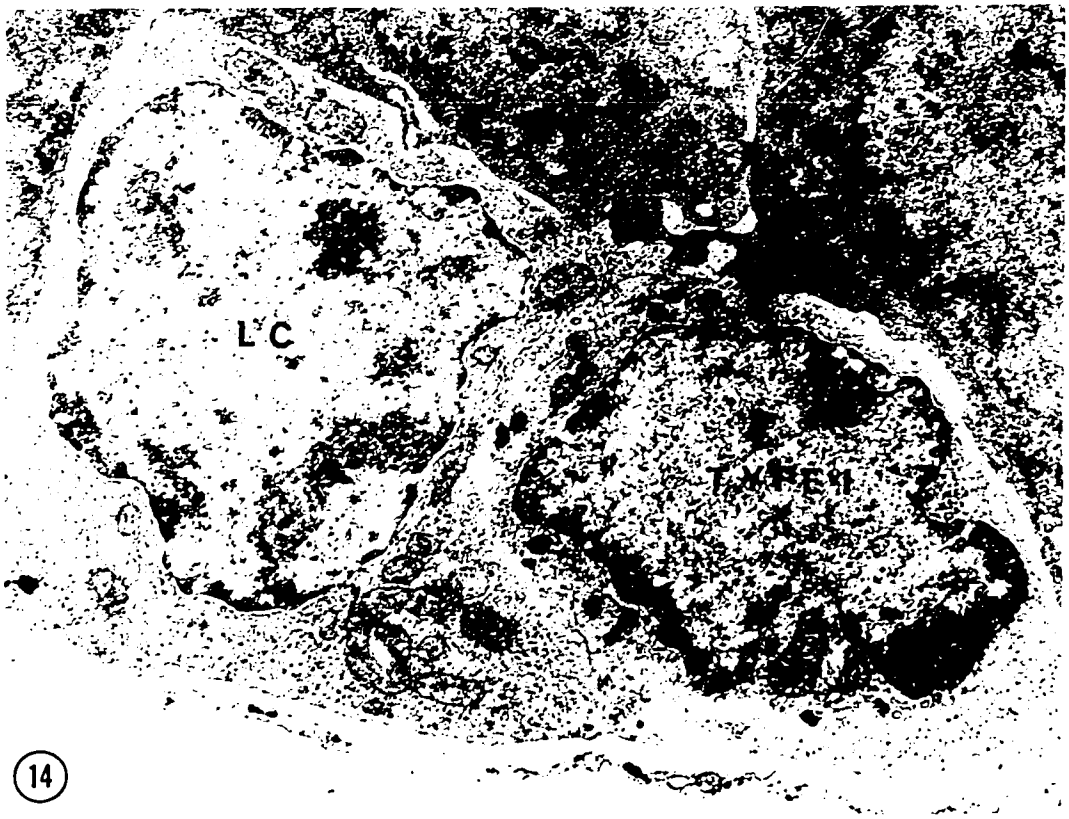

N
11
N
12
13

Figs. 14 - 15.

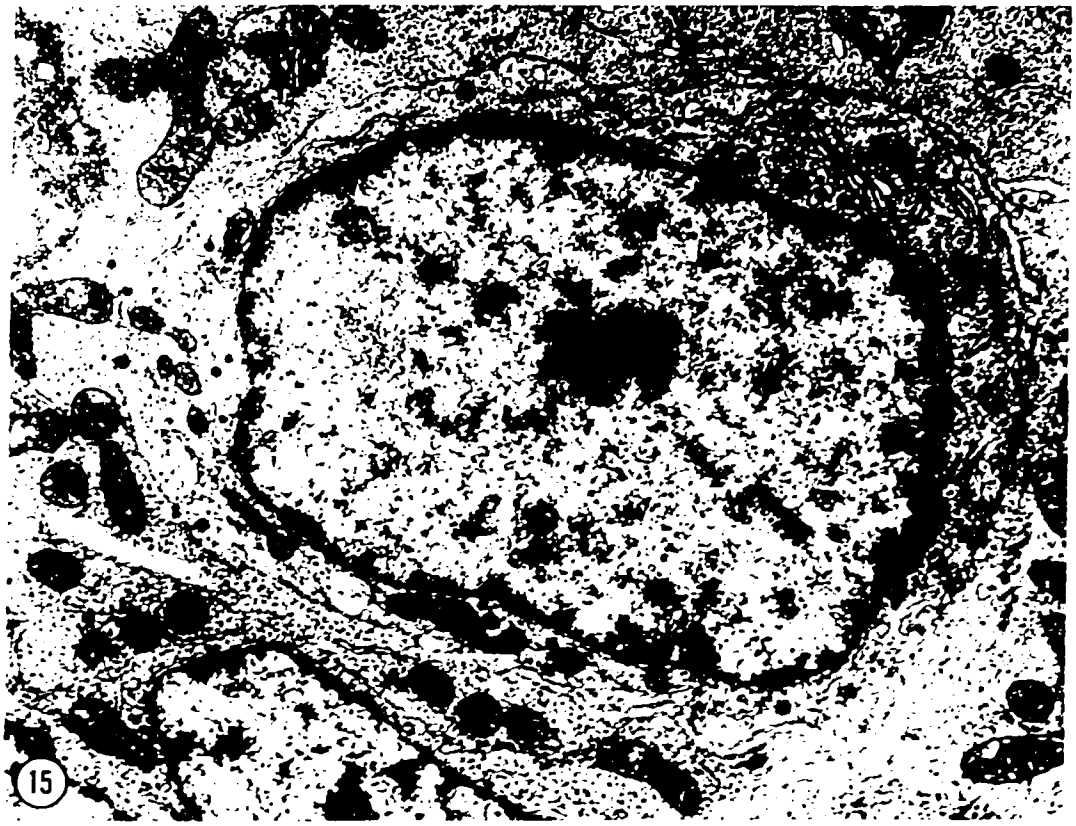
Fig. 14. Left — "Light Cell" (LC) from the base of the crypt with a large nucleus and a rather light cytoplasm rich in ribosomes, but poor in rough-endoplasmic reticulum.

Right — Biconcave granule cell (Type I) with a small number of granules; the nucleus is large and irregular. This cell may be young. 12,000 X. (Glutaraldehyde.)

Fig. 15. Small spherical granule cell (Type III) with a small number of granules. The nucleus is large, there are a few cisternae of rough-endoplasmic reticulum and in the vicinity of the Golgi apparatus (G) forming granules are observed. This cell may be young. 12,000 X. (Glutaraldehyde.)



14



15

Fig. 16. Mitotic figure (early telophase) in a columnar cell located in the lower portion of a crypt. It shows a group of lysosomes (Ly), and two spherical dense granules in the cytoplasm (arrows). The nature of these granules is not known. 30,000 X. (Glutaraldehyde.)

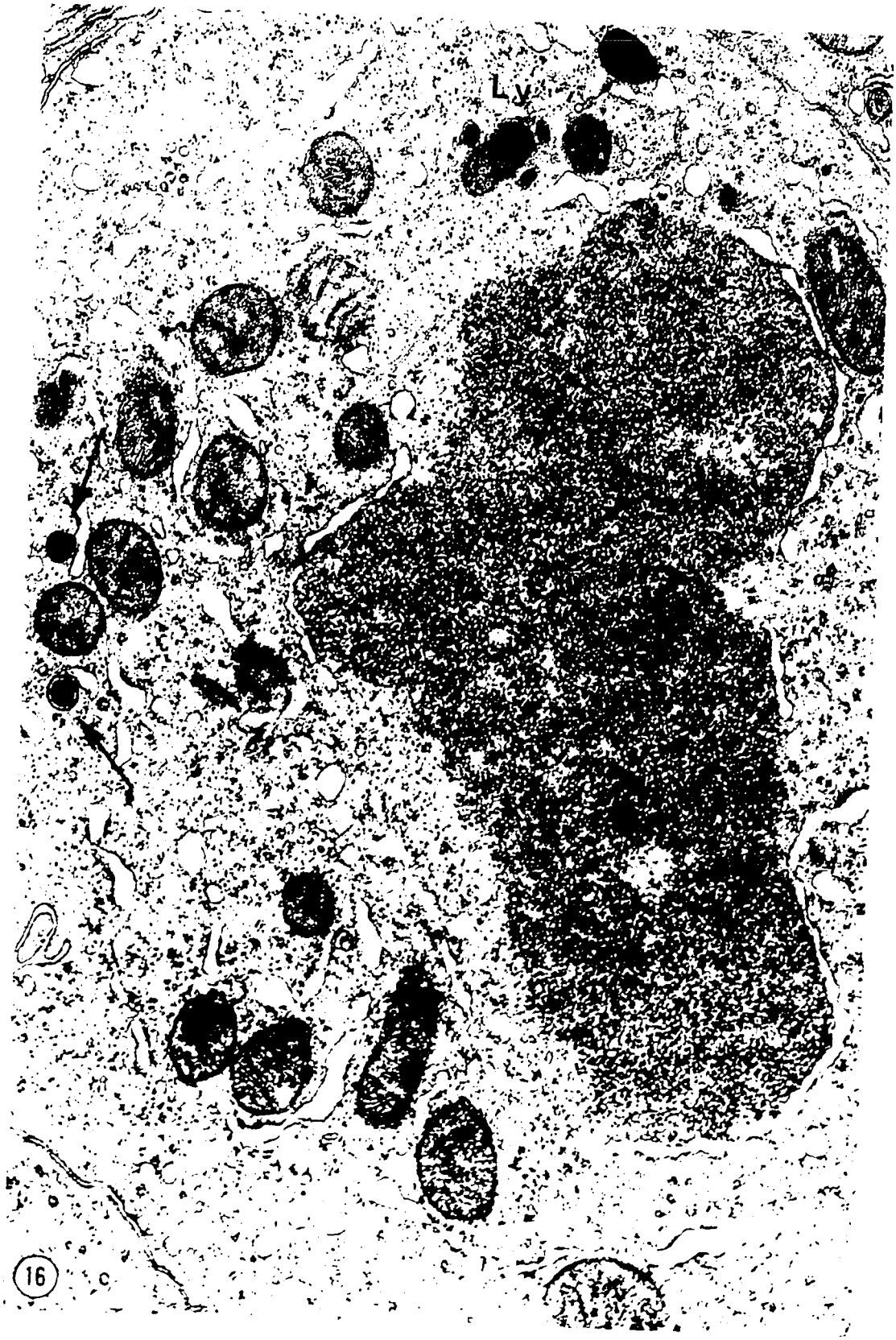

Lv
16
C

Fig. 17. Labeling of enterochromaffin cells (total cell population)

The solid line shows the percentage of labeled enterochromaffin cells at different time intervals after the start of continuous infusion of ^{3}H-thymidine. In some animals the infusion stopped at 4 days and the animals were allowed to live for another 4 or 8 days; the dotted line shows the percentage of labeled cells in these animals.

