

# ELECTRON MICROSCOPE RADIOAUTOGRAPHIC IDENTIFICATION OF SEROTONIN-SYNTHESIZING CELLS IN THE MOUSE GASTRIC MUCOSA

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## ABSTRACT

This study correlates the fine structure of mouse gastric endocrine cells with their ability to synthesize serotonin (5-HT) from 5-hydroxytryptophan (5-HTP). Mice were sacrificed 2 hr after the intravenous injection of 5-HTP-<sup>3</sup>H or 5-HT-<sup>3</sup>H. Their stomachs were processed for light- and electron microscope radioautography in a manner which retained labeled 5-HT while washing out other labeled substances. Stomachs from additional mice were incubated *in vitro* with 5-HT-<sup>3</sup>H and processed similarly.

All morphologic types of mouse gastric endocrine cells exhibited a similar facility to incorporate exogenous 5-HTP and to convert it to 5-HT which was bound intracellularly. Differences in densities of silver grains observed over endocrine cells suggested that individual endocrine cells indeed varied in their ability to synthesize and/or to bind 5-HT; such variations, however, were not reflected by differences in fine structure, with the exception that endocrine cells with few granules always contained little newly synthesized 5-HT. The newly synthesized 5-HT was associated with the intracellular granules. The gastric endocrine cells were not labeled by exogenous 5-HT-<sup>3</sup>H, whereas mast cells were labeled by either 5-HT-<sup>3</sup>H or 5-HTP-<sup>3</sup>H administration. The findings of the present study support the position that the gastric endocrine cells represent a single cell type, at least in respect to serotonin metabolism—that the argyrophil or argentaffin reactivity of these cells merely reflects their amine content at a given time.

Recent studies of the morphology, histochemistry, fluorescence, and distribution of gastrointestinal endocrine-like epithelial cells (“argyrophil,” “argentaffin,” “enterochromaffin,” “pale,” “clear”) have suggested the presence of more than one type (1-8). Forssmann and co-workers (6) have classified these cells into five morphologic entities in the rat and have suggested a probable and separate endocrine function for each type.

The present study identifies and characterizes by electron microscope radioautography all cells in the mouse gastric mucosa which can synthesize

serotonin (5-hydroxytryptamine, 5-HT)<sup>1</sup> from 5-hydroxytryptophan (5-HTP), the immediate precursor in the biosynthesis of 5-HT (9, 10). This study shows that all morphologic types of gastric endocrine cells readily incorporate exogenous 5-HTP and convert it to 5-HT, which is bound intracellularly.

<sup>1</sup> *Abbreviations used in this paper:* dpm, disintegrations per minute; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan.

## METHODS

The animals, drugs, procedures, and biochemical controls have been largely described previously (11, 12). Briefly, six adult mice, averaging 20 g in weight, were injected intravenously with 5.0 mCi of 5-HTP-<sup>3</sup>H (specific activity of 2.84 Ci/mmole). The animals were sacrificed by cervical fracture 2 hr after isotope administration; previous biochemical and light microscope radioautographic studies of animals sacrificed from 5 min to 8 days after isotope administration (11, 12) had shown that by 2 hr the isotope has disappeared from the plasma and the gastric mucosa has progressively accumulated high levels of radioactivity (from 165 to 320 dpm/g of stomach in these animals). The stomachs were immediately removed, washed for 10 min in saline at 0°C, fixed for 2 hr at 0°C in hypertonic 2.5% glutaraldehyde (0.2 M phosphate and 3% sucrose), and postfixed for 2 hr at 0°C in hypertonic 1% osmium tetroxide (Palade's [13] with 9% sucrose). They were dehydrated in graded alcohols, cleared in propylene oxide, and embedded in Epon 812 (14). As reported previously (11, 12), these procedures retain essentially all the labeled 5-HT, but wash out essentially all labeled 5-HTP and 5-hydroxytryptamine-*o*-glucuronide, the only other labeled compounds of significant concentration.

Serial thick (1 μ) and thin (silver to pale yellow) sections from all areas of the stomach and including the entire mucosa were cut with a Sorvall MT2 ultramicrotome. The thick sections were mounted on chemically clean glass slides which had been previously immersed in an adhesive gelatin solution containing 0.5 g/liter chromium alum; the thin sections were placed on parlodion-coated copper grids, which were then attached to glass slides with a scotch tape which was adhesive on both sides. Both thick and thin sections were coated with Ilford L4 emulsion by dipping, were dried, and were placed in an exposure chamber under CO<sub>2</sub> at room temperature. The thick sections were exposed for 4–14 days, developed in D19 for 4 min, stained with aqueous toluidine blue (15), and studied by light microscopy. The thin sections were exposed for 6–20 wk, developed in Microdol-X (Eastman Kodak Co., Rochester, N.Y.) for 4.5 min, stained with lead (16), and examined with a Philips EM300 electron microscope.

