THE ENDOCRINE CELLS IN THE EPITHELIUM OF THE GASTROINTESTINAL MUCOSA OF THE RAT

An Electron Microscope Study

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ABSTRACT

The authors of this study examine the question of whether the so-called enterochromaffin or argentaffin cells of the gastrointestinal tract should be considered as a single cell type. The systematic application of purely morphologic methods has led to the conclusion that the epithelium of the gastrointestinal mucosa comprises endocrine cells of several types. This conclusion is primarily based on the uneven and characteristic distribution of the various cell types along the intestinal tract, an observation precluding the interpretation that the different types correspond to diverse functional stages of the same cell. A specific endocrine function may be attributed to each of the given cell types recognized so far on account of their appearance and their localization in characteristic areas of the gastrointestinal tract. It is acknowledged, however, that a purely morphological study leaves room for doubt. The first cell type is probably responsible for the formation of 5-hydroxytryptamine. Cells of type II are morphologically comparable to the pancreatic A cells and may, therefore, be called intestinal A cells. Cell type III comprises intestinal D cells since their appearance corresponds to that of pancreatic D cells. Cell type IV might well be responsible for catecholamine production, whereas gastrin is in all probability produced in endocrine cell type V. As yet, the thorough morphological study of the gastrointestinal epithelium does not provide information as to additional distinct cellular sites of production of the several other hormones isolated from different parts of the gut.

INTRODUCTION

Enterochromaffin cells were first observed by Heidenhain (41) in the stomachs of rabbit and dog. Soon after, these cells were identified in the entire gastrointestinal tract of numerous animal species. These fascinating cells, not immediately involved in the process of digestion, were named either after those who described them (Heidenhain cells, Nicolas cells, Kultschitsky cells, Nussbaum cells, Ciaccio cells, Schmidt cells, Feyrter cells; see references 12, 20, 41, 56, 68, 69, 84, and also Plenk, 78), or according to their staining properties (enterochromaffin, argentaffin, argyrophile, pale or yellow cells). In recent publications, their designation as "enterochromaffin cells," as well as the opinion that they are a single type of serotonin-producing cells, has seemed to prevail.

Although the existence of different enterochromaffin cell types in the intestinal tract has not been established by the various authors who have studied these cells with the electron microscope, some of these authors have come to the conclusion that functional differences, at least, may well exist. Other recent publications have pointed out the existence of probably distinct endocrine cells and have established a link between the A cells of the pancreas and the site of production of enteroglucagon (21, 22, 70–74). They thus have confirmed the findings of those who had established a similar link by biochemical and immunochemical means (83, 96, 97, 100–102).

Up to a point, electron microscopy allows for the definition of the specific nature of an active secretory cell according to its morphologic individuality, particularly that of its product which is stored in characteristic granules. Thus our analysis of the ultrastructural properties of cells of endocrine appearance in the intestinal tract has led us to differentiate a series of different cells among the various endocrine type cells found scattered along the epithelium of the digestive tract. Some of these cells may not belong to the group of enterochromaffin cells. The present study suggests that at least five types of endocrine-appearing cells may be differentiated, each producing a different hormone (serotonin, glucagon, catecholamine, gastrin, or secretin). This would appear to contradict the prevailing opinion that the enterochromaffin cell is the only endocrine cell type found in the gastrointestinal mucosa and that it produces only serotonin, first recognized as a secretion product of these cells by Erspamer and Asero (17).

MATERIALS AND METHODS

Over 50 male and female albino rats weighing between 180 and 300 g were fixed by glutaraldehyde perfusion, according to our own previously published method (24), prior to fixation in buffered osmium tetroxide (66). The tissue was dehydrated in ethanol and then embedded in Epon (62). Semithin sections were studied under the phase-contrast microscope in order to locate the enterochromaffin cells which were then examined in ultrathin sections under the electron microscope. The sections were obtained with Porter-Blum and LKB ultramicrotomes and contrasted with lead hydroxide (49). The electron microscopes Zeiss EM 9 and Philips EM 300 were used.

RESULTS

Our findings indicate that the gastrointestinal tract contains at least five different types of cells each of which possesses characteristic secretory granules suggesting a specific endocrine function. By trying to establish a connection between the morphological properties of each variety of endocrine cell and its location, we have come to tentative conclusions as to the nature of five such cell types, as follows: (*a*) cells likely to account for the serotonin content of the gastrointestinal tract, type I; (*b*) cells resembling the A cells of the pancreas, type II (intestinal A cells); (*c*) cells resembling the D cells of the pancreas, type III (intestinal D cells); (*d*) cells that appear to represent the equivalent of the catecholamine cells of the sympathetic trunk and of the adrenal medulla, type IV; and (*e*) cells with a characteristic morphology of their own, suggesting that they may be gastrin-producing cells, and which we shall call type V.

The ultrastructure of all endocrine cells presents common features. Thus, the nucleus is generally oval, although it may occasionally be indented or round. A chunky or narrow border of chromatin along the nuclear periphery is seen to surround nucleoli and abundant nuclear pores. The cytoplasm is comparatively lighter than that of the surrounding epithelial cells. Mitochondria, lysosomes, and free ribosomes occur quite regularly, and so does ergastoplasm which seems to be accumulated in some regions of the cells. Bundles of filaments as well as cytoplasmic tubules generally grouped with vesicles are seen. All cells are clearly in contact with the basement membrane.

CELL TYPE I: This is the predominant gastrointestinal endocrine cell, and it is well characterized by its highly electron-opaque, polymorphous secretion granules (Figs. 1–4, 12). The predominance and distribution of these cells correspond to the presence of serotonin in the intestinal tract. Under the phase-contrast microscope this cell type is easily distinguished from the remaining cells of the endocrine system on account of its apical pole, which regularly borders on the intestinal lumen and has a straited border of microvilli resembling those of the columnar cells of the small intestine (Figs. 2 and 4). Even when found in the pylorus and other regions of the stomach, the microvilli resemble those in the small intestine, although they now contrast with the smooth surface of the neighboring cells and form a small

rough patch. Since cell type I widens considerably at its base, forming a shallow pyramid or cone, its apex is often missed in electron micrographs of thin sections, which are often confined to a small area at the base of the cell.

The Golgi complex is found in the area between the nucleus and the apical pole (Fig. 3). The secretory granules that characterize this cell are either round, oval, kidney-shaped, rodlike, or hemispherical, etc. (Fig. 12), and they consist of a homogeneous, electron-opaque substance tightly surrounded by a membrane (Fig. 12). The diameter of these granules is about 200 mμ. The granules near the Golgi complex are not always entirely filled with their granular-filamentous substance (Fig. 2), while the finding of granules that, though electron-opaque, do not entirely fill the membrane-bounded space (Fig. 4) is more unusual elsewhere.

That the secretory granules preferentially occupy the basal area of the cell can also be observed with the electron microscope and has led to their designation as "basal granulated cells" (Figs. 1 and 2) (50, 57).

This type of endocrine cell has been found in all areas of the gastrointestinal tract that we have explored, but it was particularly frequent in the duodenum. Throughout, it was found chiefly in the basal and intermediate regions of the glands.

CELL TYPE II (INTESTINAL A CELL): The cell of the second type resembles the A cell of the pancreas. This cell also is often recognizable under the phase-contrast microscope; its rounded cell body is wrapped around a slightly oval nucleus. The two shapes it can assume may call for a subdivision; when situated in the prismatic epithelium of the intestine, the intestinal A cell often assumes the tall shape of its neighboring cells, although we have never seen it reach the lumen of the gut of rat. This is also true of the rounded version of this cell, found mainly near the base of the epithelium of the gastrin mucosa, Brunner's glands, and the crypts of Lieberkühn (Figs. 5 and 6). The rounded intestinal A cell is particularly characteristic, with its spherical shape repeated by the nucleus which is sometimes indented. The cytoplasm of type II cells is slightly darker than that of type I cells. The Golgi complex is often found basally.

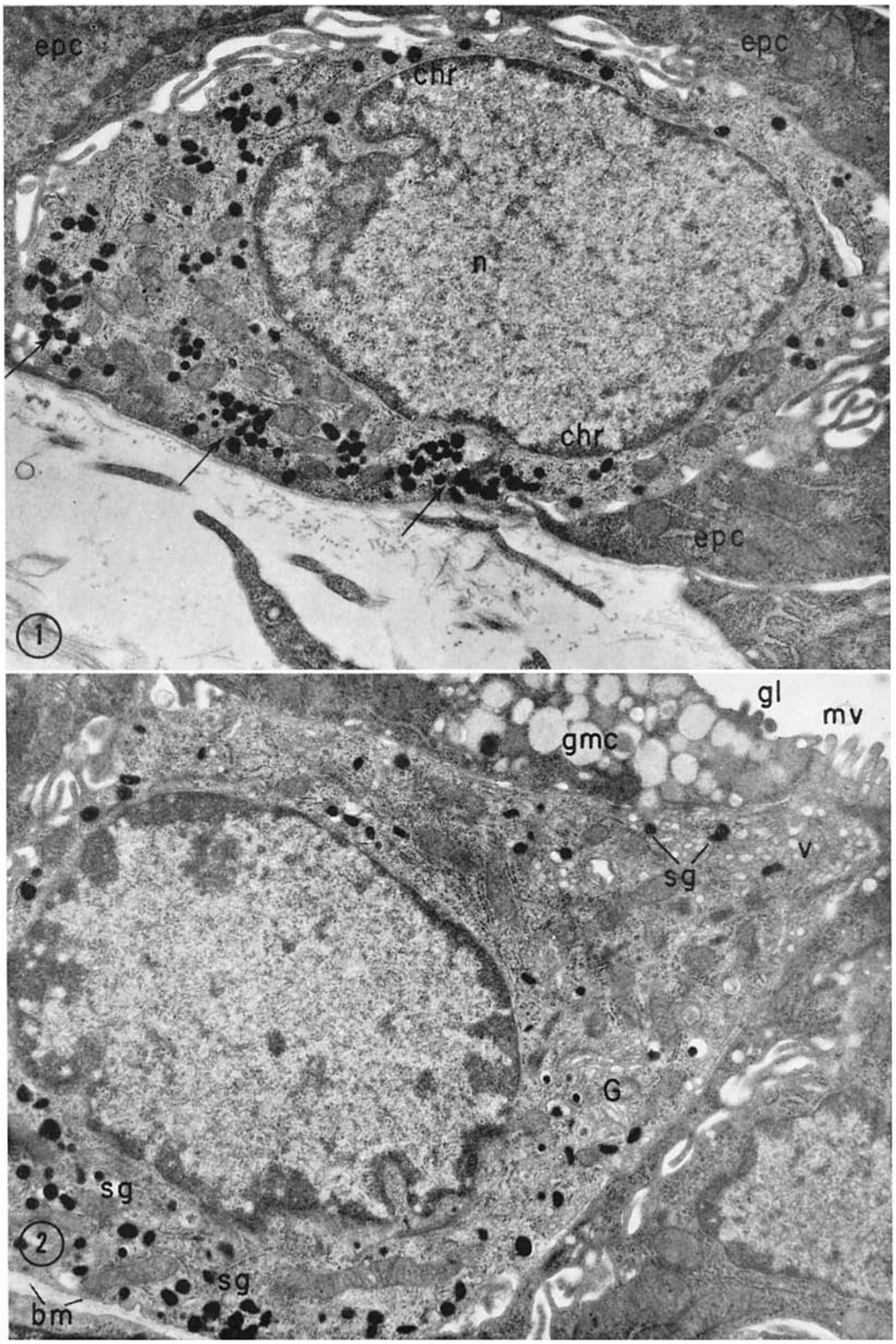
The secretion granules are characterized by their almost uniformly round shape; they vary only in diameter (500–700 mμ) and in electron-opacity (Figs. 5, 6, and 13). A fairly narrow, pale area is discernible between the membrane and the substance it encloses (Fig. 13). The cisternae of the Golgi complex often contain the same substance as the granules, but in smaller quantities (Fig. 13). The granule's preference for the basal area of a cell is particularly evident in the more elongated version of the intestinal A cell. When especially poor in granules, specimens of this type of intestinal A cell may be difficult to distinguish from the cells of type I.