The percentage of labeled cells increased throughout the period of continuous infusion. After 8 days, 80.7 percent of the enterochromaffin cells were labeled. However, 8 days after the end of a 4-day infusion, most of the labeled cells had disappeared.

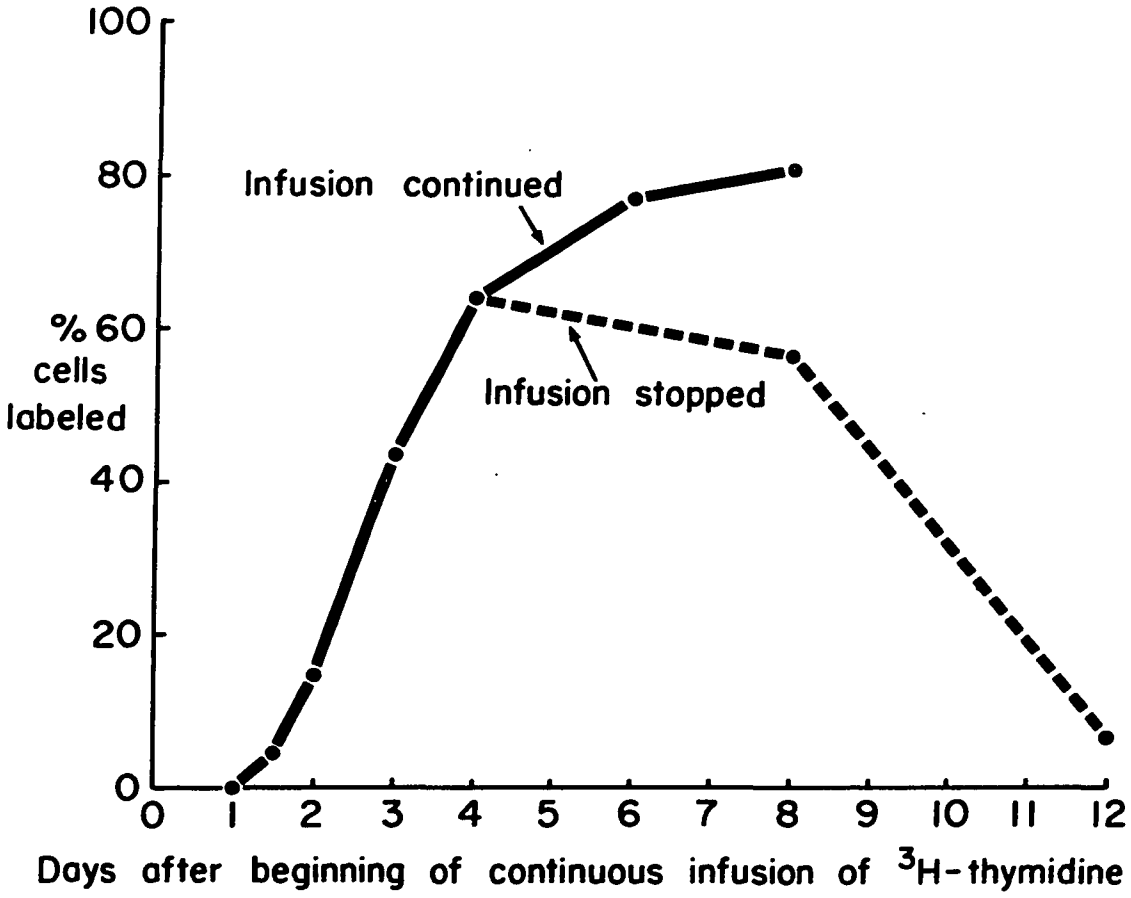

100
80
% 60
cells
labeled
40
20
0
Infusion continued
Infusion stopped
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 18. Labeling of enterochromaffin cells in crypts and villi

In crypts, labeled enterochromaffin cells appear after $1\frac{1}{2}$ days of continuous infusion and by 8 days 85 percent are labeled (solid line); 8 days after the end of a 4-day continuous infusion only 7.5 percent of the cells remain labeled (dotted line).

In villi, labeled enterochromaffin cells appear at 3 days of continuous infusion; thereafter, they behave as do those in crypts, and have almost disappeared by 8 days after the end of a 4-day continuous infusion.

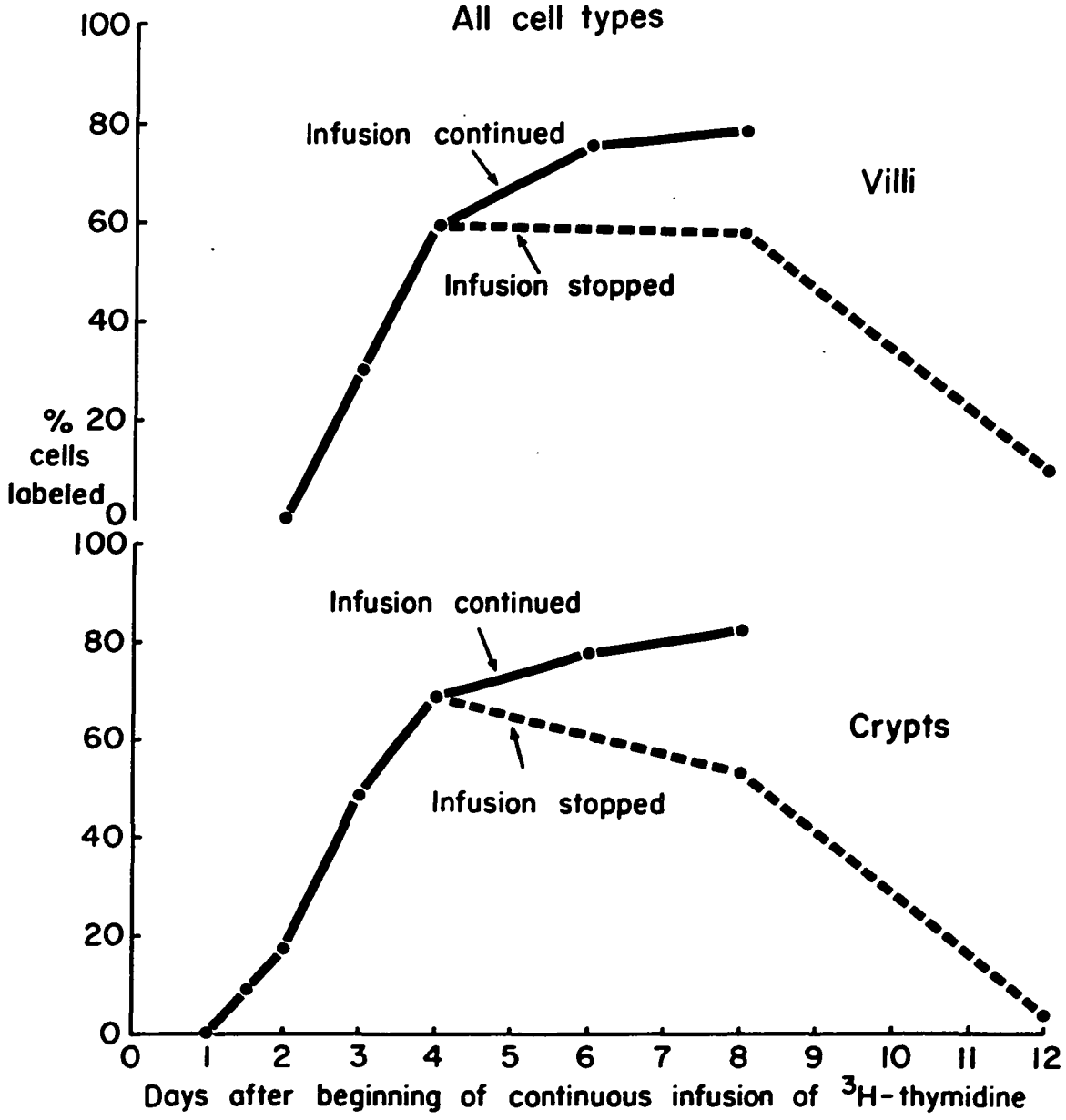

All cell types
Infusion continued
Infusion stopped
Villi
Infusion continued
Infusion stopped
Crypts
% cells labeled
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 19. Labeling of biconcave granule cells

These cells appear labeled at $1\frac{1}{2}$ days of continuous infusion, and at 8 days 82.1 percent are labeled (solid line); 8 days after the end of a 4-day infusion only 7.2 percent of the cells are labeled.