In order to determine where exogenously administered 5-HT accumulates within the gastric mucosa, the following experiments were performed. Seven mice were injected intravenously with 1.0 mCi of tritiated 5-HT (specific activity, 5.6 Ci/mmole) 1 hr after the intraperitoneal injection of the monoamine oxidase inhibitor, pheniprazine (10 mg/kg; Lakeside Laboratories, Inc., Milwaukee, Wis.). The animals and tissues were then treated as described above. In a final group of experiments, the stomach and small intestine were rapidly removed from

eight mice, half of which had received pheniprazine 1 hr before cervical fracture. The tissues were minced and were incubated in oxygenated Krebs' solution at 37°C. After 30 min of equilibration, tritiated 5-HT was added to the medium to a concentration of  $1.7 \times 10^{-7}$  M (1.0 Ci/ml). After incubations for up to 2 hr, the tissues were processed as described above.

## RESULTS

In sections from animals injected with 5-HTP-<sup>3</sup>H, both light- and electron microscope radioautography produced excellent localization of label in gastric endocrine (Figs. 1–9) and mast (Fig. 10) cells with little background activity (Figs. 1–11). No other types of mucosal cells were labeled; mucosal axons with granulated vesicles were unlabeled (Fig. 11). Labeled cells were observed throughout the gastric glands below the pits (Fig. 1); most labeled cells were located in the lower portions of the glands while relatively few were observed in the necks (Fig. 1).

Although the washing and hypertonic fixation procedures were used primarily to retain 5-HT while washing out other labeled substances, the resultant fixation was quite satisfactory with the exception that mitochondria often appeared swollen and exhibited myelin changes (Figs. 2–9). The fine structural appearance of endocrine granules, however, appeared independent of any mitochondrial changes within the same cell.

Although individual endocrine cells were sometimes difficult to classify by morphologic criteria, cells resembling each of the five morphologic types described by Forssmann and coworkers (6) in the rat were readily identified and found to exhibit similar degrees of labeling: (a) the type 1 or enteroserotonin cell, thought to be a source of 5-HT (Figs. 2, 3), (b) the type 2 or intestinal A cell, thought to be a possible source of glucagon (Fig. 4), (c) the type 3 or intestinal D cell (Fig. 6), (d) the type 4 or enterocatecholamine cell (Fig. 8), and (e) the type 5 cell, which has been thought to be the gastrin-producing cell (Figs. 6, 7). Each morphologic group of endocrine cells exhibited a similar incidence of heavy, moderate, light, and inapparent labeling. Most endocrine cells were labeled. Those endocrine cells which could not be readily classified as one of the five morphologic types also exhibited similar degrees of labeling as did the classified ones.

The silver grains were generally associated with

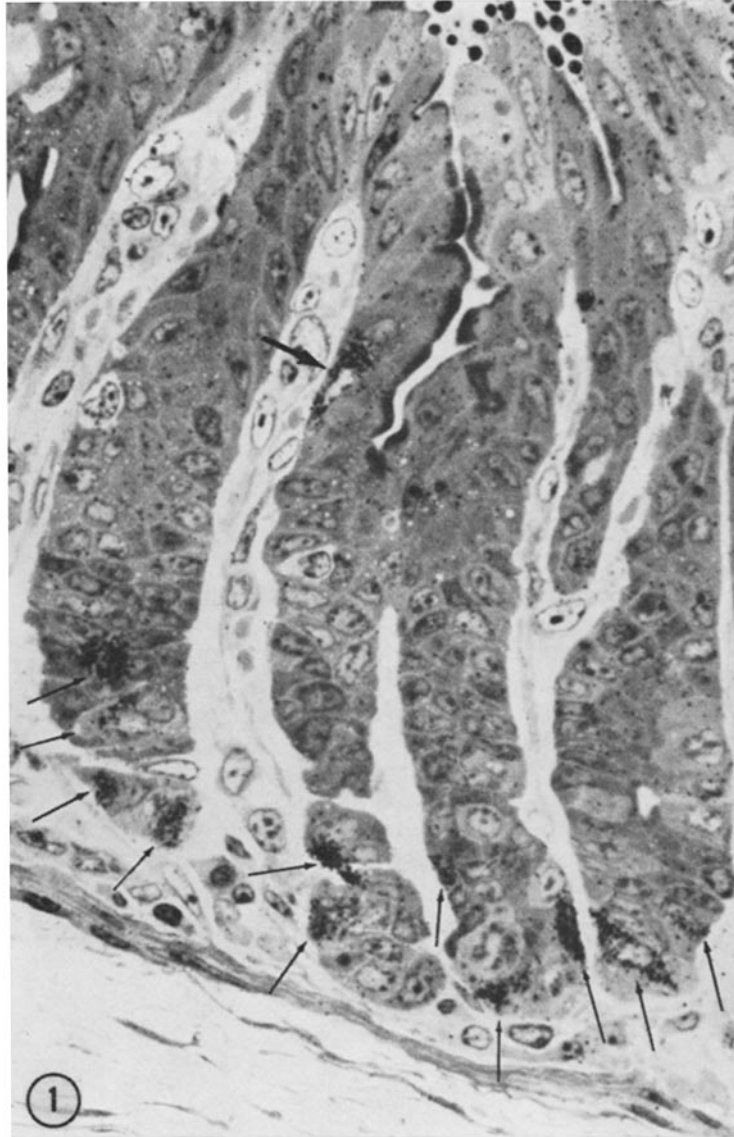


FIGURE 1 This thick mucosal section, stained with toluidine blue, was obtained from the distal portion of a mouse stomach. Note the prominence of labeled cells (arrows) in the lower portions of the glands and the essential absence of label in the pits and surface epithelium. The labeled cell above (heavy arrow) is located in the neck of a gland, just where the gland joins the base of a pit. The dark structures in the gastric lumen represent bacteria. Approximately  $\times 1060$ .