We have been able to locate the intestinal A cell in the epithelium of the gastric mucosa, the duodenum, the glands of Brunner, the jejunum, the ileum, and the colon ascendens. It occurs especially often in the cardia and fundus ventriculi. Our findings indicate that it is situated almost exclusively in the deepest regions of the crypts of Lieberkühn, the pyloric, and stomach glands.

CELL TYPE III (INTESTINAL D CELL): The third cell type is difficult to distinguish from the highly similar D cell of the pancreas. It has an elongated shape, as does also cell type IV (see below), but it is smaller than any of the other

FIGURE 1 Cell type I (enteroserotonin cell?) from a pyloric gland found in the base of the epithelium. Nowhere does it reach the glandular lumen. Note the groups of dense, polymorphous secretory granules prominent at the cell base (arrows). The cytoplasm is lighter than in the surrounding epithelial cells (*epc*). The nucleus (*n*) shows a narrow chromatin border (*chr*). × 17,500.

FIGURE 2 Cell type I in a pyloric gland; this cell, unlike that of Fig. 1, shows the connection with the glandular lumen (*gl*). The basal part of the cell lies on the basement membrane (*bm*). The apical pole of this cell contains numerous vesicles (*v*) and a few secretory granules (*sg*). When the granules are found near the Golgi complex (*G*), they are often immaturely shaped. The small apex of the cell tapers into a short microvillous fringe (*mv*). Two neighbouring cells, one of which is a glandular mucous cell (*gmc*), can be seen. × 19,000.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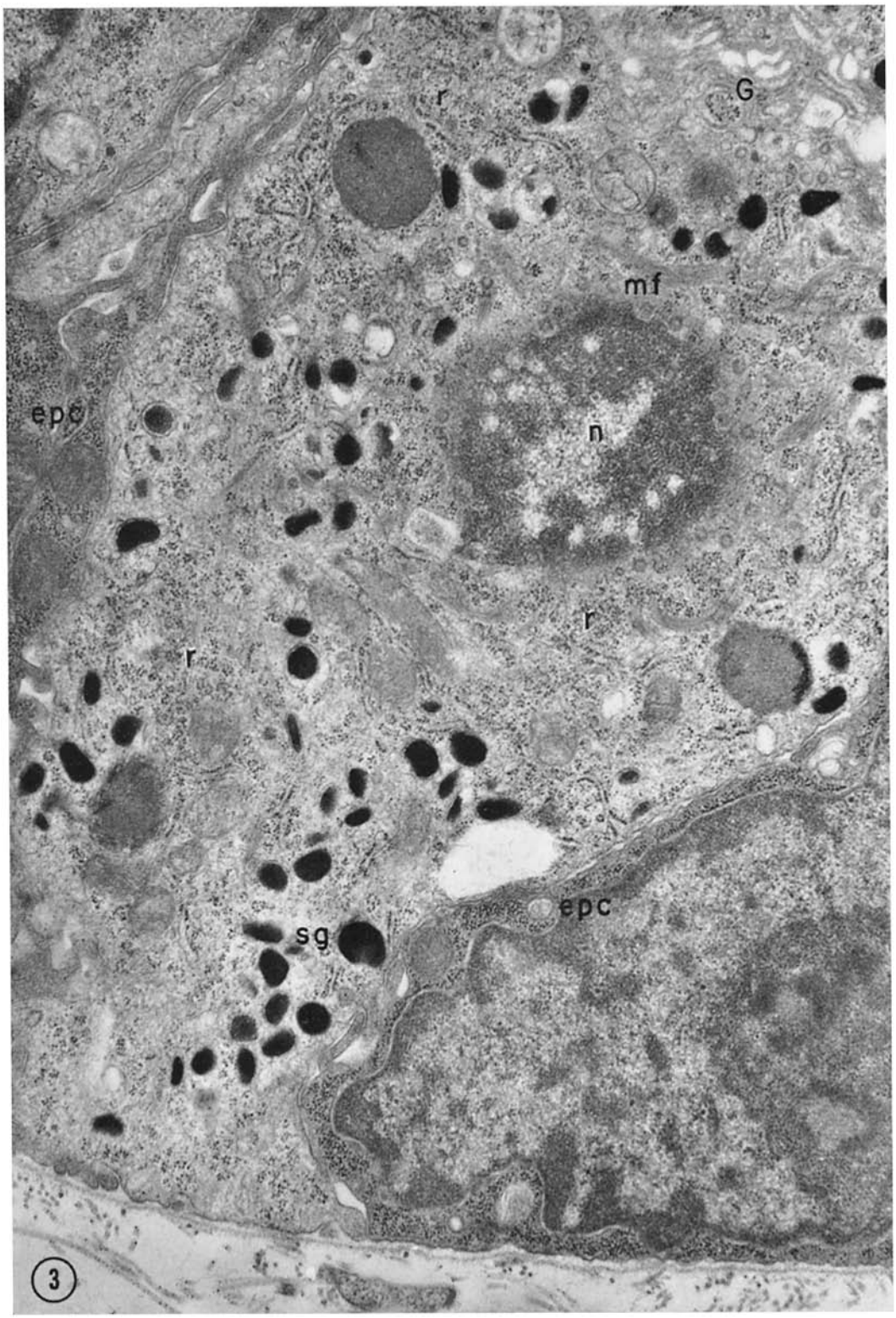


FIGURE 3 Cell type I from a crypt of Lieberkühn in the duodenum, containing numerous, dense, polymorphous secretory granules (*sg*). The cytoplasm is relatively clear and contains ribosomes (*r*), microfilaments (*mf*), and a Golgi apparatus (*G*). Pores of the nucleolar envelope can be seen in the tangentially sectioned nucleus (*n*). Further, we see several neighboring epithelial cells (*epc*). × 20,000.