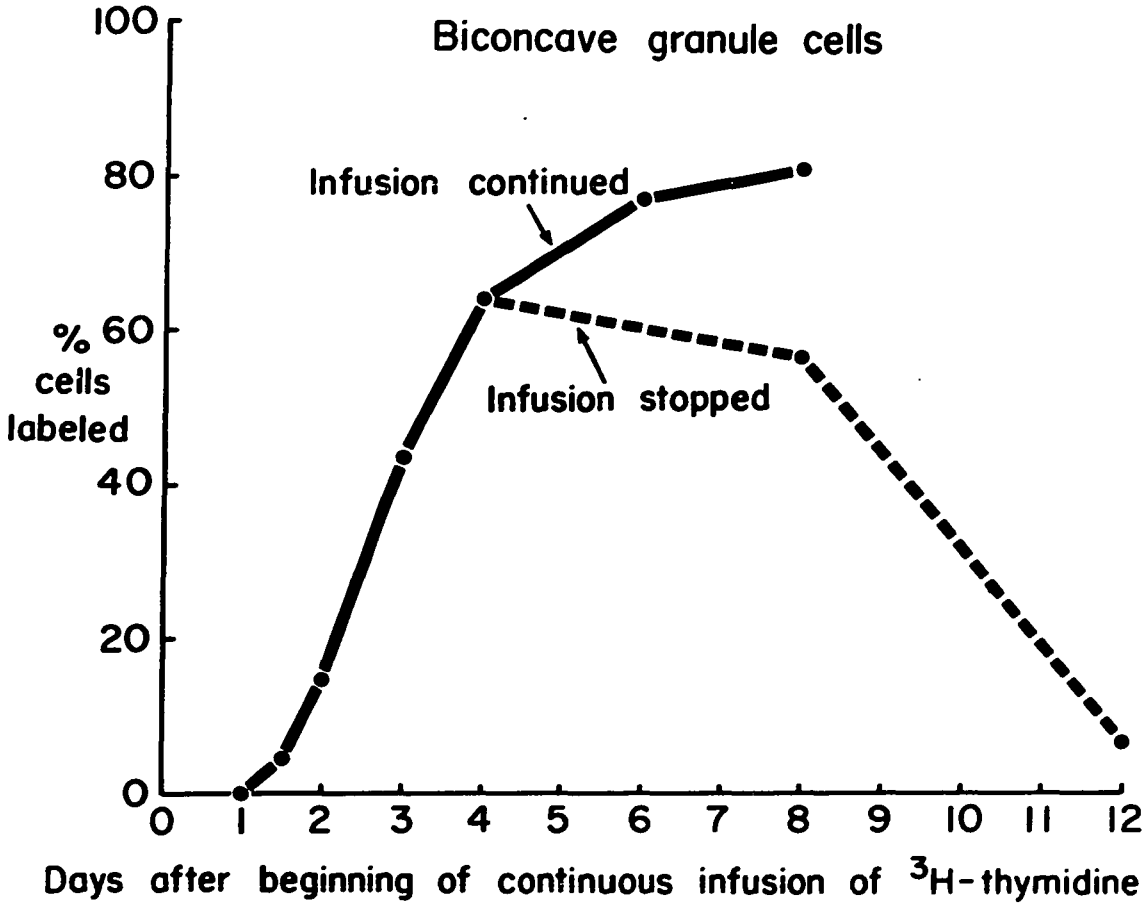

Biconcave granule cells
Infusion continued
Infusion stopped
% cells labeled
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 20. Labeling of biconcave granule cells in crypts and villi

These cells appear labeled in crypts at $1\frac{1}{2}$ days and in villi at 3 days, and reach 85 percent in crypts and 76 percent in villi 8 days after the start of the continuous infusion (solid line); 8 days after the end of a 4-day infusion only 7.5 percent labeled cells were found in crypts, and 7.0 percent in villi.

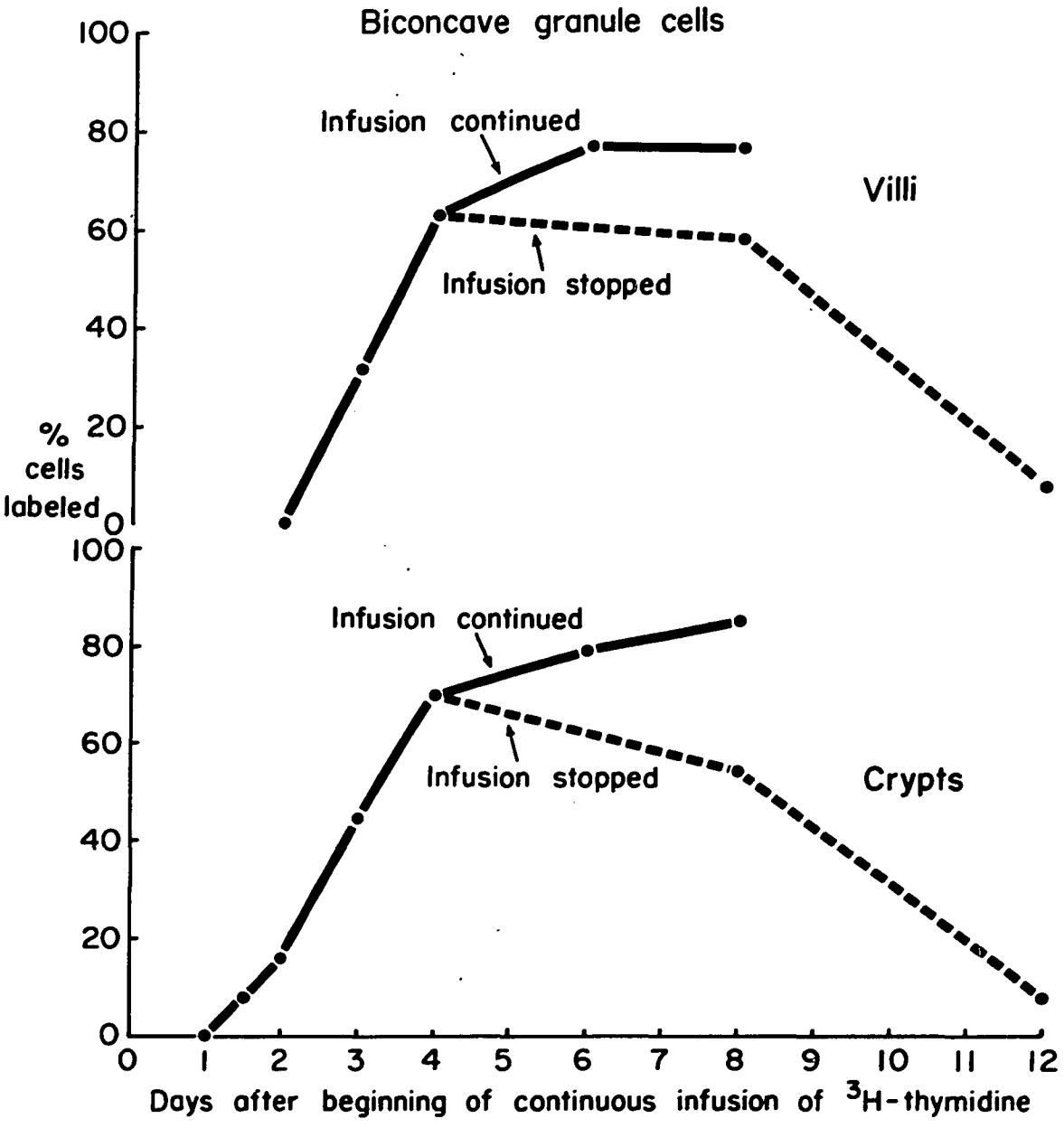

Biconcave granule cells
Infusion continued
Infusion stopped
Villi
% cells labeled
100
80
60
40
20
0
Infusion continued
Infusion stopped
Crypts
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 21. Labeling of biconcave granule cell subgroups (Ia, Ib, Ic)

Ia cells appear labeled at $1\frac{1}{2}$ days and most of them are labeled at 6 days of continuous infusion;

Ib cells appear labeled at 2 days and 80 percent are labeled at 8 days of continuous infusion;

Ic cells appear labeled at 3 days and 77 percent are labeled at 8 days of continuous infusion.

8 days after the end of a 4-day infusion, labeled Ia cells have disappeared; labeled Ib cells represent about 10 percent; and labeled Ic cells form about 7 percent.

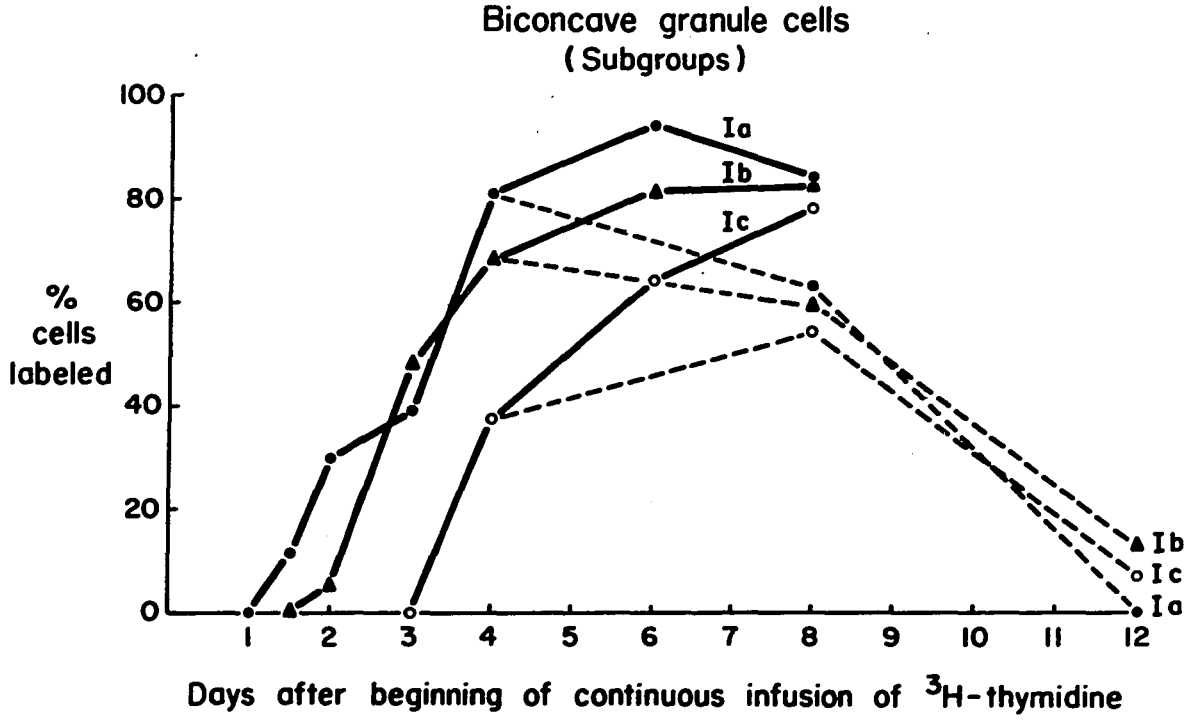

Biconcave granule cells
(Subgroups)
100
80
60
40
20
0
% cells labeled
Ia
Ib
Ic
Ib
Ic
Ia
1
2
3
4
5
6
7
8
9
10
11
12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 22. Labeling of spherical granule cells

These cells appear labeled at $1\frac{1}{2}$ days and at 8 days after the start of the continuous infusion 77.2 percent are labeled (solid line); only 5.8 percent were found labeled 8 days after stopping the 4-day infusion (dotted line).