the granules in both endocrine (Figs. 2-9) and mast (Fig. 10) cells. Cells with few granules were only lightly labeled (Figs. 9, 11); heavy labeling was restricted to cells with numerous granules (Figs. 3, 4, 6, 7). Occasional endocrine cells exhibited grains over the nuclei which were more than  $0.2 \mu$  away from granules (Fig. 4).

Sections from mice injected with  $5\text{-HT-}^3\text{H}$  and

from tissues incubated with  $5\text{-HT-}^3\text{H}$  in vitro revealed heavy labeling of mast cells (and of the myenteric plexus). However, no labeling of gastric endocrine cells was observed, although the injection of smaller amounts of  $5\text{-HTP-}^3\text{H}$  was known to produce heavy labeling of these cells by light microscope radioautography (12).

Sections from control mice which had not been

injected or had received cold 5-HTP revealed no labeling.

#### DISCUSSION

The gastric endocrine cells, which resemble peptide-synthesizing endocrine cells (5), have been thought to be able to produce pharmacologically active amines and peptides, such as serotonin (5-HT) (12, 17-19), histamine (20-22), catecholamines (4, 5, 18, 19, 22), gastrin (3, 5, 23-25), and possibly glucagon (6, 26). Several studies in recent years have suggested that these cells represent more than one cell type (1-8). Forssmann and coworkers (6) have recently categorized rat gastric endocrine cells into five types according to their fine structure and have suggested a probable and separate endocrine function for each type. Only type 1, the "enteroserotonin" cell, was thought to represent the true enterochromaffin cell, which synthesizes serotonin. Glucagon, catecholamine, and gastrin synthesis were attributed respectively to three other cell types.

The present study correlates the fine structure of mouse gastric endocrine cells with their ability to synthesize serotonin from exogenous 5-HTP, the immediate precursor in the biosynthesis of 5-HT (9, 10). The design of this study resulted from a previous experience (11, 12) which demonstrated that by 2 hr after the intravenous injection of radioactive 5-HTP into mice, label was no longer present in the plasma; the gastric mucosa had progressively accumulated high levels of radioactive 5-HT, which was only slowly released; and the fixation and embedding procedures retained labeled 5-HT while washing out other labeled substances, which were quantitatively identified. Thus, silver grains in the present study were thought to reflect the presence of only 5-HT and to be representative of bound mucosal 5-HT synthesized from the injected 5-HTP.

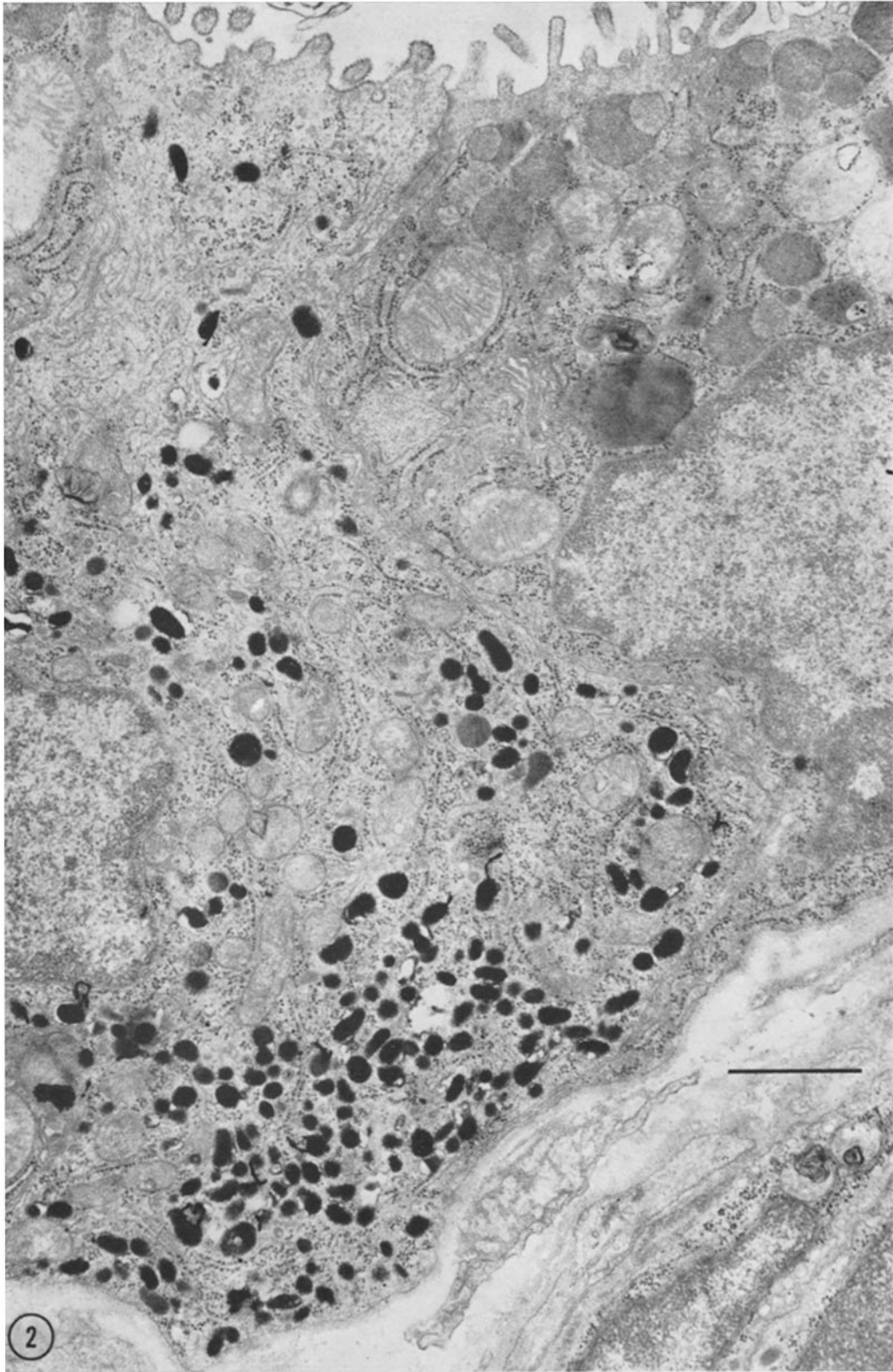
The present study shows that all morphologic types of mouse gastric endocrine cells exhibit a similar facility to incorporate exogenous 5-HTP