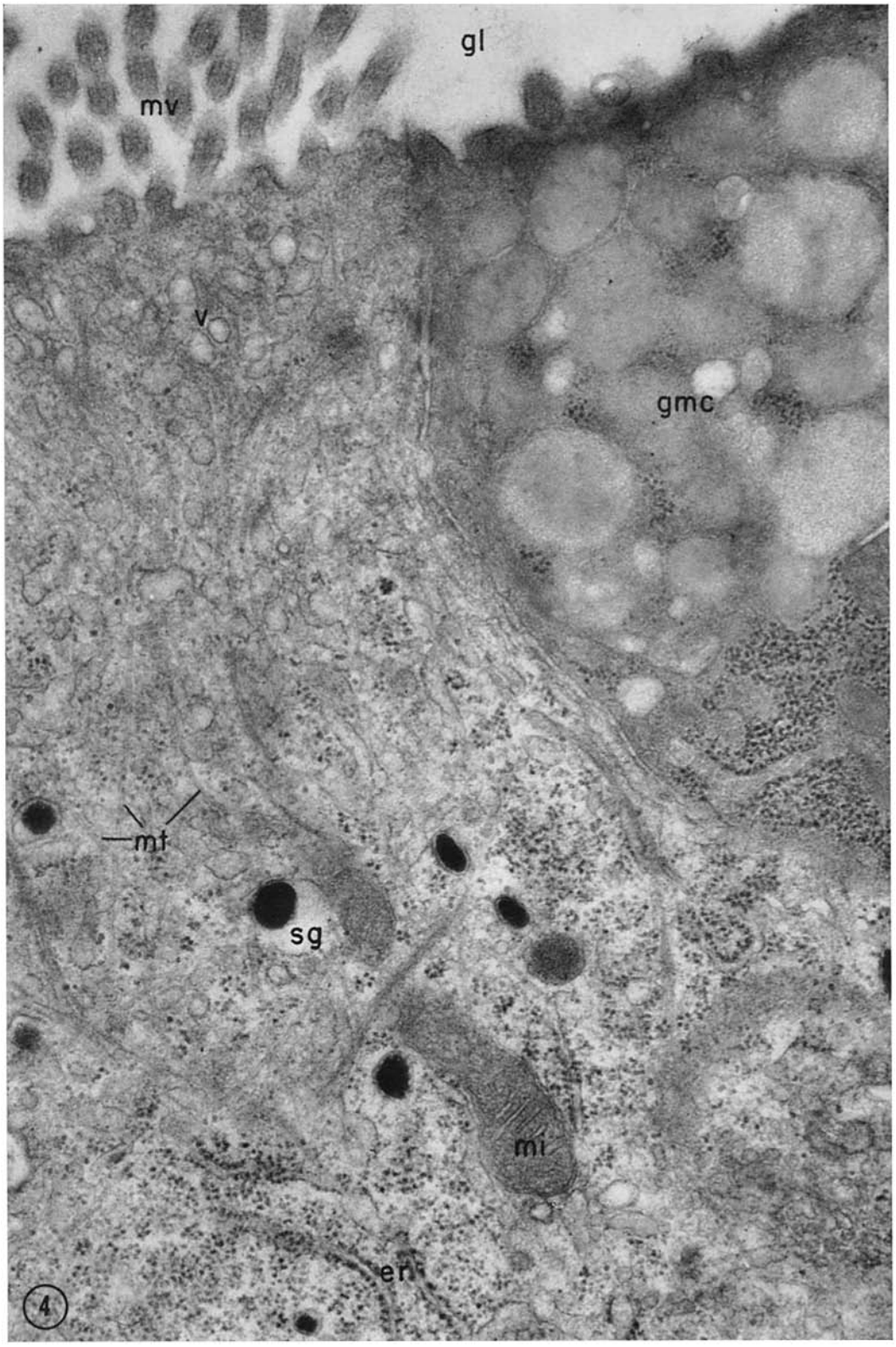
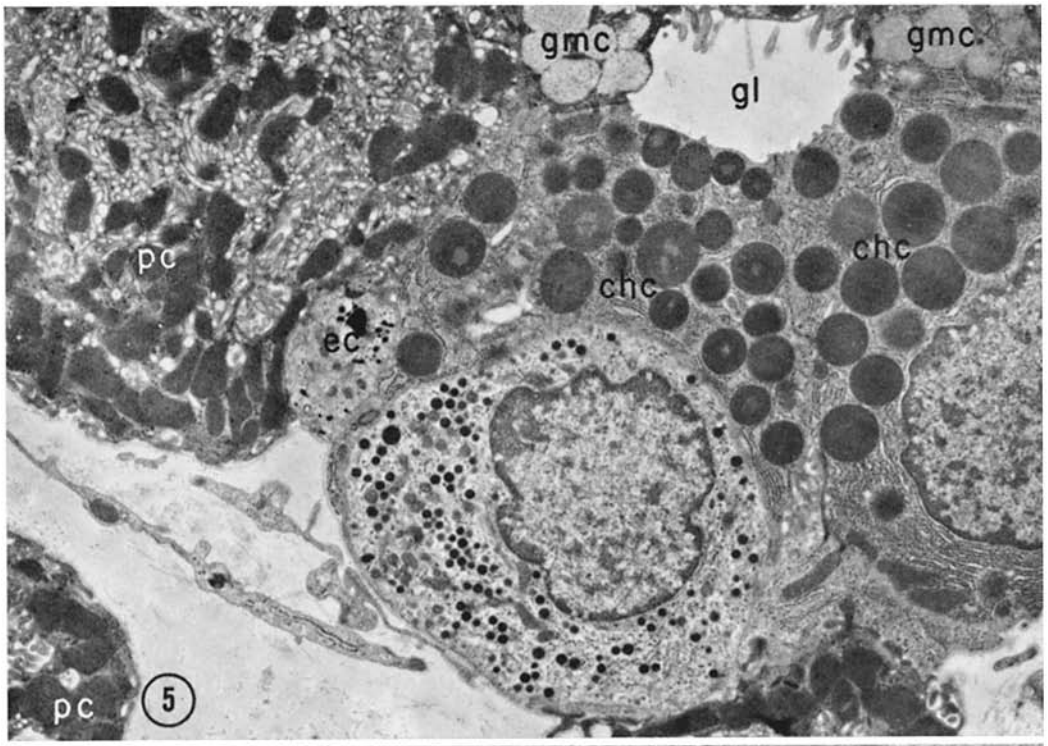


FIGURE 4 Apical region of a cell type I similar to that shown in Fig. 2. Numerous obliquely sectioned microvilli (*mv*), as well as vesicles (*v*) and microtubules (*mt*), are seen. An occasional secretory granule (*sg*) is either small or not entirely filled. In the upper right of the picture lies a glandular mucous cell (*gmc*). Ergastoplasm, *er*; mitochondria, *mi*; glandular lumen, *gl*. × 37,500.



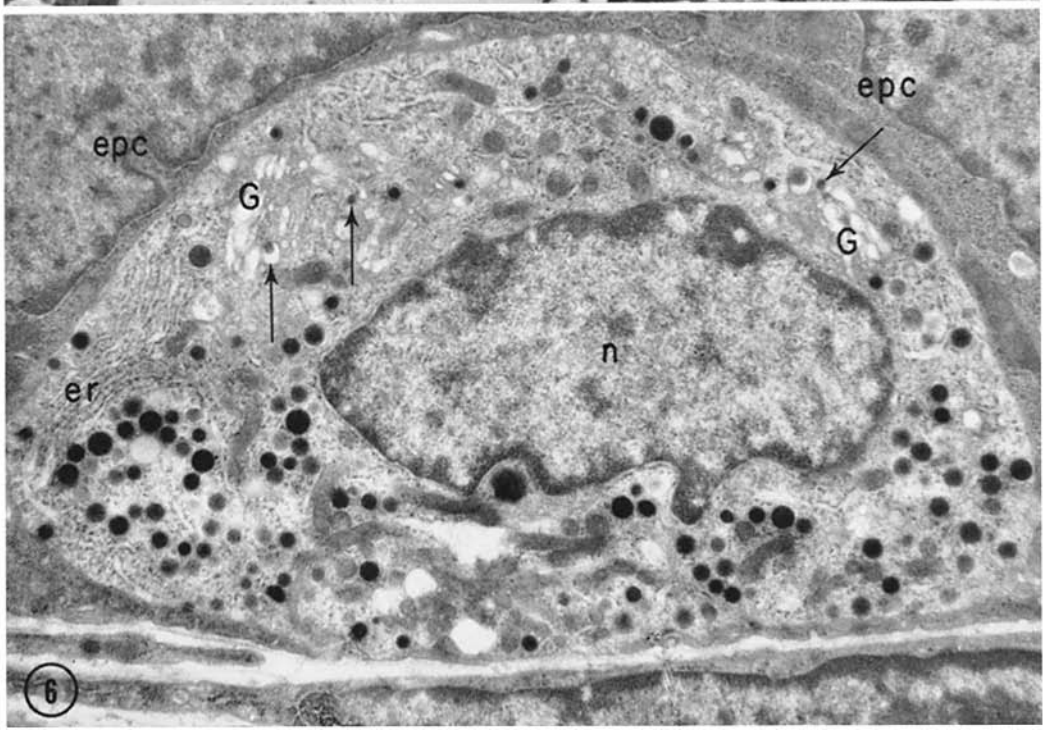


FIGURE 5 Intestinal A cell (cell type II) in a fundic gland near the pars cardiaca ventriculi. Chief cells (*chc*), parietal cells (*pc*), glandular mucous cells (*gmc*), and a small portion of a cell type I (*ec*) are also observed. The cytoplasm of the intestinal A cell is slightly lighter than that of the surrounding cells and is filled with round, dense secretory granules. Glandular lumen, *gl*. × 6,000.

FIGURE 6 The intestinal A cell (cell type II) of a crypt of Lieberkühn in the duodenum is characteristically rounded and contains round secretory granules. In the Golgi apparatus (*G*) some progranules are present (arrows). Ergastoplasm, *er*; nucleus, *n*; epithelial cells, *epc*. × 10,000.

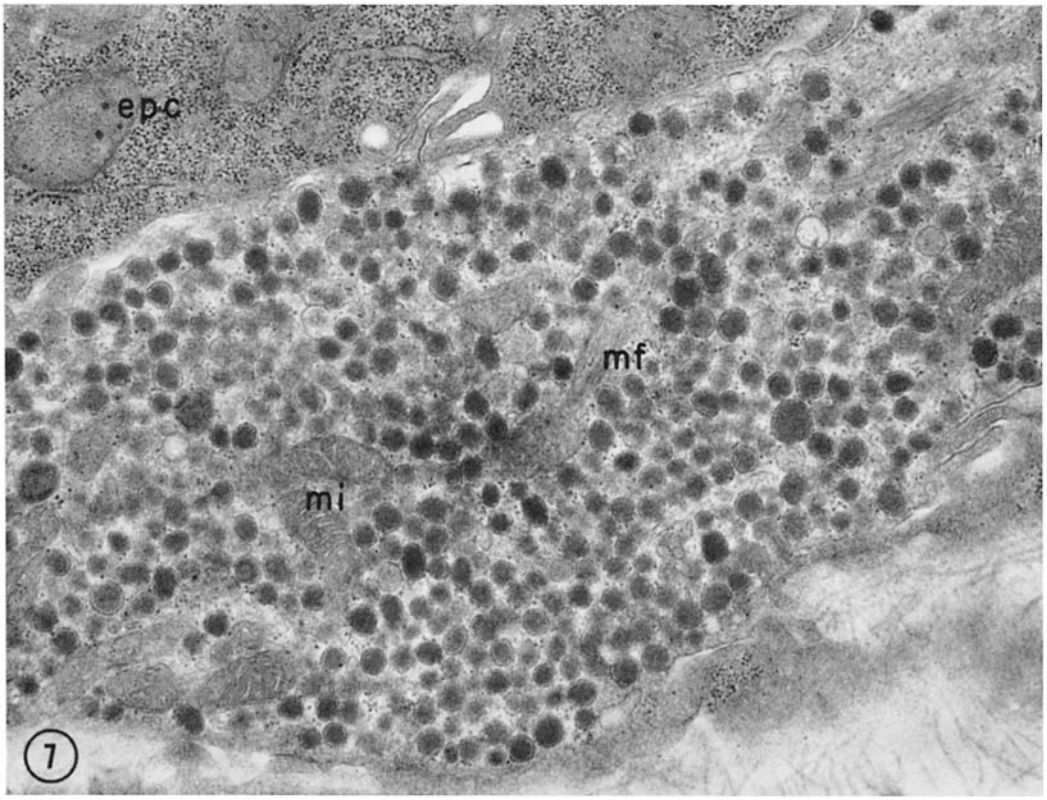


FIGURE 7 Part of an intestinal D cell (cell type III) from a pyloric gland, filled with numerous secretory granules, occasional mitochondria (*mi*), and cytoplasmic microfilaments (*mf*). In the upper part of the picture lies a glandular epithelial cell (*epc*). × 22,000.

intestinal endocrine cells. The intestinal D cell has not so far been found in contact with either the pyloric lumen or the lumen of the crypts of Lieberkühn.