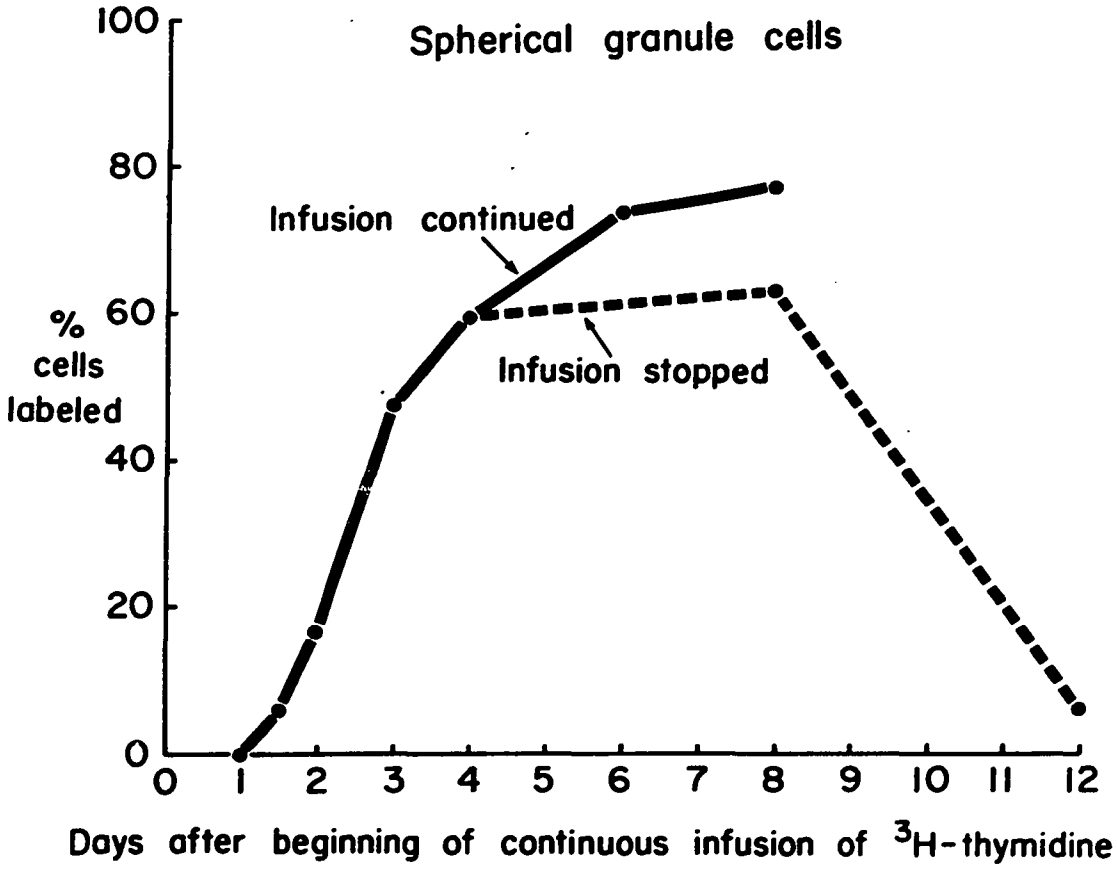

Spherical granule cells
100
80
60
40
20
0
% cells labeled
Infusion continued
Infusion stopped
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 23. Labeling of spherical granule cells in crypts and villi

Like the biconcave granule cells, spherical granule cells appear labeled at $1\frac{1}{2}$ days in crypts, and at 3 days in villi, and 8 days after the start of the continuous infusion 75 percent are labeled in crypts, and 80 percent in villi (solid lines); 8 days after the end of the 4-day infusion, all labeled cells disappeared from crypts, and 11.3 percent remain in villi (dotted lines).

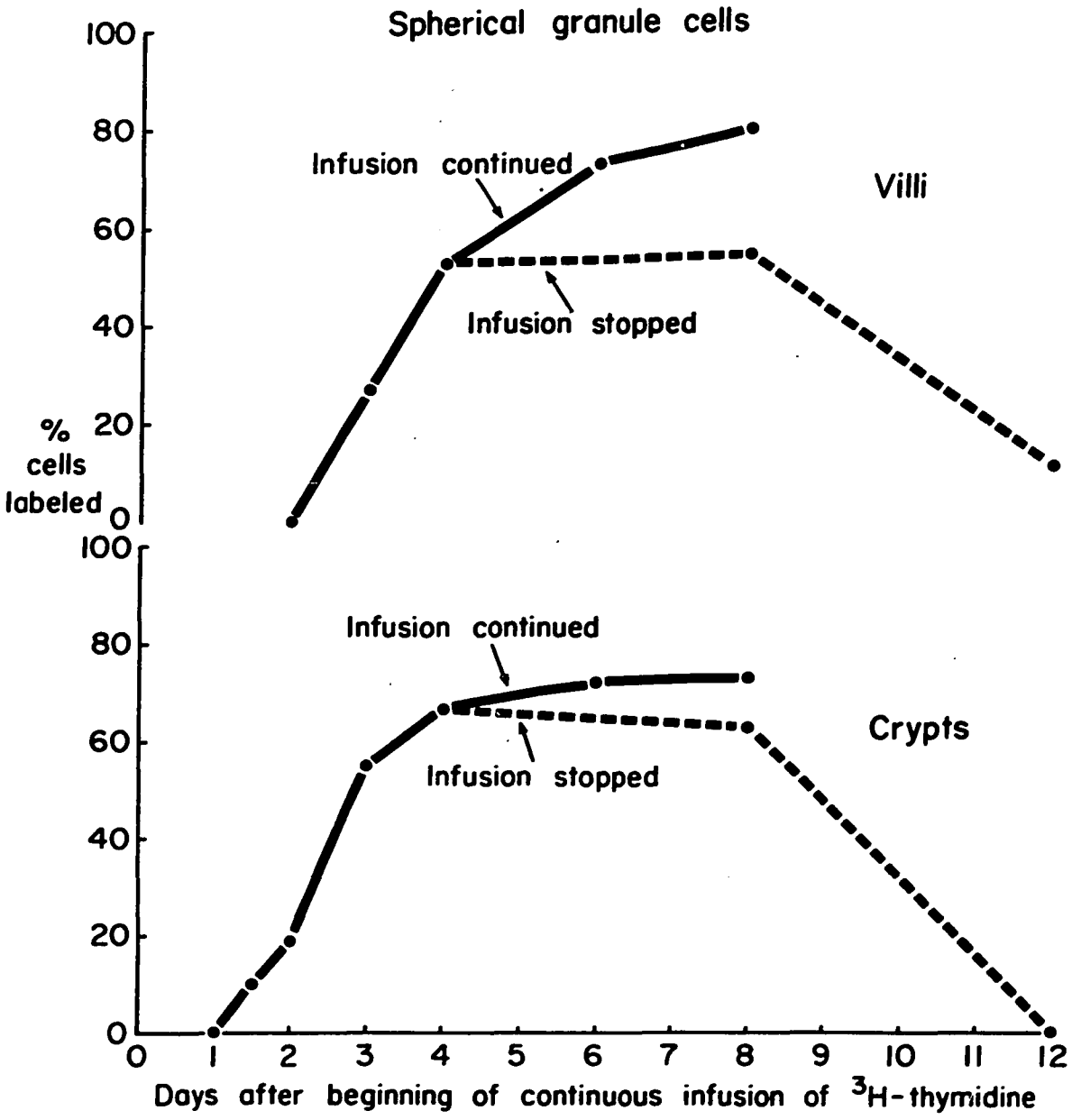

Spherical granule cells
100
80
60
40
20
0
% cells labeled
Infusion continued
Infusion stopped
Villi
100
80
60
40
20
0
Infusion continued
Infusion stopped
Crypts
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 24. Labeling of large spherical granule cells (Type II) in crypts and villi

They also appear labeled in crypts at $1\frac{1}{2}$ days, and in villi at 3 days; 8 days after the start of the continuous infusion all cells recorded were labeled (solid line); 8 days after the end of a 4-day infusion, labeled cells are no longer found in crypts and villi.