and to convert it to 5-HT which is bound intracellularly. The different densities of silver grains observed over endocrine cells suggest that individual endocrine cells indeed vary in their ability to synthesize and/or bind 5-HT, at least at a given time; however, such variations were not reflected by differences in fine structure, with the exception that endocrine cells with few granules always contained little newly-synthesized 5-HT. This study, however, cannot assess the extent to which individual endocrine cells normally synthesize 5-HT *in vivo*. This amount depends not only on the cell's facility to convert 5-HTP to 5-HT, which probably reflects its content of the enzyme aromatic L-amino acid decarboxylase (27), but also on its exposure to exogenous 5-HTP and/or on its ability to hydroxylate tryptophan. Such information is not known. Under the conditions of the present experiment, for example, adrenal chromaffin cells synthesize and bind large amounts of 5-HT which is slowly released (11, 12); however, the adrenal medulla normally contains only a little 5-HT (28), probably because of its inaccessibility to 5-HTP or a limited ability to hydroxylate tryptophan (12). Since human carcinoid tumors can readily hydroxylate tryptophan (29), normal gastrointestinal endocrine cells from which these tumors are derived probably possess this ability as well. However, any differences in the ability of individual endocrine cells to hydroxylate tryptophan and thereby to synthesize serotonin would not be detected in the present study.

Despite these limitations, the demonstration in the present study that all morphologic types of gastric endocrine cells exhibit a comparative facility to synthesize 5-HT supports the previous contention of Gershon and Ross (12) and of other investigators (30-35) that gastric endocrine cells represent a single cell type, at least in respect to serotonin metabolism—that the argyrophil or argentaffin reactivity of these cells merely reflects their amine content at a given time. After the intravenous injection of 5-HTP into mice, Gershon

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FIGURE 2 This weakly labeled endocrine cell, located near the base of a pyloric gland, corresponds to the type 1 or "enteroserotonin" cell of Forssmann et al. (6) in the rat. Its principal morphologic features include the presence of dense pleomorphic granules, usually with a closely applied membrane, and its extension to the glandular lumen. Although most investigators have described such granules in enterochromaffin cells (3, 5-8), not all have thought that these cells extend to the lumen (5). Scale line for Figs. 2-11 = 1  $\mu$ . Approximately  $\times 21,000$ .