Although the cytoplasm of this cell type is paler than that of the surrounding cells (Figs. 7 and 14), it nevertheless appears darker than that of any of the endocrine cells hitherto described, chiefly as a result of the great number of secretory granules it contains. The Golgi complex is poorly developed and generally lies close to an indentation in the nuclear membrane.

Different intestinal D cells contain different numbers of secretory granules, which are closely packed in some cells (Figs. 7 and 14) and yet are so scarce in other cells that it is not always easy to determine the type of the cell in question. The granules are characterized by their quite uniform size varying from 150 to 250 mμ and by their homogeneous appearance. They are separated from their membranes by an extremely narrow space, about 100 A across (Fig. 14). The membrane of these granules often has a discontinuous, nibbled-at appearance (Fig. 14), and only rarely does it enclose a considerably reduced quantity of secretory product (Fig. 7).

The intestinal D cells are found mainly in the stomach; they occur less frequently in the duodenum, in the jejunum, and in the ileum. In the gastric glands, they are generally found in proximal areas.

CELL TYPE IV: The ultrastructure of type IV endocrine cells is similar to that of the chromaffin cells of the sympathetic trunk. In all observations made so far, the roundish cell body has not been found to reach the lumen of the gut (Figs. 8, 9, and 15). As yet, these cells have only been observed in the gastric mucosa. The Golgi complex generally lies near the poles of the nucleus.

The characteristic secretory granules are gener-

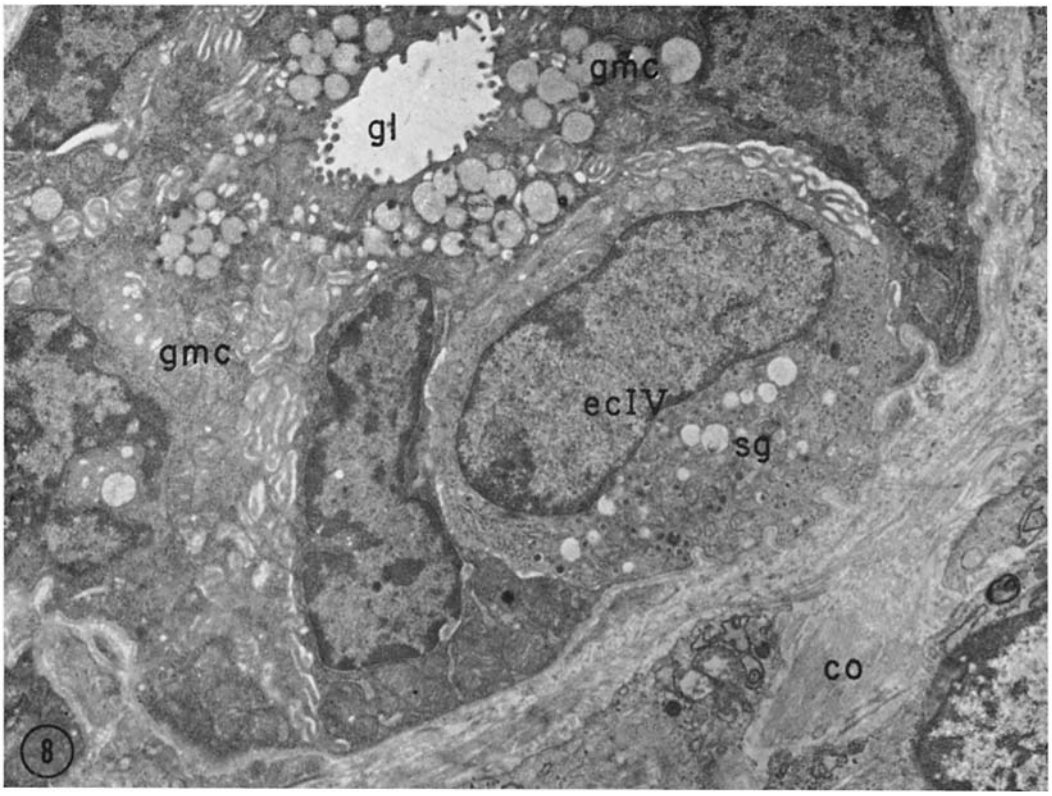


FIGURE 8 Basal region of a pyloric gland showing an endocrine cell of type IV (*ec* IV). On the basal cell side one can see an area filled with different secretory granules (*sg*). Only the glandular mucous cells (*gmc*) extend to the glandular lumen (*gl*). Collagen (*co*) and cells of the lamina propria are seen on the lower right of the micrograph. × 6,000.

ally confined to a small area in the basal region of the cell (Fig. 8). Some of them are small (about 150 mμ) and contain a granular filamentous substance which is separated from the surrounding membrane by a narrow space (Figs 9 and 15). Some of these granules are unusually dense and are surrounded by the narrowest clear spaces (Fig. 15). Other granules are considerably larger (between 300 and 800 mμ) and contain small, eccentric accumulations of granular-filamentous material (Fig. 15). In numerous sections, the largest of these bubble-shaped granules do not seem to contain any secretory product at all; yet if we examine the same granules in consecutive sections, a blob of secretory product invariably turns up.

Type IV endocrine cells have only been found in the gastric mucosa particularly near the pylorus. Identification is difficult whenever a section does not include the basal area of the cell, since secretion granules are very scarce in the remaining parts of the cell; the cells are then easily mistaken for undifferentiated digestive cells, as type IV cells are generally found in the epithelium of the gastric glands.

CELL TYPE V: In the pylorus we find a large number of probable endocrine cells. They differ from the previously described ones by the dark color of their cytoplasm and by the appearance of their secretory granules (Figs. 10, 11, and 16). These granules are fairly uniform in diameter (300–500 mμ) and are clearly bound by a membrane. Their contents vary greatly, however; some scarcely contain any substance at all, others are evenly filled with a light granular and filamentous material (Figs. 10, 11, and 16). The secretory granules may be so dense as to resemble those of the intestinal A cells. Thus, the variability in the

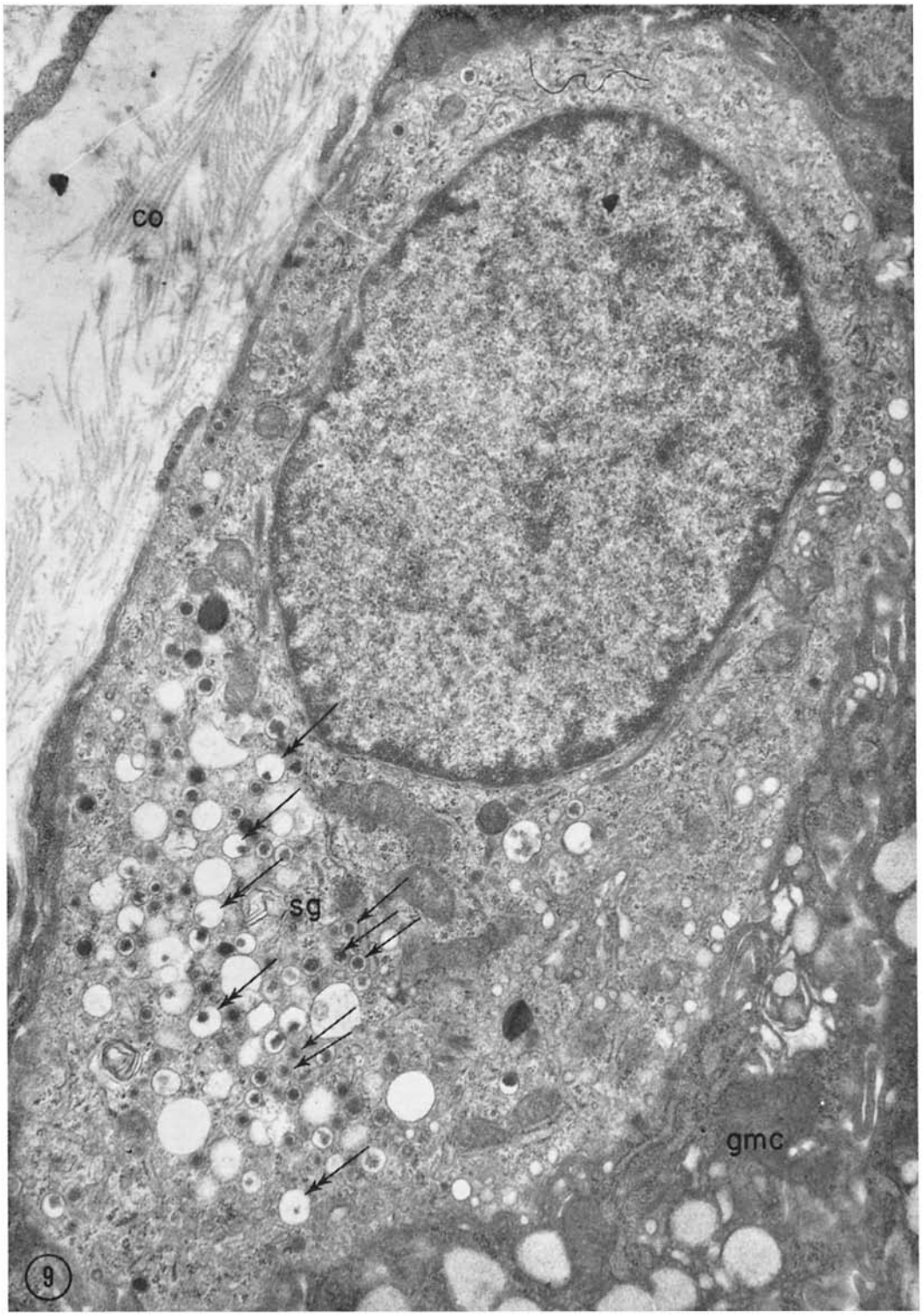


FIGURE 9 Cell of type IV in a pyloric gland (enterocatecholamine cell). The cytoplasm is paler than that of the adjacent glandular cells and contains an area of secretory granules (*sg*), some of which are big (double-headed arrows), others extremely small and entirely filled with the secretory product (single-headed arrows). Glandular mucous cells, *gmc*; lamina propria with collagen, *co*. × 15,000.

density of the granules, ranging from nearly complete transparency to complete opacity, becomes characteristic for this type of endocrine cell. The granules lie scattered about without any regular distribution pattern, so that it is impossible to relate them to any of the cytoorganelles.