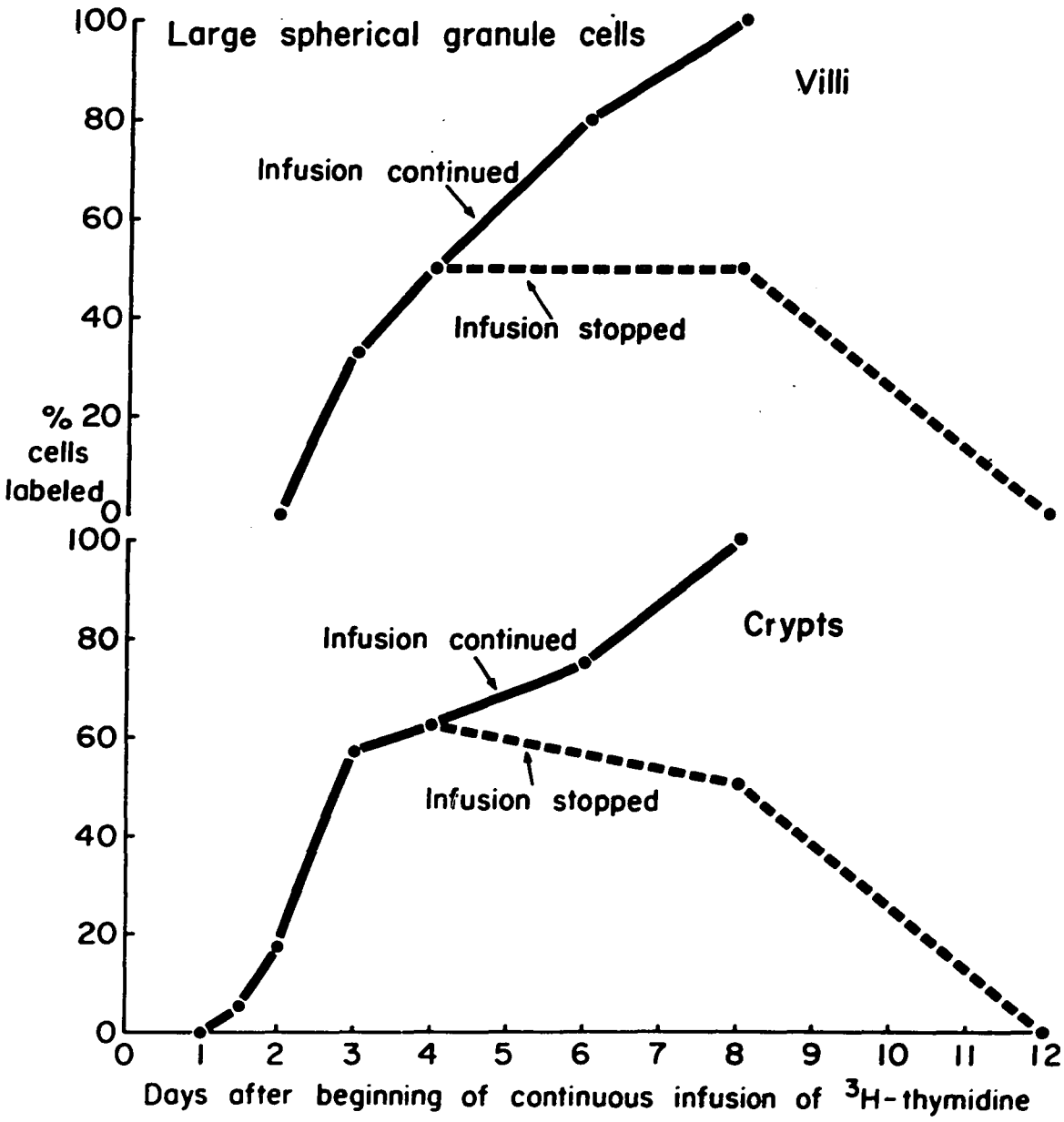
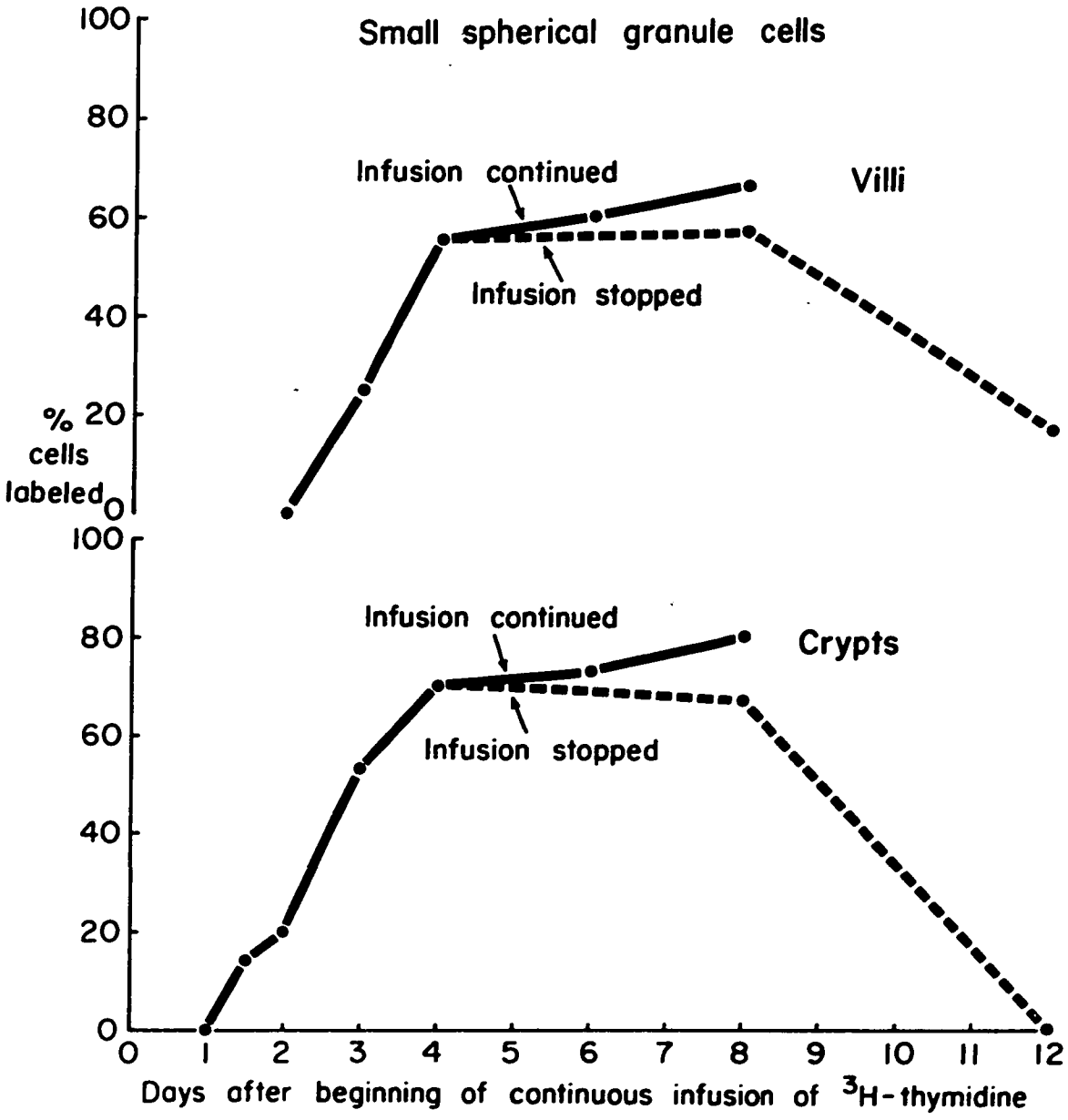

Large spherical granule cells
Villi
Infusion continued
Infusion stopped
% cells labeled
100
80
60
40
20
0
Crypts
Infusion continued
Infusion stopped
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

Fig. 25. Labeling of small spherical granule cells (Type III) in crypts and villi

They appear labeled in crypts at $1\frac{1}{2}$ days, and in villi at 3 days; 80 percent in crypts, and 66 percent in villi are labeled 8 days after the start of the continuous infusion.

8 days after the end of the 4-day infusion these cells have disappeared from the crypts but 16.6 percent labeled cells are still in villi.


Small spherical granule cells
Infusion continued
Villi
Infusion stopped
% cells labeled
100
80
60
40
20
0
Infusion continued
Crypts
Infusion stopped
0 1 2 3 4 5 6 7 8 9 10 11 12
Days after beginning of continuous infusion of ^{3}H-thymidine

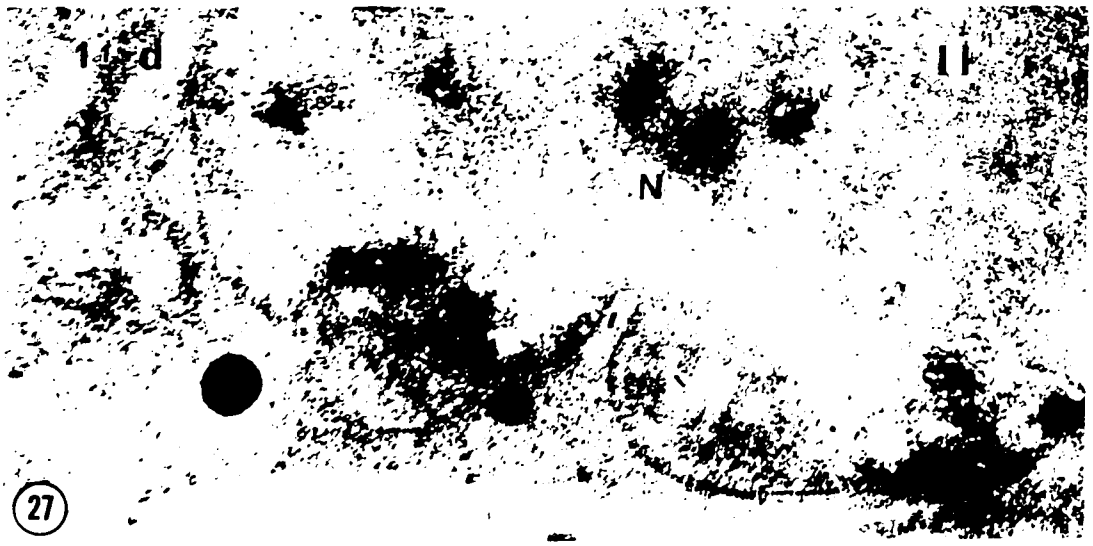
Figs. 26 - 28. Young enterochromaffin cells labeled by $1\frac{1}{2}$ days of continuous infusion of ^{3}H-thymidine.

Fig. 26. Biconcave granule cell (type I, subgroup Ia) in crypt. The number of characteristic granules is small. The nucleus is large. Four silver grains over the nucleus indicate that this cell is labeled. 20,000 X. (Formaldehyde.)

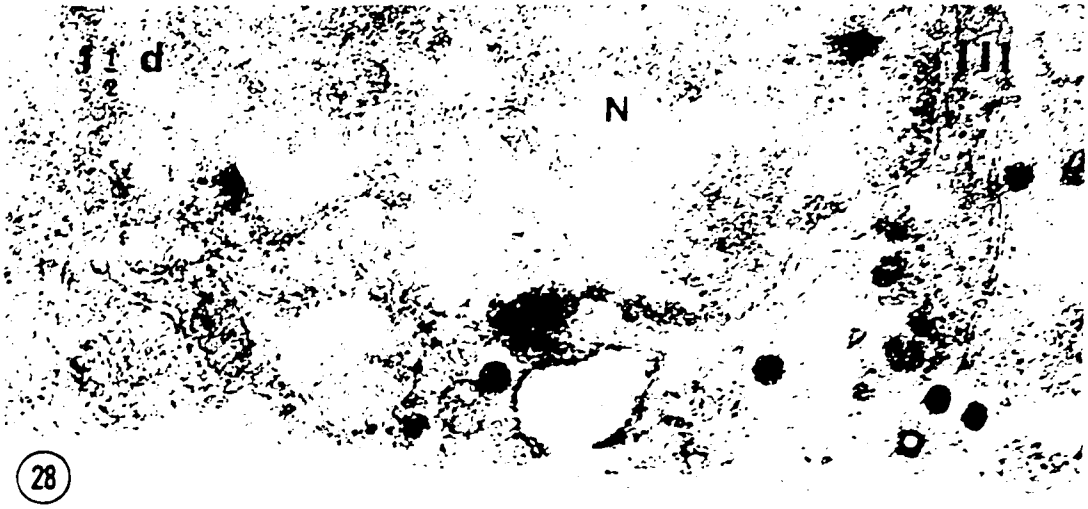
Fig. 27. Large spherical granule cell (type II) in crypt, with heavy labeling of the nucleus. Two granules are in the basal cytoplasm. This cell also exhibited a few similar granules in the apical cytoplasm. 20,000 X. (Formaldehyde.)

Fig. 28. Small spherical granule cell (type III) in crypt. Three silver grains are over the nucleus. A few granules are in the basal cytoplasm. 20,000 X. (Formaldehyde.)