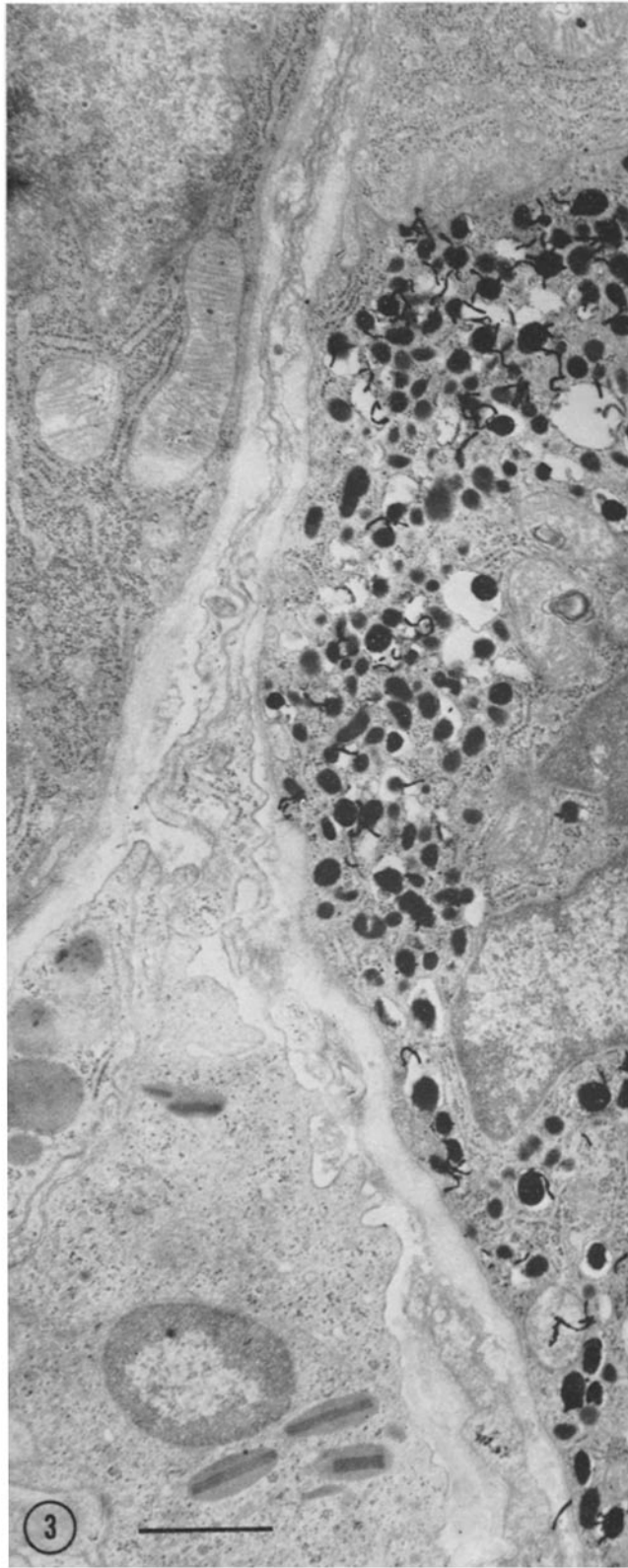
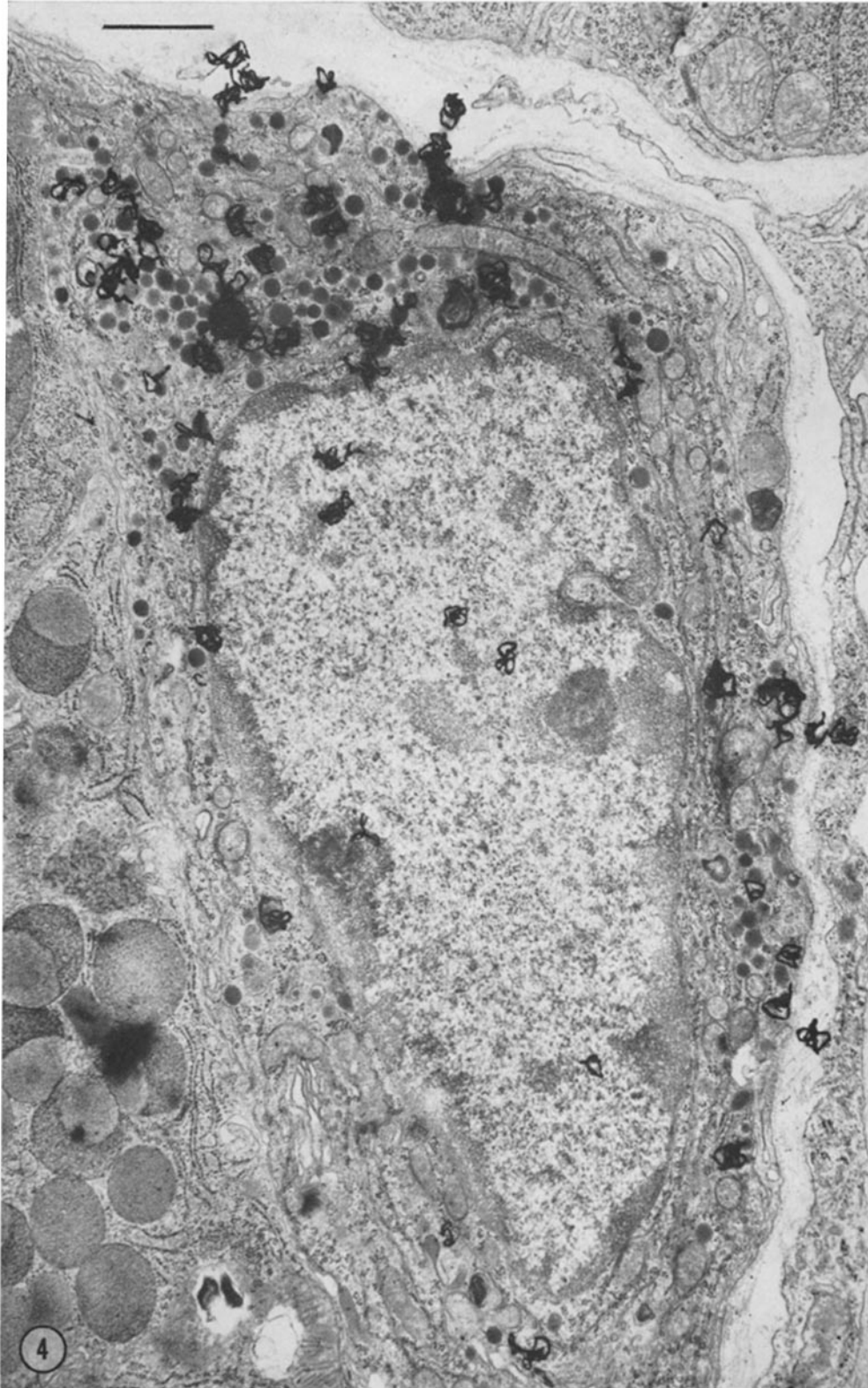