Apart from the pyloric region, the type V endocrine cells have also very occasionally been found in the cardia and the upper part of the duodenum. All areas of the pyloric glands, even their outer surface, contained cells of this type (Figs. 10 and 11). These cells generally extend from the basement membrane to the glandular lumen and may be limited by microvilli as are the cells of type I (Fig. 11). The shape of the granules is independent of their proximity to the glandular lumen. In the gland's distal regions, the elongated cells attain the height of the prismatic epithelial cells.

DISCUSSION

In the past, many studies have pointed out differences in structure and in staining properties of the so-called enterochromaffin cells. Nevertheless, since the publication of Erspamer and Asero (17) who recognized the secretory product of these cells as 5-hydroxytryptamine (see also Gershon and Ross, 26), the prevailing opinion has been that the enterochromaffin cells are of a single cell type (2, 5, 6, 77, 90, 91, 93, 94). Few authors, such as Schofield et al. (85), consider the enterochromaffin cell system as a heterogeneous group of cells including two main categories.

Similarly, electron microscopic studies either refer to a single type of enterochromaffin cell (11, 42, 43, 86, 107, 108), or else compare "argentaffin" cells with "argyrophile" cells (99). Authors who note differences in shape and in granular content of the enterochromaffin cells account for them as different stages in formation or degradation of secretory granules (80, 81). However, their conclusions and hypotheses have often been based on techniques insufficient to justify such an interpretation. Moreover, their findings (80, 81) are illustrated by drawings. Our findings do not confirm the opinions of either group of authors.

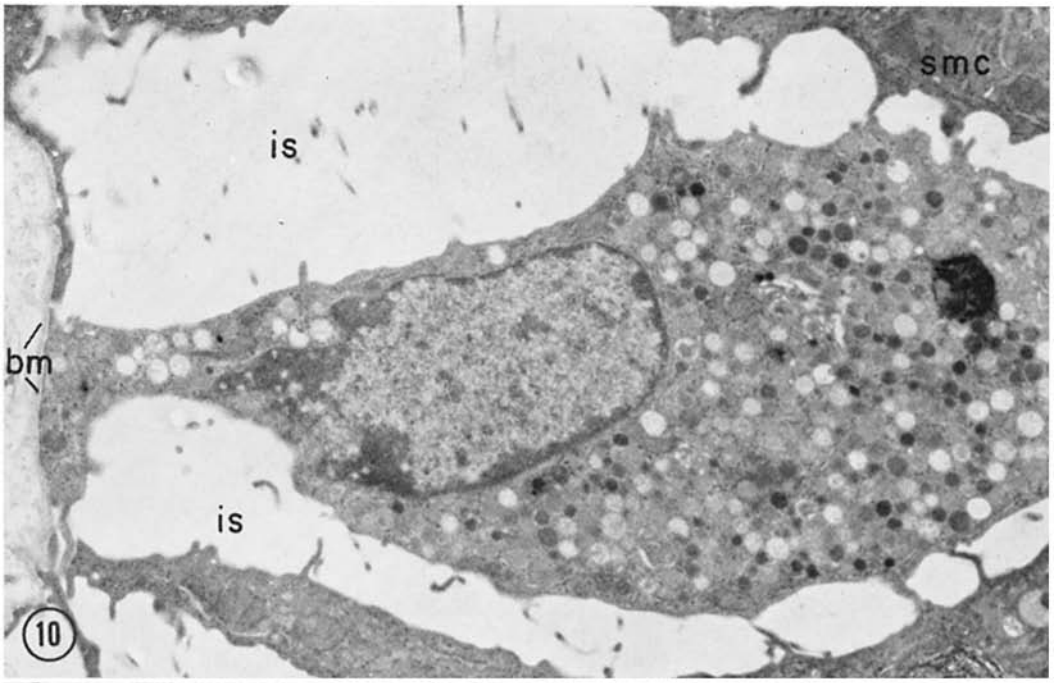
The several endocrine cells which we have pointed out here may not all belong to the group of enterochromaffin cells, as some of them may not be demonstrable by the methods employed for their study. Indeed, the term enterochromaffin cell may well be applicable only to one of the five cell types described here, most likely to type I.

The fact that different endocrine cells are located in different and characteristic areas of the digestive tract would seem to justify the assumption that each of the cell types, some of them described here for the first time, constitutes a type of its own. Localization of the endocrine cells was established after thorough examination of material derived from specific areas of the digestive tract fixed by perfusion. Particular attention had to be paid to localization in the stomach. Also, we wish to reject the theory that these cell types represent various functional stages of the same cell, on the following grounds. (*a*) As just mentioned, the different cell types occur more or less frequently according to the area under investigation, and some of them are found in specific regions only. (*b*) Some cells are in contact with the intestinal lumen, where they terminate in a fringe of microvilli (cell types I and V). Others are rounded, smooth-surfaced cells, preferentially located at the base of the epithelium (cell types II, III, IV). Transformation between two groups of such diverse appearance is highly unlikely. (*c*) There is no evidence of cells representing intermediary stages between the different cell types.

CELL TYPE I: This cell can be traced from

FIGURE 10 Cell type V (gastrin-producing cell ?) in the surface epithelium of the pylorus mucosa. The cytoplasm seems to be of the same density as that of the surrounding surface mucous cells (*smc*). The cell is completely filled with its numerous secretory granules and its nucleus. Note the enlarged intercellular space in the surface epithelium (*is*). The footlike processes of the epithelial cells are seen along the basement membrane (*bm*). × 6,600.

FIGURE 11 Cell of the fifth endocrine type found in a basal region of the pyloric gland. The cell is relatively big, trimmed with a small border of microvilli (*mv*), and has a large base. Except in the apical area of the cell, its cytoplasm contains numerous secretory granules of varying density. Nucleus, *n*; region of the Golgi apparatus, *G*; adjacent glandular mucous cells, *gmc*; glandular lumen, *gl*. × 6,300.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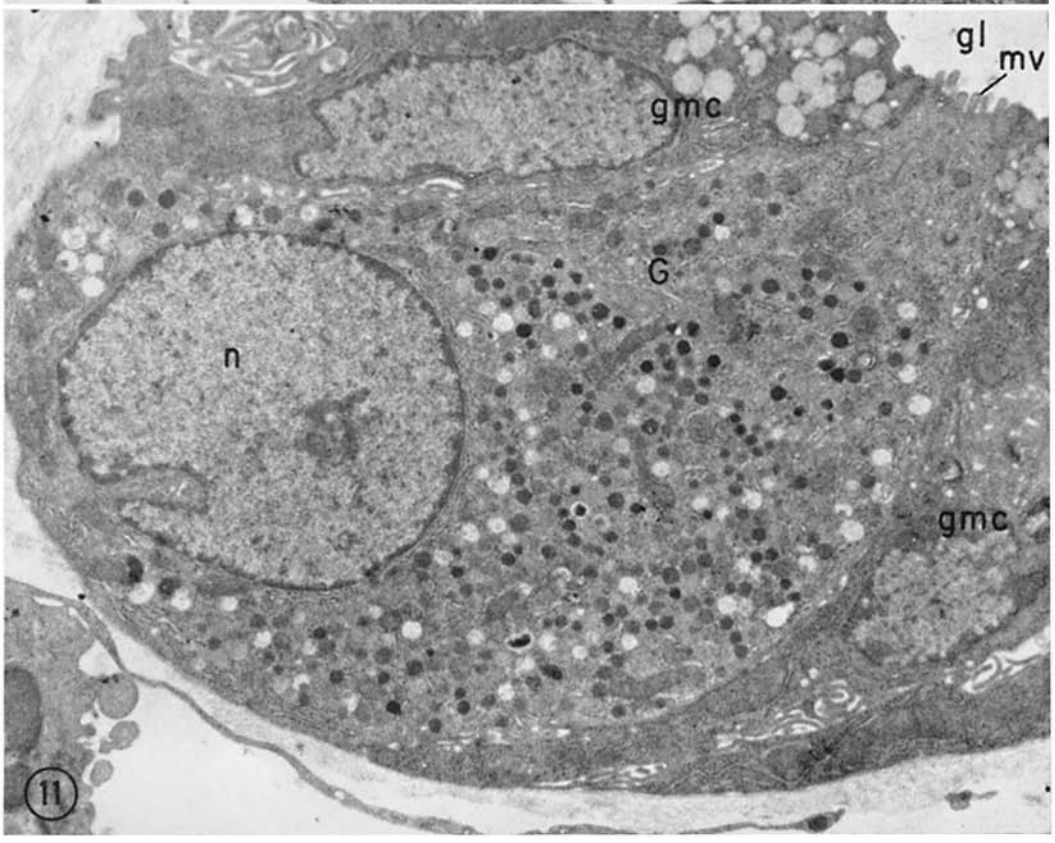
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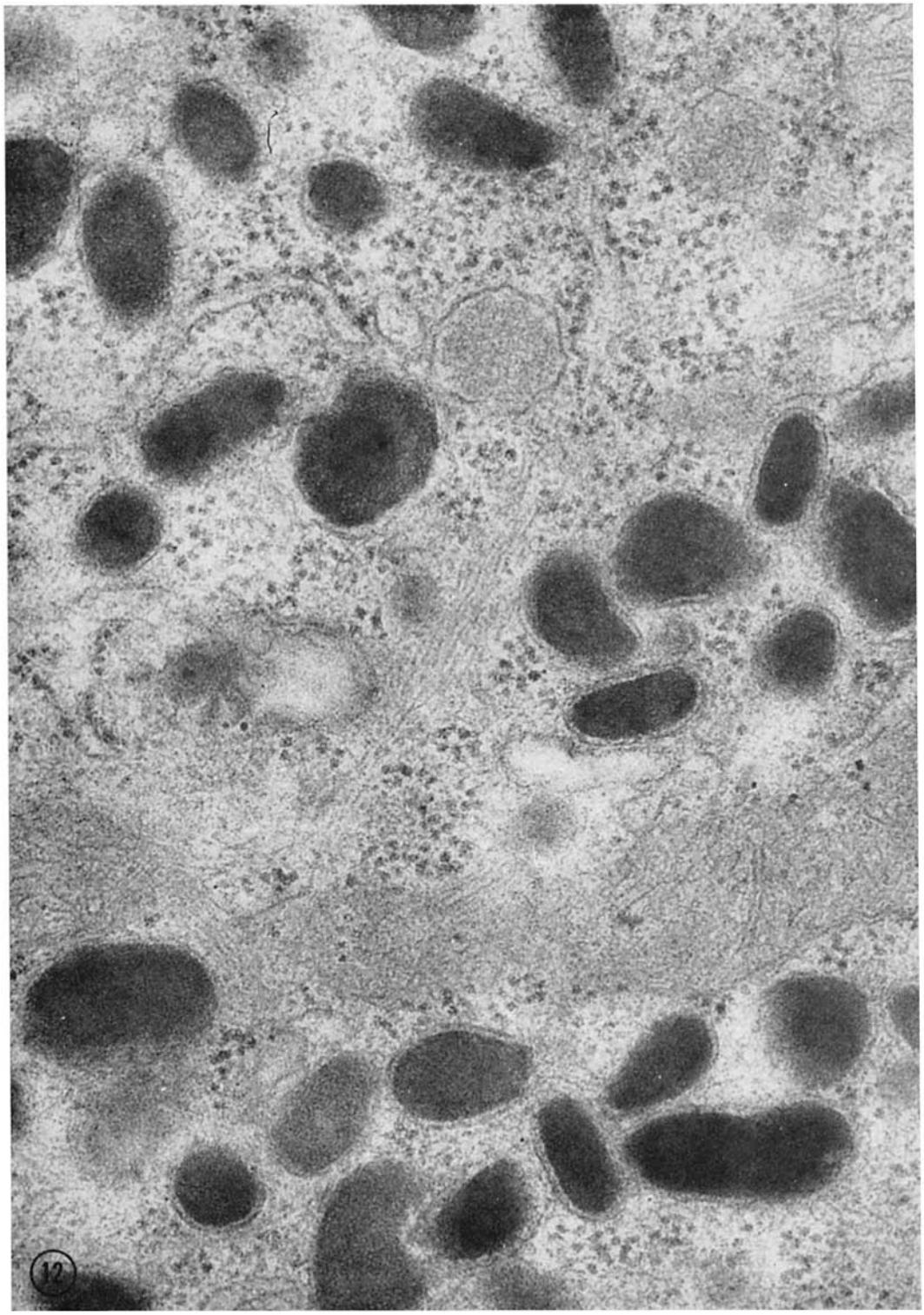