26



27

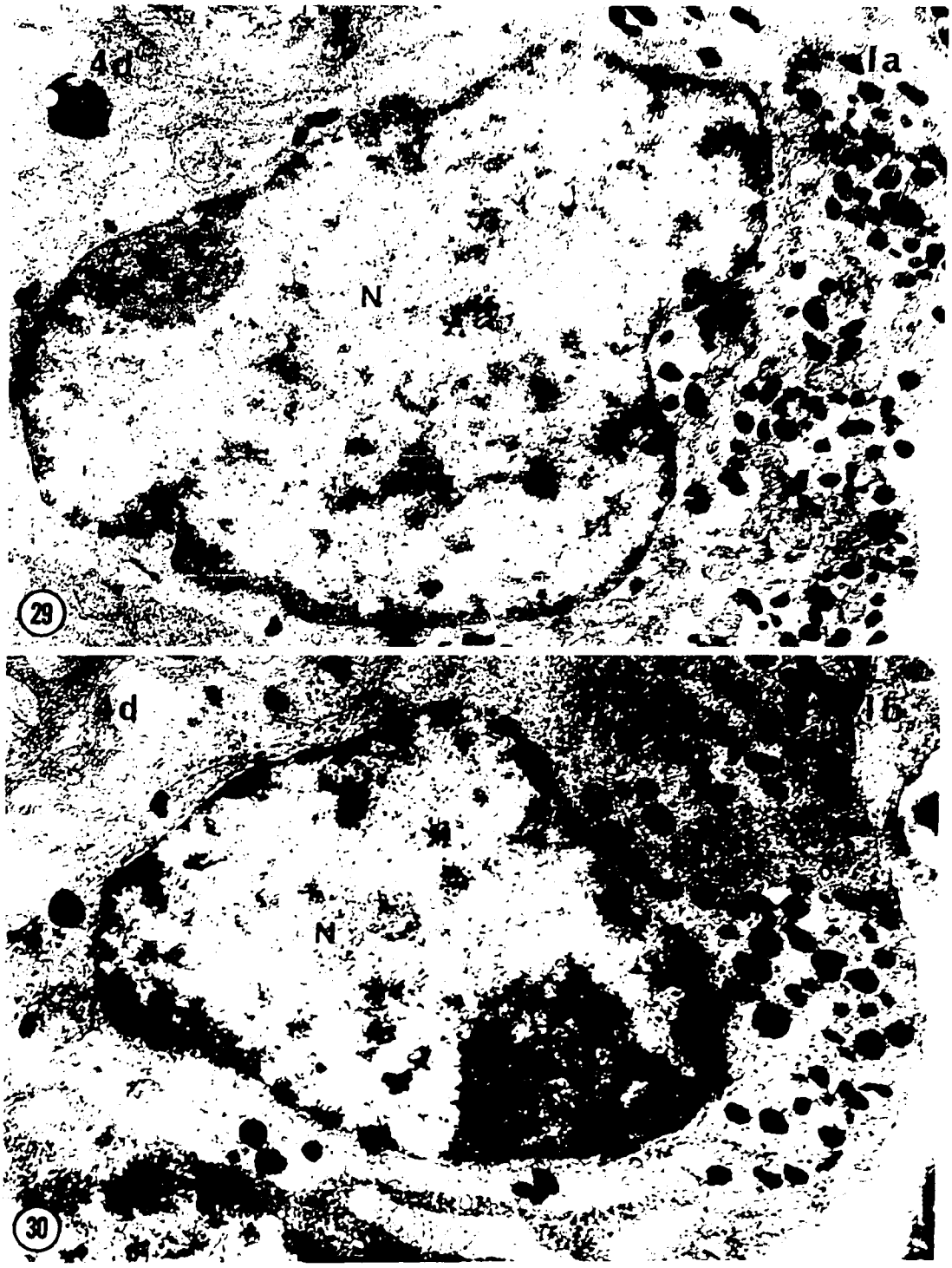


28

Figs. 29 - 30. Biconcave granule cells (type I) labeled by 4 days of continuous infusion.

Fig. 29. Type Ia cell in crypt, with a considerable number of granules, almost all in the basal cytoplasm. A lysosome (Ly) is present. 20,000 X. (Glutaraldehyde.)

Fig. 30. Type Ib cell in crypt. Some of the round shaped granules are less dense than biconcave granules. 20,000 X. (Glutaraldehyde.)


4d
1a
N
29
d
1b
N
30

Figs. 31 - 32. Spherical granule cells labeled by 4 days of continuous infusion.

Fig. 31. Large spherical granule cell (type II) in crypt. 20,000 X. (Glutaraldehyde.)

Fig. 32. Small spherical granule cell (type III) in crypt. 20,000 X. (Glutaraldehyde.)

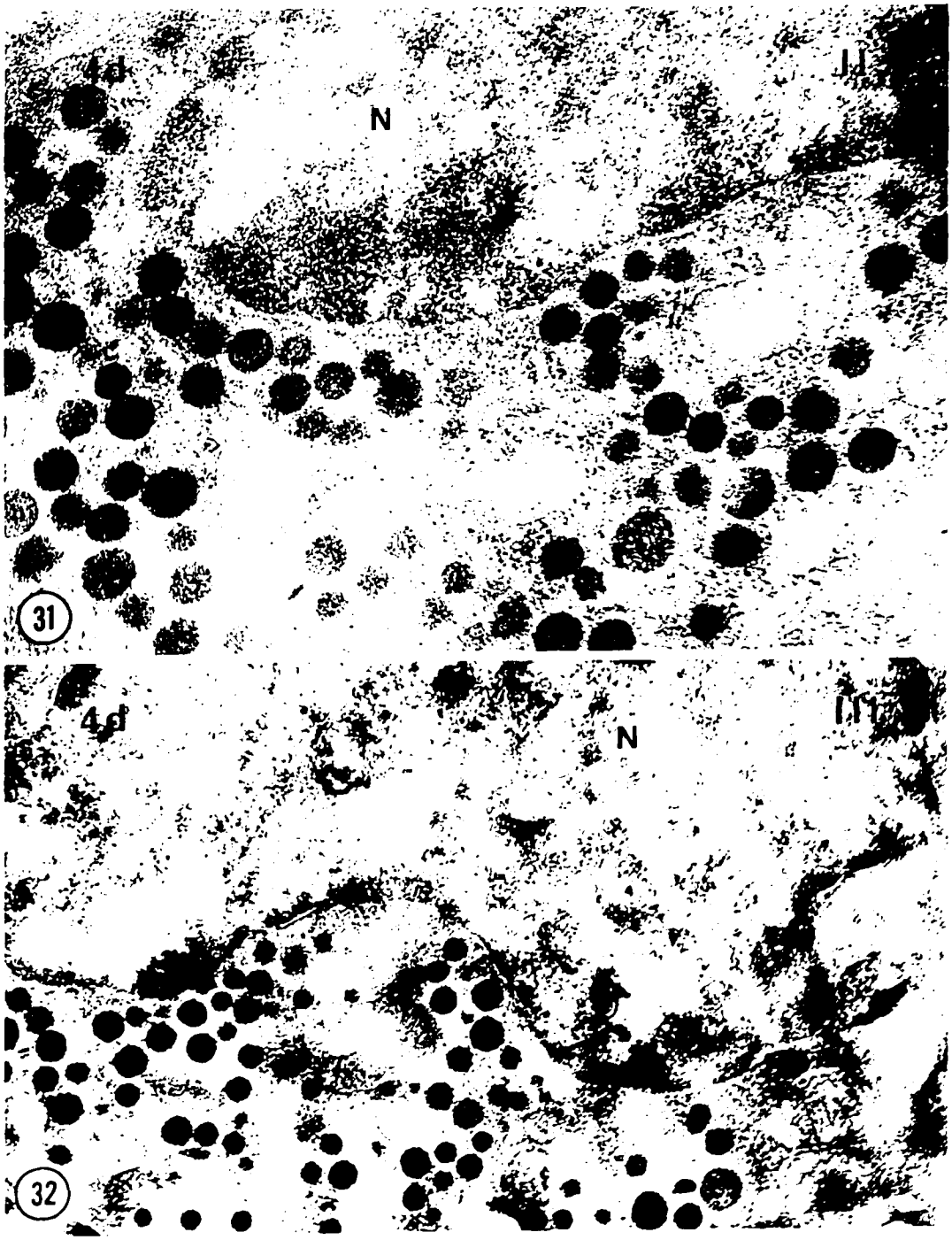

4d
N
II
31
4d
N
II
32

Fig. 33. Labeled large spherical granule cell (type II) in villus; 4 days of continuous infusion. 20,000 X. (Glutaraldehyde.)