FIGURE 3 This heavily labeled type 1 (enteroserotonin) cell was located near the cell in Fig. 2 in the same section. Despite their morphologic similarities and subjection to identical experimental procedures, these two neighboring cells demonstrated a marked difference in their apparent content of newly synthesized 5-HT. Approximately  $\times 18,000$ .



**FIGURE 4** This heavily labeled cell, located in the lower portion of a pyloric gland, resembles the type 2 or intestinal A cell of Forssmann et al. (6). Note the uniform, round, electron-dense granules and the abundant profiles of endoplasmic reticulum and Golgi complex. The significance of the nuclear labeling, observed over other types of endocrine cells as well, is not known. Approximately  $\times 17,000$ .

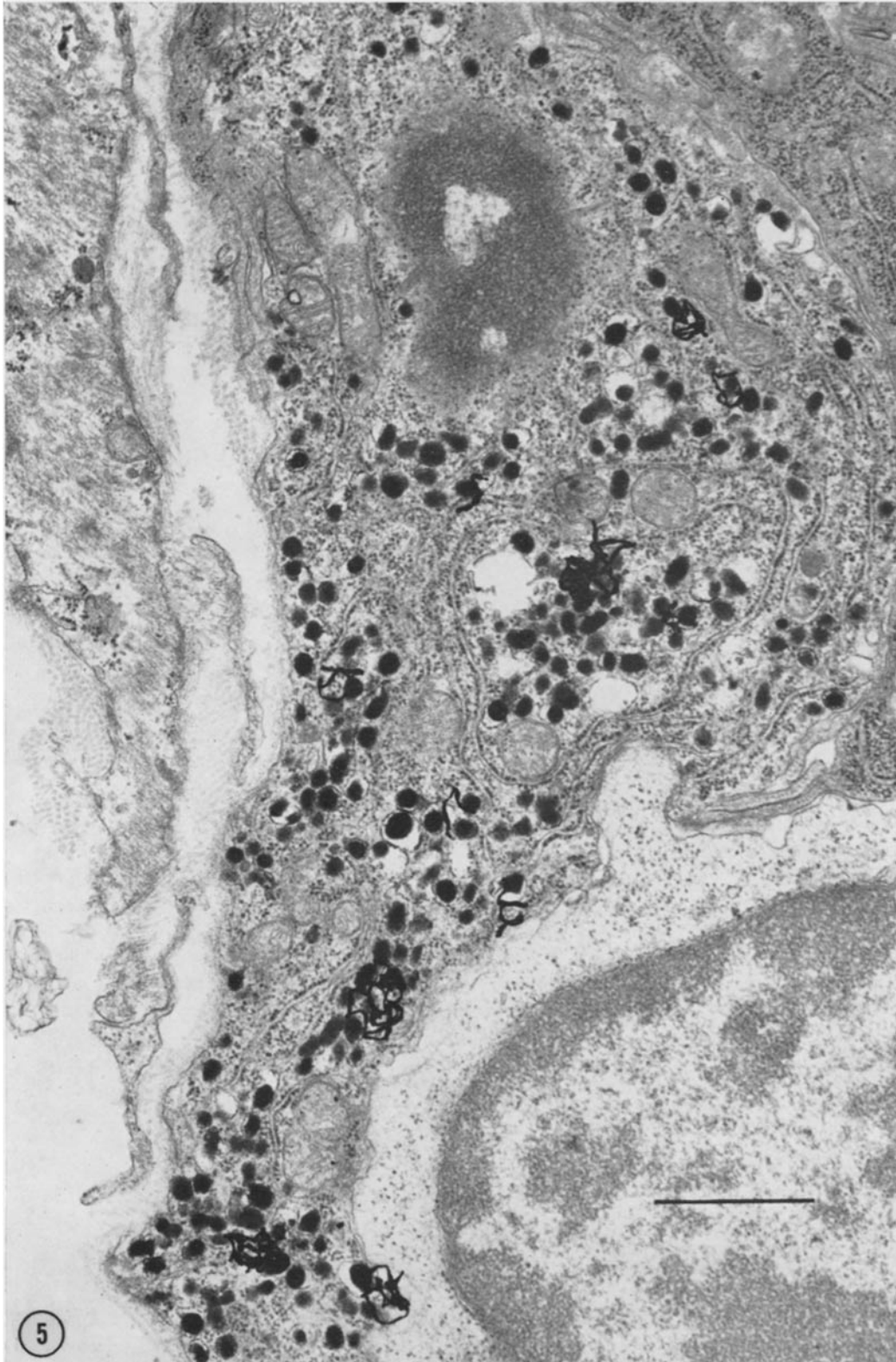