FIGURE 12 Small portion of the cytoplasm of a cell type I in the duodenal mucosa, showing the dense, polymorphous secretory granules at a higher magnification. The granules consist of a tightly-bound membrane enclosing the secretory product. × 64,000.

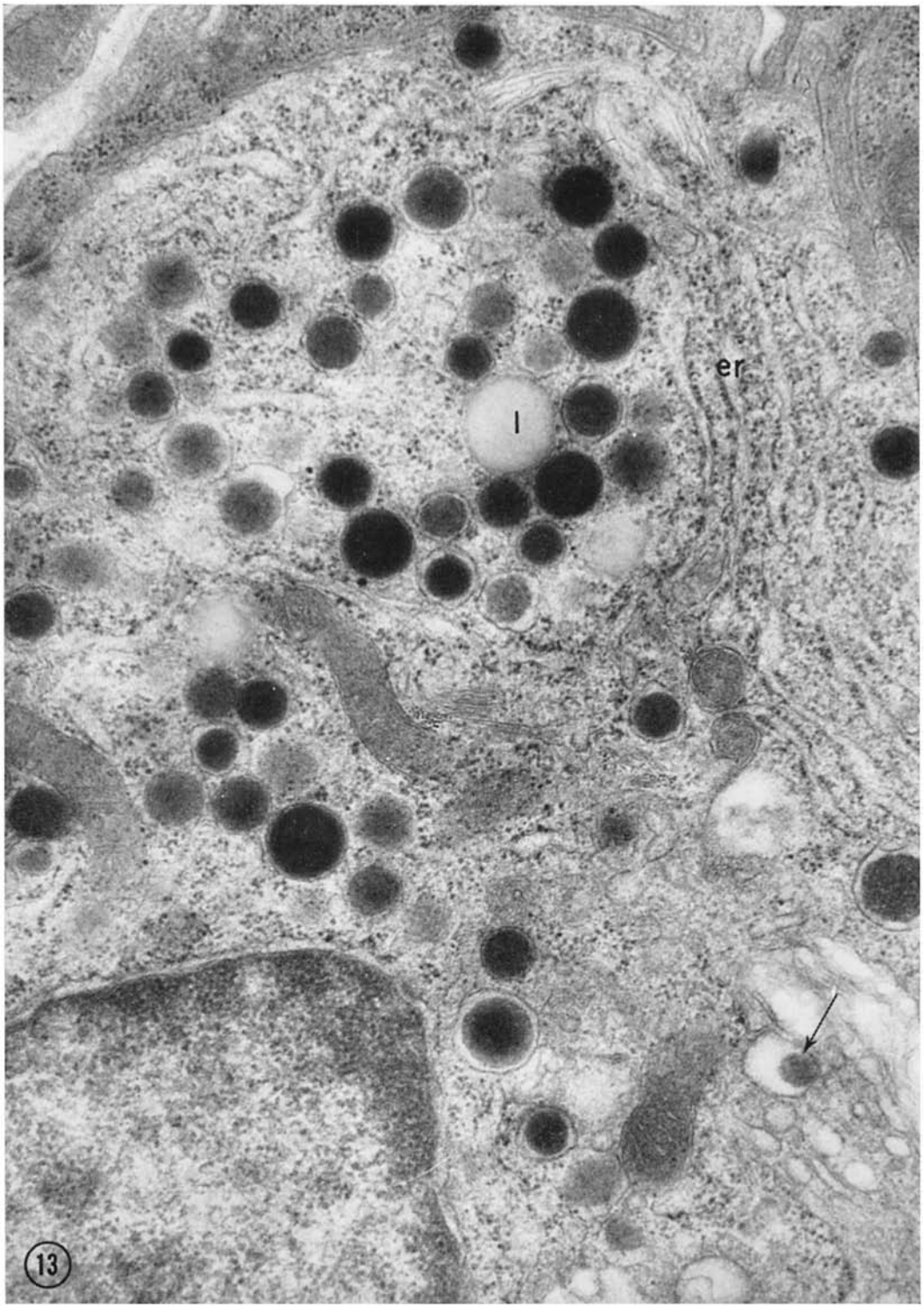

FIGURE 13 Intestinal A cell seen in Fig. 6 but at higher magnification. Each of the numerous, round secretory granules of different diameter is enclosed by a distinctive membrane. A progranule can be seen in the Golgi apparatus (arrow). Lipid droplet, *l*; ergastoplasm, *er*. × 35,000.

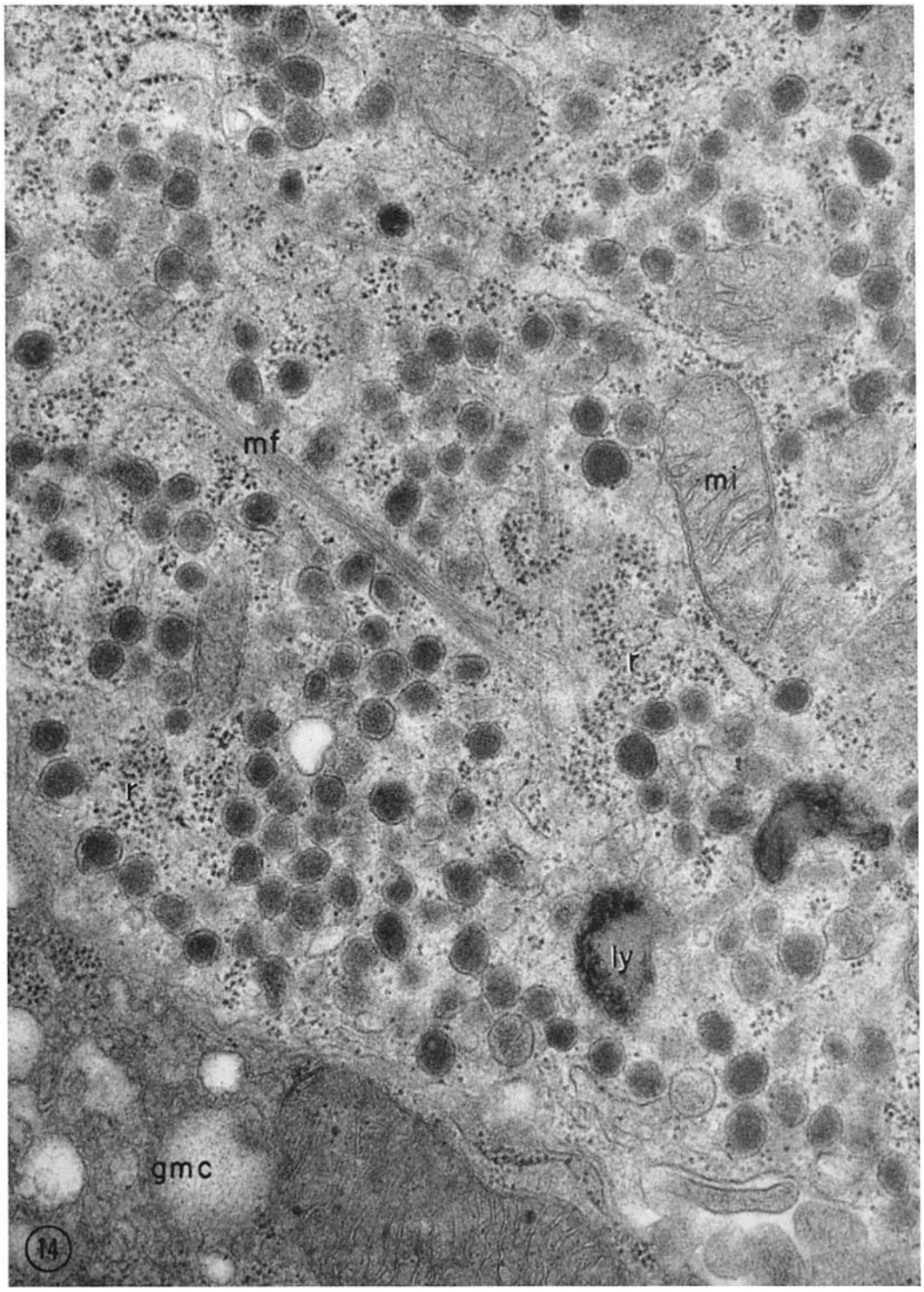

FIGURE 14 Intestinal D cell from the pyloric mucosa, showing numerous secretory granules with a small gap between the "nibbled-at" membrane and the enclosed substance. The cytoplasm is lighter than that of the adjacent glandular mucous cell (*gmc*) and contains ribosomes (*r*), mitochondria (*mi*), microfilaments (*mf*), and lysosomes (*ly*). × 40,000.