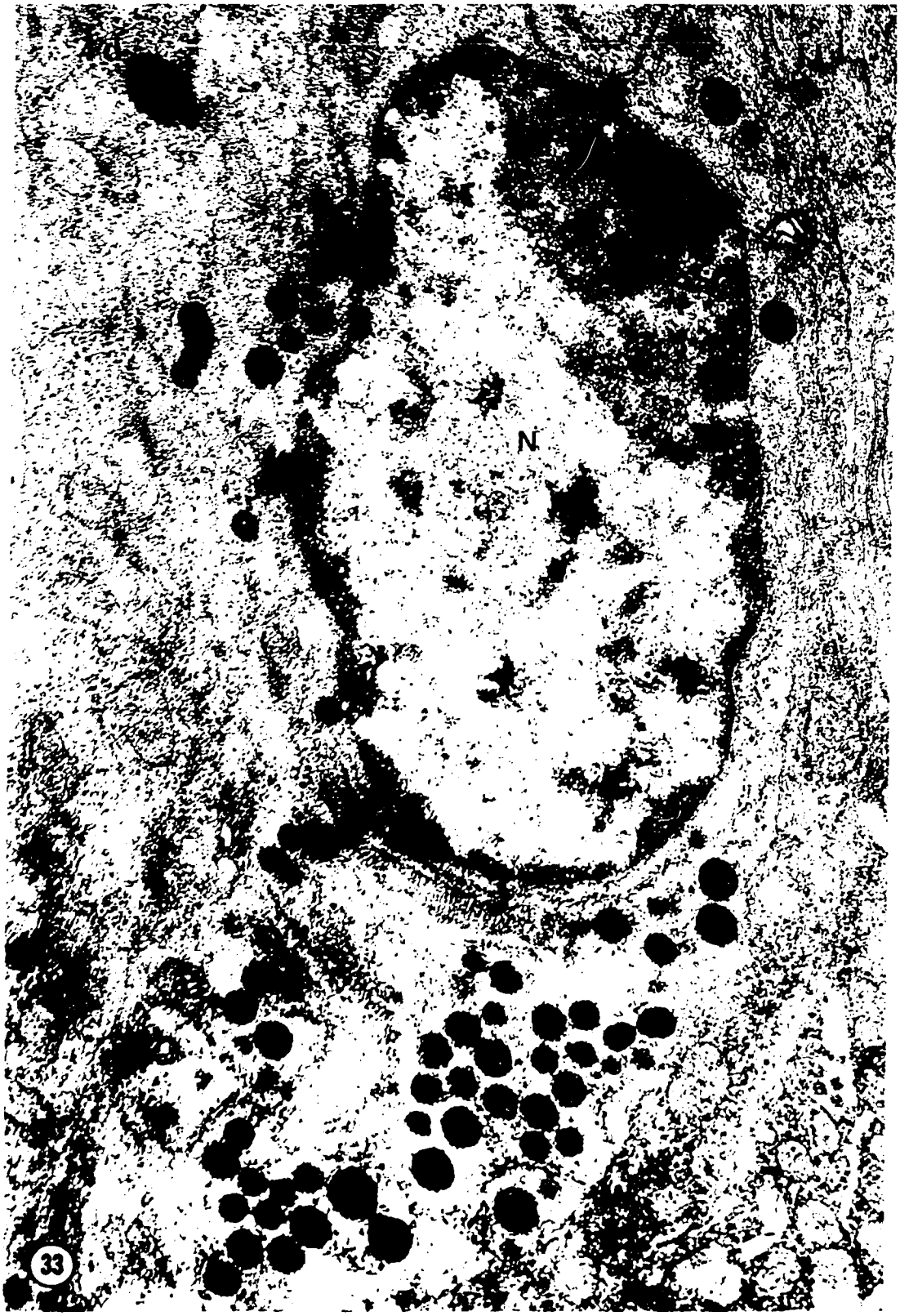
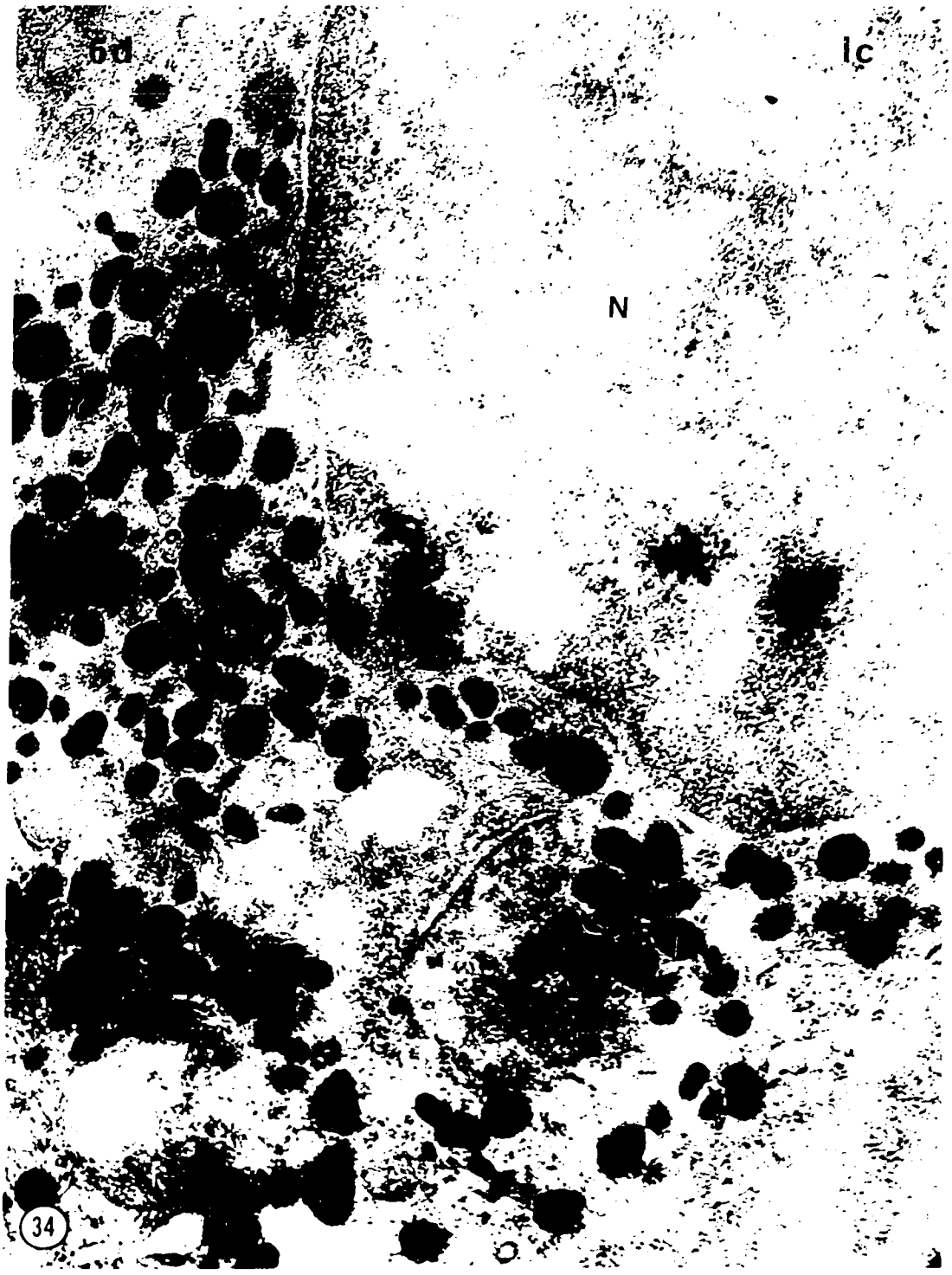

N
33

Fig. 34. Biconcave granule cell (type Ic) in villus; 8 days of continuous infusion. This cell has biconcave granules and many "particulate granules" with less density and opaque cores. Some of the biconcave granules also present opaque cores. 30,000 X. (Formaldehyde.)


bd
lc
N
34

Figs. 35-36. Spherical granule cells labeled by continuous infusion.

Fig. 35. Small spherical granule cell (type III) in crypt; 6 days of infusion. 20,000 X. (Formaldehyde.)

Fig. 36. Large spherical granule cell (type II) in villus; 8 days of infusion. "Empty-appearing vesicles" are present in the basal cytoplasm. 20,000 X. (Formaldehyde.)


6d
N
35
8d
N
36

Figs. 37 - 38. Enterochromaffin cells labeled by a 4-day continuous infusion, in animals sacrificed 4 days later.

Fig. 37. Biconcave granule cell (type Ic) in villus with most of the granules with "particulate" texture. 30,000 X. (Formaldehyde.)

Fig. 38. Spherical granule cell in crypt, with very large granules; this cell may be type V. The granules are either dense or "empty-appearing vesicles" (arrows), with forms transitional between both. 30,000 X. (Formaldehyde.)

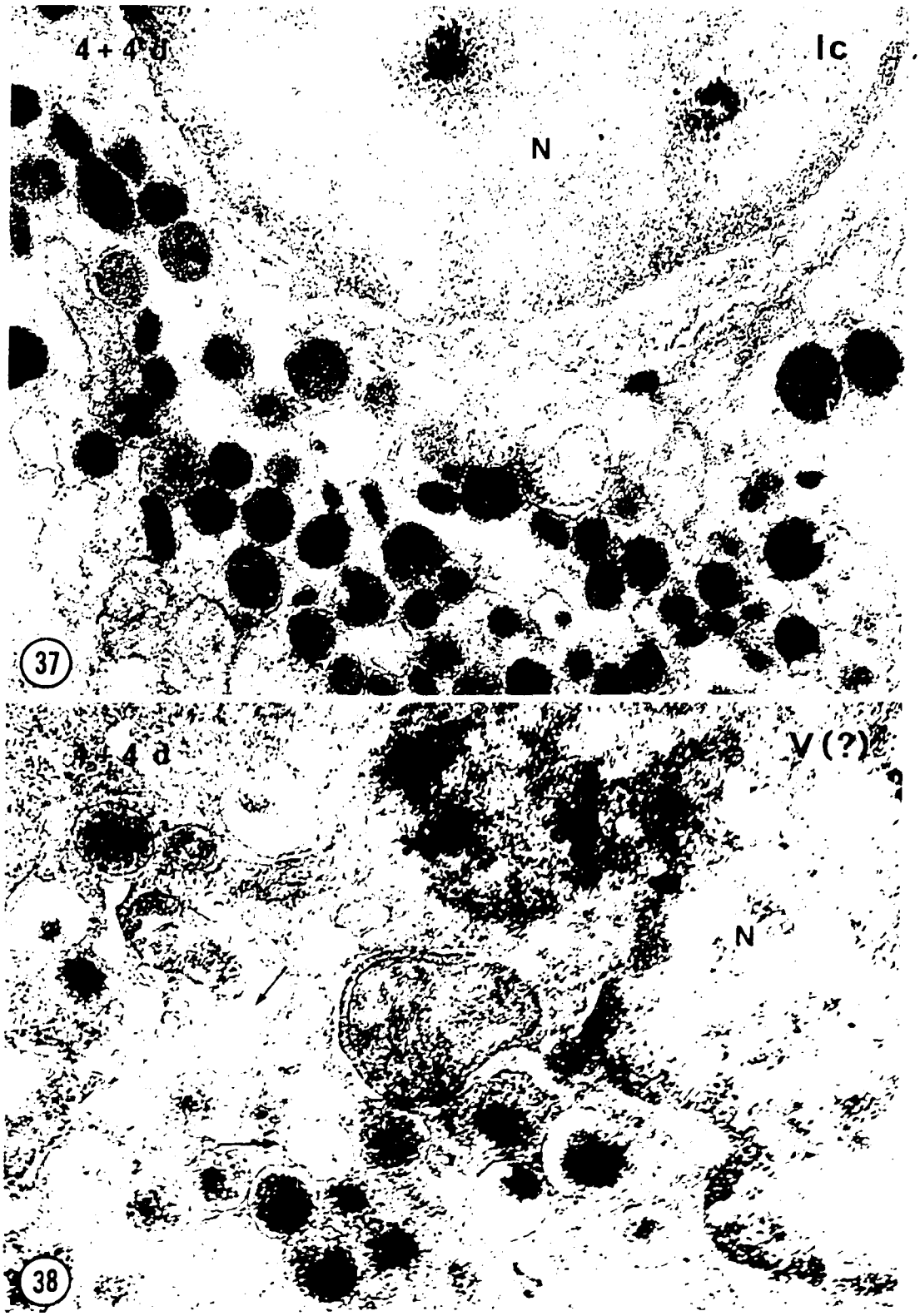
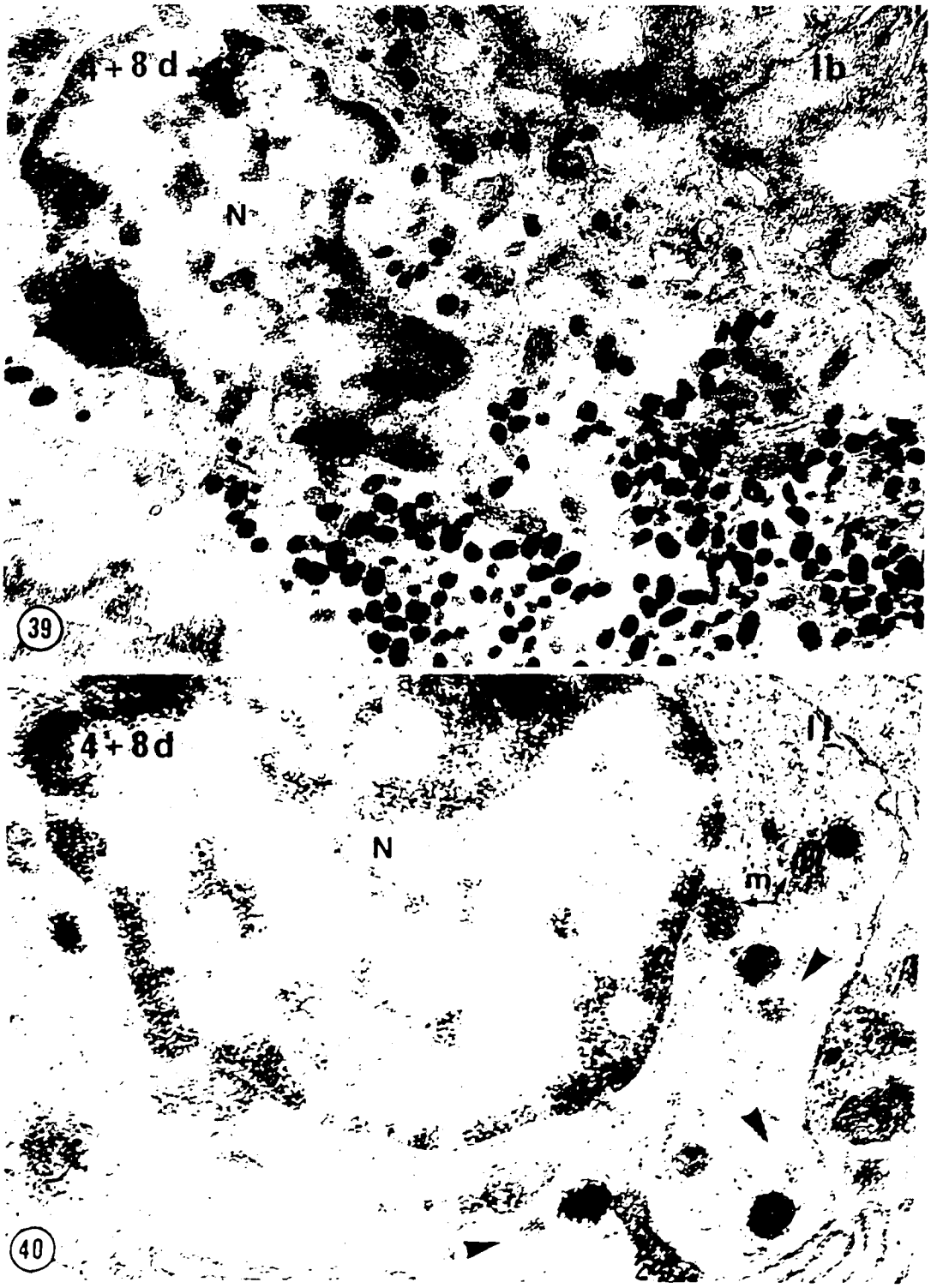