FIGURE 5 This moderately labeled cell, located near the base of a pyloric gland, represents either a type 1 or 2 endocrine cell (6). Note the association of silver grains with the granules. The adjacent pale cell below is a lymphocyte. Approximately  $\times 24,000$ .



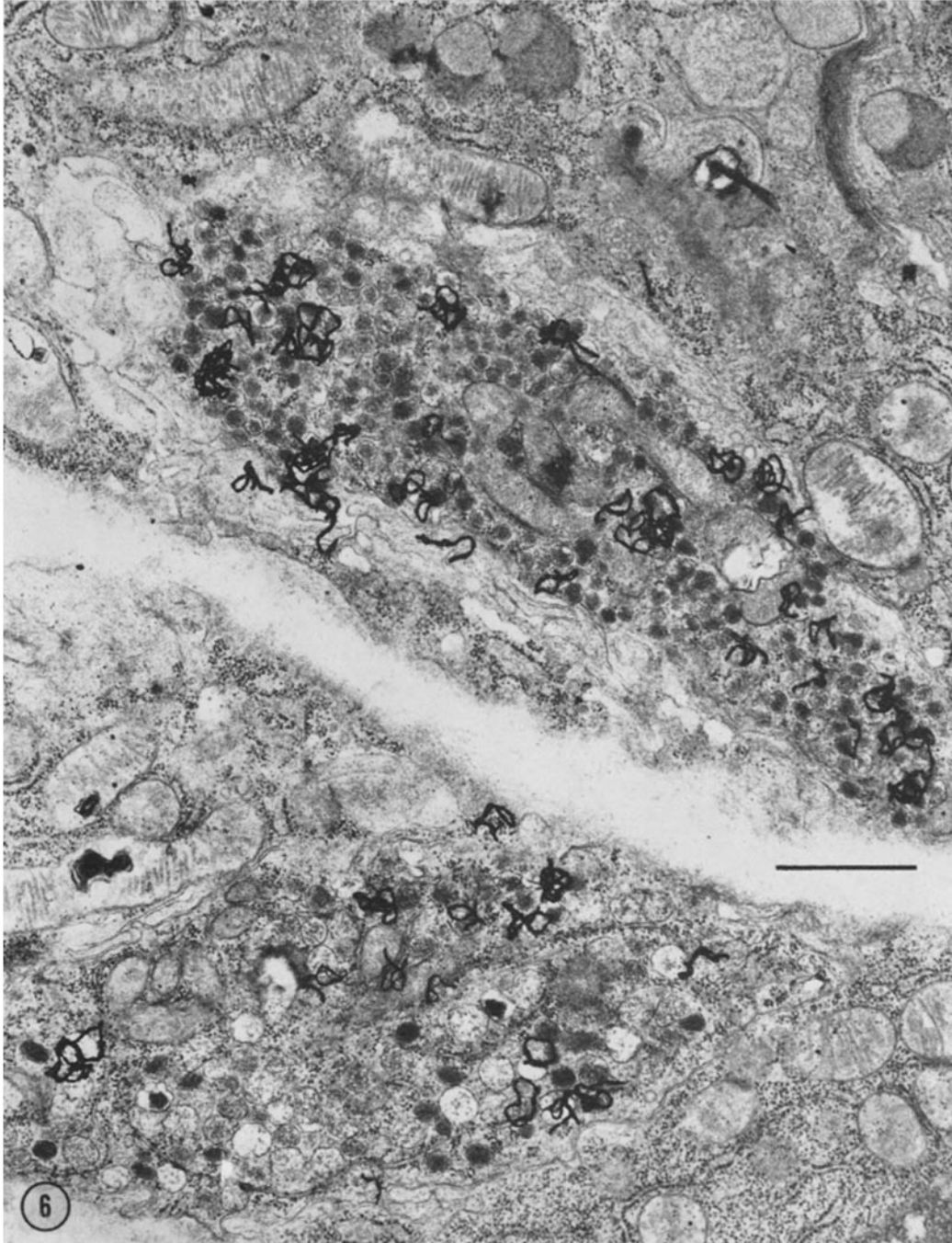


FIGURE 6 These two heavily labeled endocrine cells were located in the lower portion of a pyloric gland. The cell above contains numerous granules characteristic of the type 3 or intestinal D cell (6). They appear round, regular, and homogeneous and their granular matrix is usually separated from their membranes by a narrow lucent space. The cell below represents a type 5 cell, thought to be the source of gastrin (6). Its round granules are fairly uniform in size, but the appearance of their contents varies extensively. Some appear electron-lucent while others are as dense as those in type 1 or type 2 cells. Approximately  $\times 20,000$ .