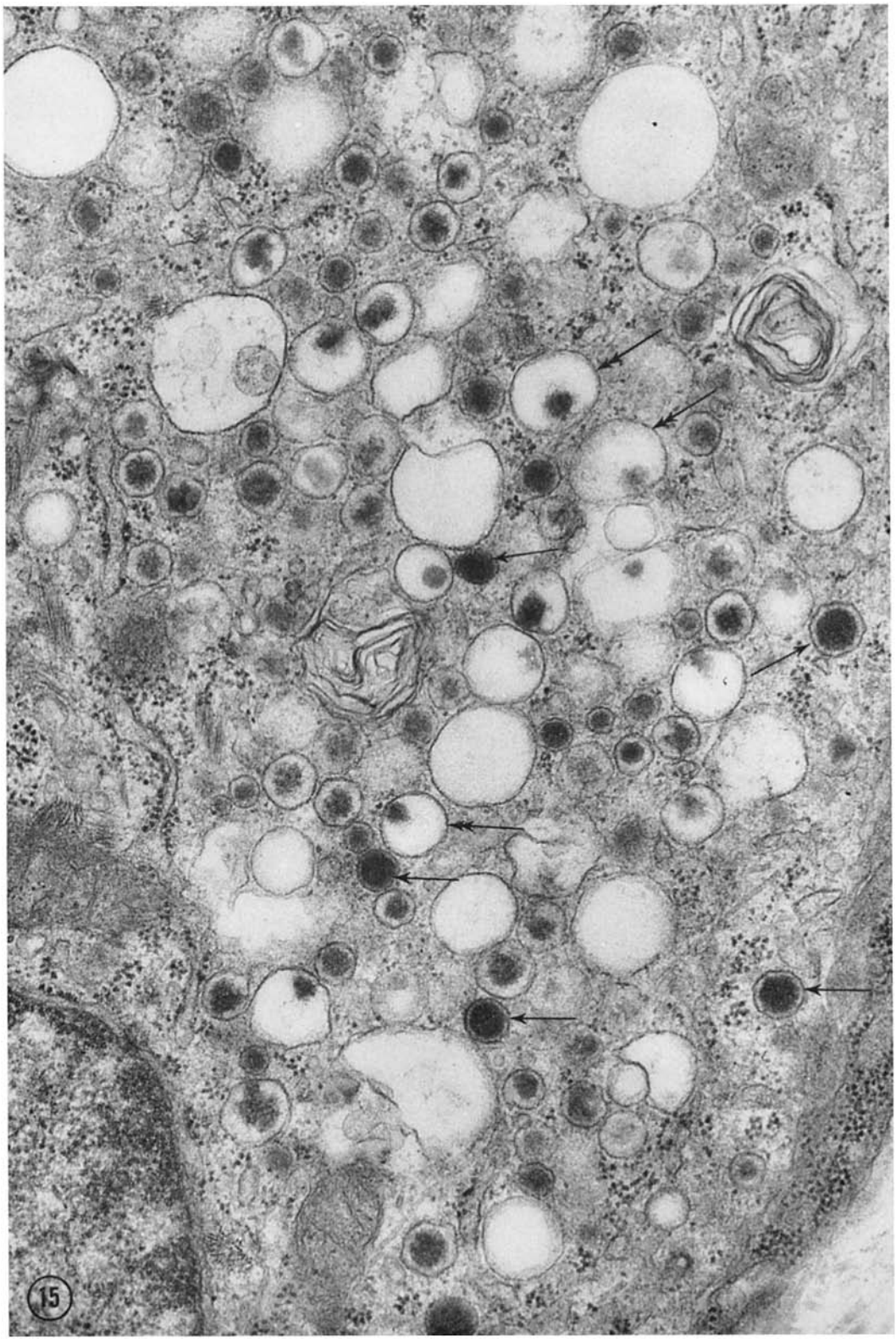

FIGURE 15 Cell type IV shown in Fig. 11 but at a higher magnification. There are two sorts of secretory granules: (*a*) small, dense granules tightly-bound by a membrane (single-headed arrows), and (*b*) larger granules often incompletely filled with secretory substance or containing a lightly packed, generally eccentric substance (double-headed arrows). × 40,000.

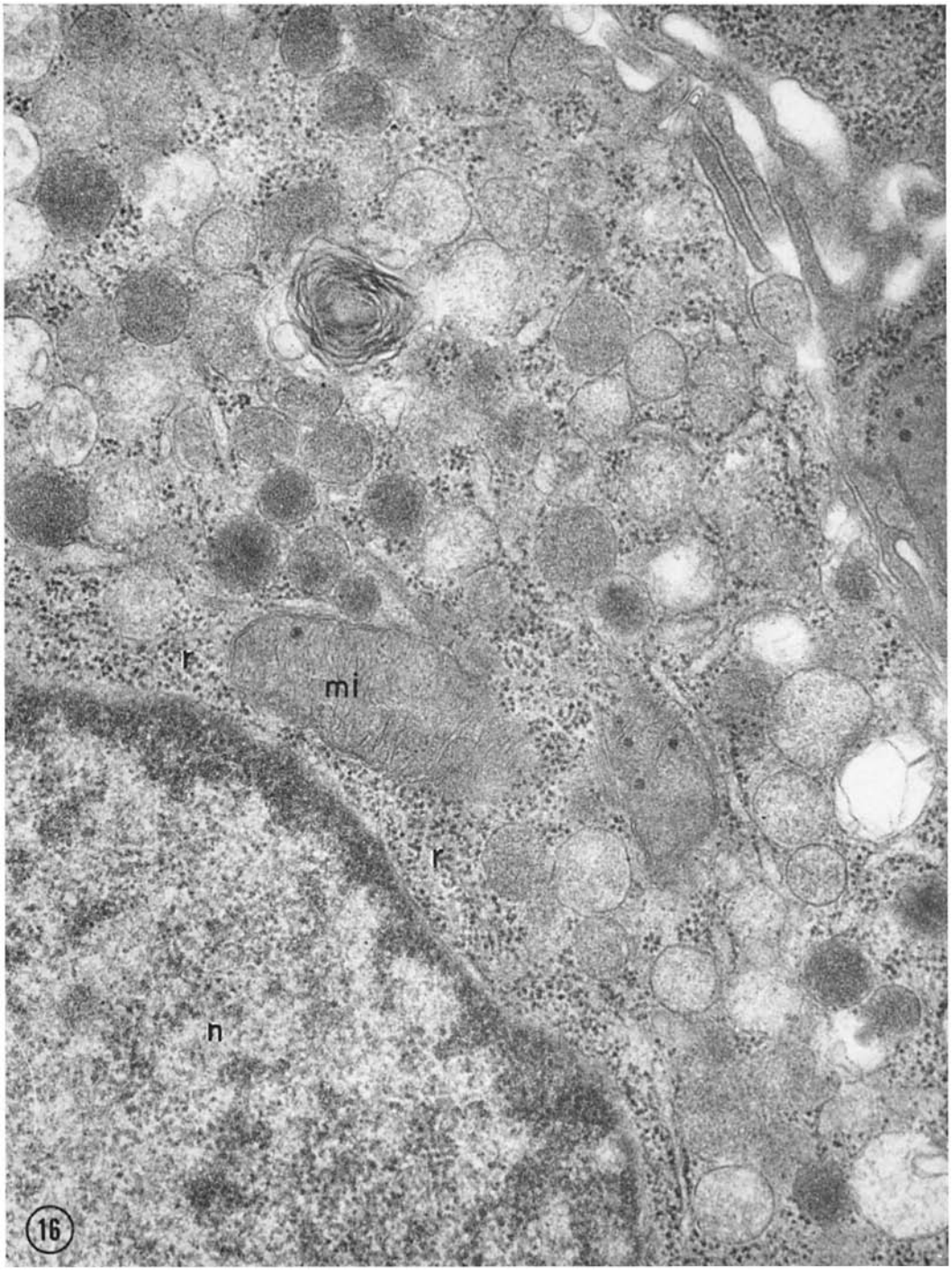

FIGURE 16 Cell type V showing several granules of approximately the same diameter but containing a substance of varying density. Nucleus, *n*; mitochondria, *mi*; ribosomes, *r*. × 40,000.

the stomach to the colon, and it is the most frequently occurring endocrine cell in the small intestine. Its hormone content may be serotonin as identified by Erspamer and Asero (17) after they had succeeded in extracting this hormone from the stomach of rabbit and had observed similar staining properties *in situ* and after isolation. Feldberg and Toh (18) have established that the distribution of serotonin coincides with the location of the enterochromaffin cells. Many others confirmed that 5-hydroxytryptamine is the secretory product of the enterochromaffin cells by fluorescent and simple staining methods (3), alkaline thionidoxyl reaction (76), and ninhydrin vapor reaction (45). The general opinion is, therefore, that the enterochromaffin cells produce serotonin. Evidence to the contrary, however, has also been found. For example, reserpine, which provokes the release of serotonin from the gut, is reported to have been administered to different regions of the intestinal tract with varying results (109) Moreover, the effectiveness of reserpine depends on the animal species concerned; in the rat, the decrease in stainable enterochromaffin cells after treatment with reserpine is practically nil (77). This was confirmed ultrastructurally by the failure of type I cells to degranulate after high doses of reserpine (personal observation).

Despite these inconsistencies, we nevertheless feel that the weight of the presently available evidence is in favor of identifying cell type I with enterochromaffin cells, and both with the site of serotonin production. These cells probably give rise to the serotonin-producing carcinoid tumors (27, 39, 59, 87, 95).