4 + 4 d
Ic
N
37
4 d
V (?)
N
38

Fig. 39 - 40. Old enterochromaffin cells labeled by a 4-day continuous infusion in animals sacrificed 8 days later.

Fig. 39. Biconcave granule cell (type Ib) in villus, with granules of different densities, and many round in shape. 20,000 X. (Formaldehyde.)

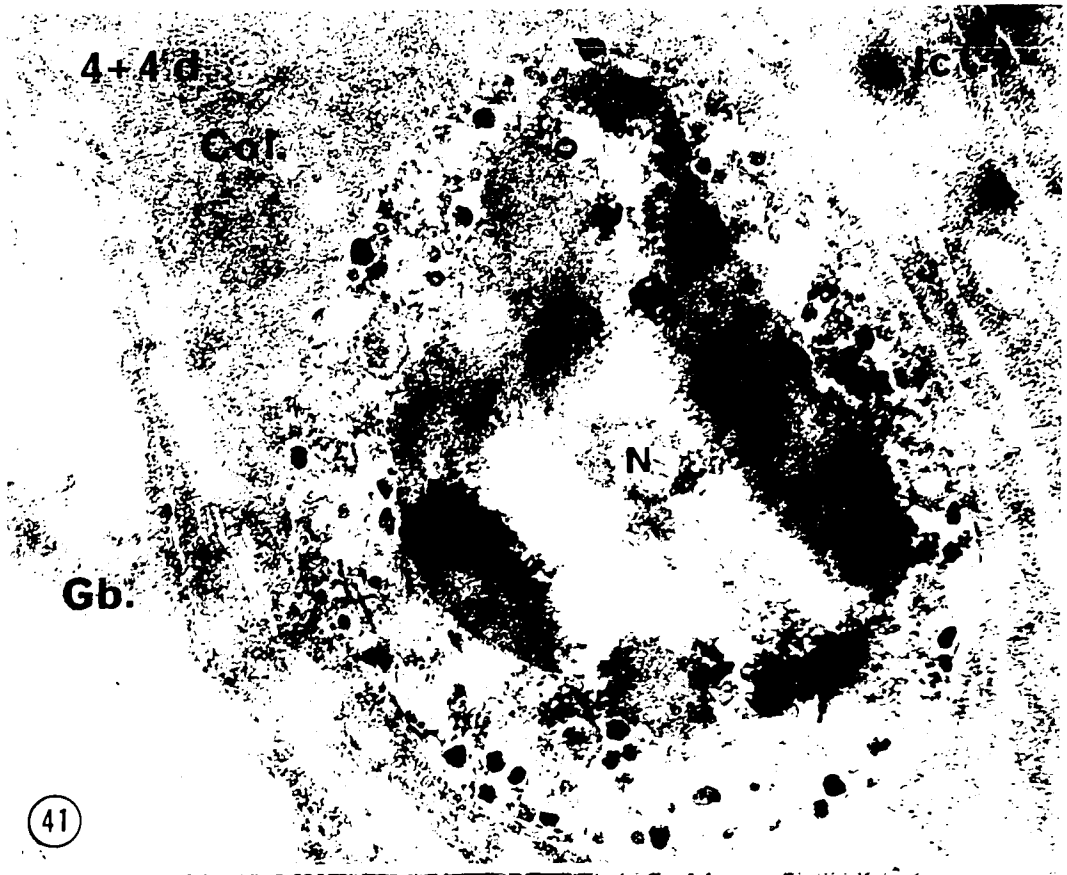
Fig. 40. Large spherical granule cell (type II) in villus, with dense granules and "empty-appearing vesicles" (arrows). Mitochondria are small (m). 20,000 X. (Formaldehyde.)


4+8d
lb
N
39
4+8d
l
N
m
40

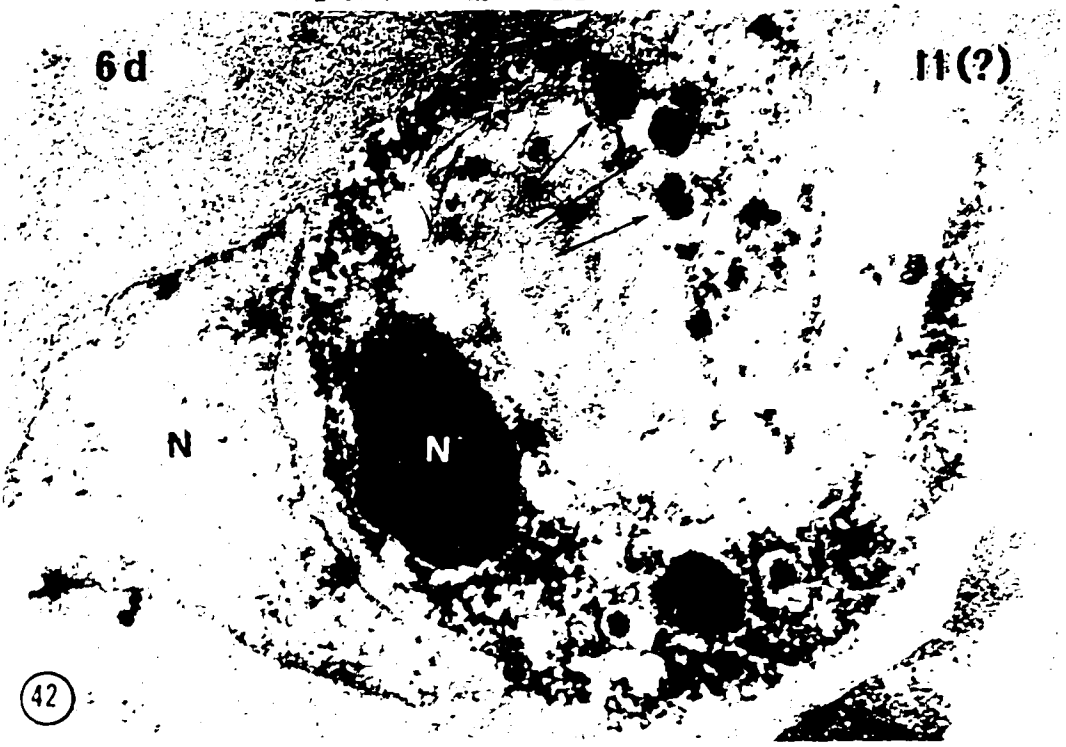
Figs. 41 - 42. Labeled "degenerating cells" from the bottom of the crypt.

Fig. 41. Biconcave granule cell (type Ic ?) with many granules which seem in dissolution. Dense patches of chromatin forms the rest of the nuclear material (N), and the nuclear envelope has disappeared. This cell seems to be phagocytosed by a columnar cell (Col.) located in cell position 7 above the Paneth cells zone. A portion of a goblet cell (Gb.) is seen (4-day infusion in animal sacrificed 4 days later). 20,000 X. (Formaldehyde.)

Fig. 42. Large spherical granule cell (type II) (?) with a complex structure and dense-looking granules (arrows) in the cytoplasm. Portions of nuclear material with silver grains are observed (N). 6 days of continuous infusion. 20,000 X. (Formaldehyde.)



41



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