CELL TYPE II (INTESTINAL A CELL): Ultrastructural evidence of the presence in the intestinal tract of enteroglucagon-producing cells, i.e. intestinal A cells, was discussed in previous papers (21, 22, 70–74). The presence of the intestinal A cell was also suspected as a result of biochemical experiments (51, 64, 96, 97, 100, 101). After using silver impregnations, as well as other techniques, Ferner (19) and Solcia and Sampietro (92) were induced to conclude that the endocrine cells of the intestinal epithelium and of the islets of Langerhans in the pancreas are analogous.

Ultrastructurally, the A cells are easily identified in the pancreas as well as in the intestine. Light microscopic methods are far less suitable; on the one hand, they may lead to the identification of the argyrophile cells as A cells (19, 58), and on the other, as D cells (25). The latter are probably A_1 cells as defined by Hellerström et al. (44).

Many contradictory results obtained with light microscopy can be explained when the presence of these argyrophile cells is considered. The relationship of this cell to the glucagon-like hormone of the digestive tract explains the lack of response to experiments influencing serotonin metabolism in the argyrophile cells (77, 109). The argyrophile cell, however, is considered by most authors to be an enterochromaffin cell undergoing modifications brought about by the secretory process.

A recent publication by Carvalheira et al. (9) distinguished two types of endocrine cells in the gastrointestinal epithelium. Considerable importance was attributed to the cholinesterase content of these cells. The cells with low cholinesterase content seem to be equivalent to our enteroserotonin cells, and the cholinesterase-rich cells probably correspond to intestinal A cells, judging from electron micrographs. The assumption that these cells produce gastrin is surely unfounded, since the ileum (see reference 14) does not contain this hormone and since these cells have been found by Carvalheira et al. (9), as well as by ourselves, in other gastrin-free areas of the gastrointestinal mucosa (see also below). Nor do these cells seem to correspond to those described by Solcia et al. (95) as gastrin-producing cells.

We were able to identify this second cell type in cats, both young and old, in guinea pigs, and in rabbits (22), as well as in man (70). Further studies comparing the pancreatic and the intestinal A cells of diverse species may well reveal that these two cells belong to a single cell type, varying only from species to species.

With electron microscopy, only Toner (99) has so far clearly identified what we call the first and second endocrine cell types, which he terms argentaffin and argyrophile cells, respectively. Without discussing their functional significance, and without reference to the analogy between pancreatic and intestinal argyrophile cells, Toner (99) comes to the important conclusion that two fundamentally different cell types are involved.

Among the numerous cells qualified by Ito and Winchester (46) as argentaffin, both A and D cells may be identified, and they are therefore probably to be classified as argyrophile cells. This interpretation must remain hypothetical, however, since neither Ito and Winchester (46) nor we have con-

ducted light and electron microscopic experiments simultaneously. Ito and Winchester (46) are of the opinion that a single cell type is involved. We cannot subscribe to their opinion, for the reasons mentioned.

The only electron microscopic studies mentioning any relationship between argyrophile cells and the formation of glucagon are those of Helander (43) and our own.

The presence of a glucagon-like hormone in the gastrointestinal tract has been established independent of histological methods. The ultrastructural approach may well contribute to the solution of the problems related to the origin of intestinal "glucagon" (Samols et al, 83; and Samols and Marks, 82).

CELL TYPE III: Since the third endocrine cell type of the intestinal tract is analogous to the D cell of the pancreas, we must refer briefly to this cell, which is generally considered a third type of pancreatic cell (7, 8, 67). Few are the authors who see in it a variant of A cells (52) and who accordingly name it A_1 cell (44). It remains difficult to answer the question of whether the D cell is to be considered a separate cell type or not, because experimental data are not available as yet.

Similarly, nothing definite is known to date about the function of the pancreatic D cell. From considerations of the Zollinger-Ellison-syndrome (110), Cavallero et al. (10) come to the conclusion that the D cells may produce gastrin (see also references 4 and 88). The localization of these cells throughout the entire gastrointestinal tract does not, however, coincide with the areas of gastrin production pointed out by physiologists and biochemists (compare with cell type V). These cells may well contain secretin or some other polypeptide, although there is no reason to believe that these hormones are present in the pancreatic D cells.

In the dog, Krawitt et al. (55) have found secretin in the duodenum, mainly in the areas between the crypts and the villi (55). These authors assume that the formation of secretin is in no way connected with the basal granulated cells, as suggested by Wermel and Kacharova (106), but that this hormone is produced in the cells of the stroma or in the epithelium of the villi.

Solcia et al. (95) also considered the possibility of a connection between the production of gastrin and the D cells (which they have recently distinguished from the enterochromaffin cells). The endocrine cells in their electron micrographs can only be qualified as D cells, with a certain amount of hesitation. We disagree with these authors, because in our opinion cell type V is involved in the production of gastrin (see below) and has nothing to do with the D cell. We prefer the hypothesis of Ito and Winchester (46) who consider the intestinal A and D cells to be cells of a similar type yet representing different functional stages. Our findings seem to justify such a hypothesis inasmuch as our sections of the intestinal tract showed that A and D cells are distributed in, or almost, identical manner and proportions.

The D cell is particularly characteristic in the cat, in whose stomach and pancreas it could clearly be recognized (22, 70).

CELL TYPE IV: This cell type may also be an enterochromaffin reacting cell, although it occurs far less frequently in the stomach. Already in 1907, Ciaccio (12) commented on the similar staining properties, after treatment with dichromate solutions, of enterochromaffin and adrenal medullary cells, and he concluded that the enterochromaffin cells contain adrenalin. Masson (65) and Hamperl (38) also are partisans of the "brenzcatechin" theory, which was, however, refuted by Gomori (28) and completely neglected after the discovery by Erspamer and Aspero (17) of 5-hydroxytryptamine in the enterochromaffin cells. More recently, Lillie (60) has tried to revalidate the brenzcatechin theory.

Existing micrographs of endocrine cells type IV provide insufficient evidence as to the nature of the content of the granules, whether noradrenalin or another similar substance. Resemblances in the granules' morphology do, however, seem to indicate that the cells of this type might contain catecholamines, since very similar cells were observed under similar technical conditions by Siegrist et al. (89) in the sympathetic trunk. The occurrence of catecholamine could be explained in conjunction with this cell type (33, 34). Häggendal (33) and Håkansson and Owman (34) have already pointed out the possibility of dopamine being produced in what they called enterochromaffin-like cells (16).

CELL TYPE V: This cell type is especially characteristic, since the morphology of its granules distinguishes it from all other known pancreatic, adrenal-medullary, and other endocrine cells. Particular attention was paid to this cell type, since despite thorough searching these cells could

be found only in the antrum pylori and adjacent duodenal regions, as well as very occasionally in the cardia ventriculi. Therefore, the question of whether these cells produce gastrin necessarily arises.

Numerous authors have revealed the presence of gastrin in the antrum pylori (13, 15, 40, 47, 53, 54, 103–105) and in the cardia (13, 29, 61), but not in either the corpus or fundus, and very occasionally in the duodenum where in the case of the cat its activity represents 20% of its total activity in the antrum pylori (14).

In the epithelium of the pylorus, gastrin was also found at the level of the glandular lumen. The normal pancreas contains no gastrin (35, 37), but gastrin may, however, occur in pathological states (1, 30, 32, 36). These findings seem to contradict the opinion of Solcia et al. (95), who consider the D cell in the stomach and pancreas responsible for the production of gastrin. Moreover the presence of cells rich in cholinesterase amongst the enterochromaffin cells (endocrine cells) does not justify the assumption that they produce gastrin, since gastrin is only produced in the specific areas already mentioned (9).

As far as we are concerned, the evidence that endocrine cell type V is responsible for gastrin production seems to be sufficient to warrant classification of this cell as an intestinal gastrin cell. Thus, this cell type is readily affected by fasting and feeding (unpublished data) and, furthermore, these cells are found in far greater quantities in stomachs of patients with peptic ulcers than in the normal stomach (22*a*, 23). The correlation between the growth of an ulcus ventriculi and the formation of gastrin is, or course, well known (75). Some of these problems can perhaps be resolved by the examination of gastrin-producing tumors, such as those associated with the Zollinger-Ellison-syndrome.[1]

At present, ultrastructural studies available on pancreatic tumors (31, 63, 79) do not permit a definitive conclusion as to the classification of the cell types found in these tumors.

There remains yet another question; how can a cell type similar to that in the antrum pylori suddenly appear in the pathological, gastrin-producing pancreas? The common embryological origins of the pancreas and pylorus provide a possible explanation, a hypothesis that has already been expressed by Tauber (98).

[1] *Note added in proof:* Since the manuscript has been accepted for publication, we had the occasion to see photomicrographs from Prof. W. Creutzfeld, Göttingen, Germany, showing equivalent cells to type V, but taken from a Zollinger-Ellison-syndrome in man.

CONCLUSIONS

Our morphological analysis of the intestinal endocrine cells necessarily included a systematic survey of all areas within the gastrointestinal tract of the rat. Our findings clearly refute the widely held opinion that these endocrine cells form a single cell type. It would seem that prior differentiation as to enterochromaffin, argentaffin, argyrophile, and other designations may well refer to specific cell types of which some may correspond to one of the types reported in this study. Although our methods have been purely ultrastructural, they have led us to conclusions that lie beyond the usual scope of morphologic investigations, giving rise to new interpretations. We are well aware of the fact that conclusions based on morphologic methods alone have, in the long run, usually revealed certain errors, and we too will no doubt be unable to avoid these errors. We hope, however, to have indicated new areas for future investigations. We feel that the general conclusion of our study is that each of the biochemically identified hormones in the gastrointestinal tract is probably produced in a specific area, and that we are therefore to consider the epithelium of the intestinal tract as a complex endocrine organ that fulfils numerous functions. More specifically, we have ascertained that certain cells may well be of the long-sought-for areas where the glucagon-like product of the intestinal tract might be formed and stored. We do not attribute the formation of gastrin to a cell similar to the pancreatic D cell, but rather to a new cell type which we term cell type V. Finally, we wish to say, in view of the numerous publications that speak of a so-called "organ of pale cells" (19, 80), that the pale cells may not be involved in endocrine frunction at all. In that case, these cells might not be degranulated, but rather represent undifferentiated epithelial cells (48) that may represent a reserve state for several types of cells.

We must also ask ourselves whether each cell type is responsible for one hormone only, or, if in cases of similar polypeptide sequences, one cell type might not be involved in the formation of several biochemically related secretion products.

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