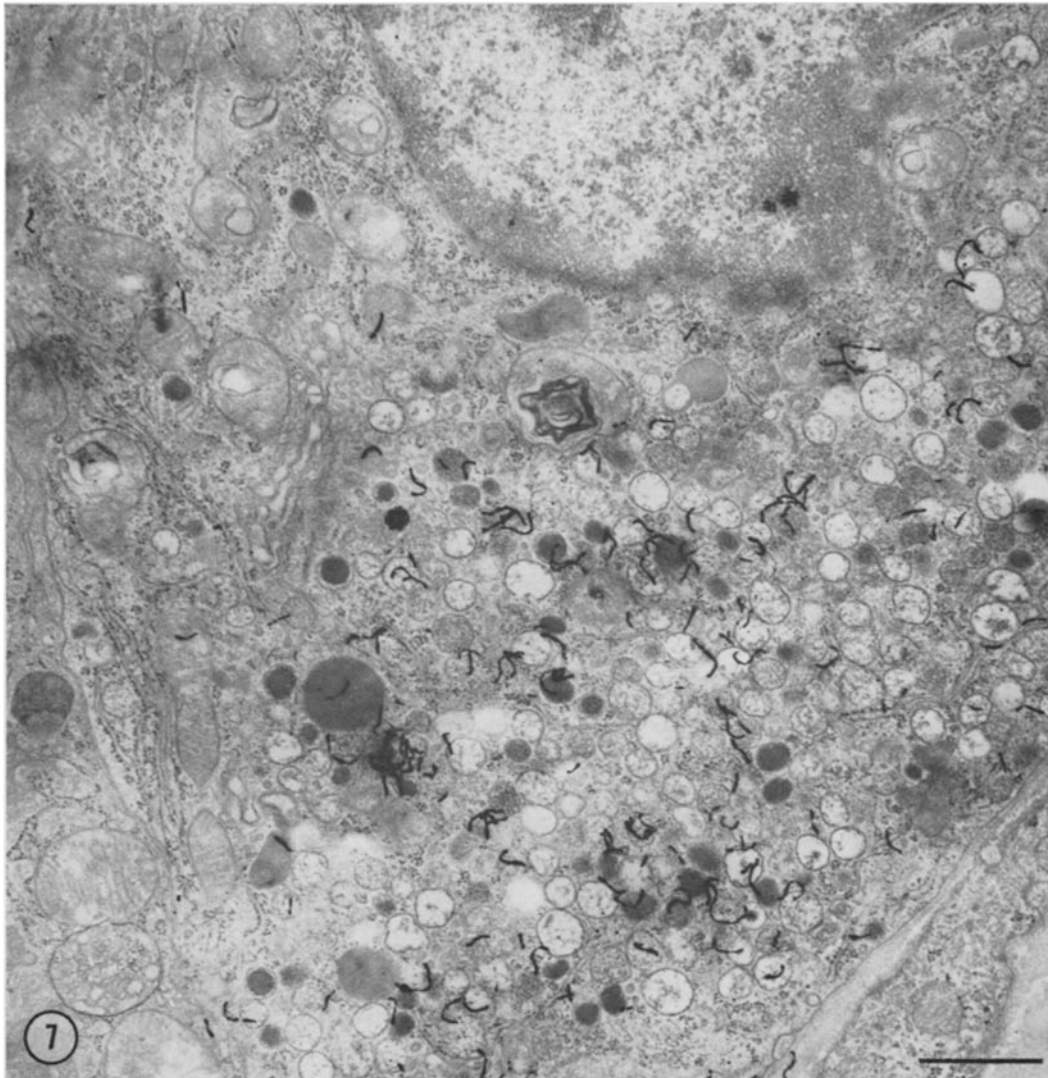


FIGURE 7 This heavily labeled type 5 (gastrin) cell was located in the pyloric glands. Note the many round, membrane-enclosed granules with contents of varying electron density; the numerous free ribosomes; moderate profiles of rough endoplasmic reticulum which form small ergastoplasms; and the paracnuclear Golgi profiles. The silver grains are associated with the granules. Approximately  $\times 16,000$ .

and Ross first observed newly synthesized 5-HT predominantly within nonargentaffin argyrophils, but at later periods after injection, a progressively larger percentage of labeled cells gave positive argentaffin reactions (12). (By 2 hr after injection, the period selected for the present study, approximately half of the labeled cells were argentaffin.) They suggested that endocrine cells which contained little 5-HT and were therefore argentaffin-

negative were capable of binding most of the newly synthesized 5-HT, and as such cells accumulated more 5-HT, they became argentaffin-positive but depleted their available binding sites for additional 5-HT. This hypothesis was consistent with the previous knowledge that the intestine's capacity to synthesize 5-HT from 5-HTP was virtually unlimited, but that its capacity to bind 5-HT was certainly limited (36). Hammarström

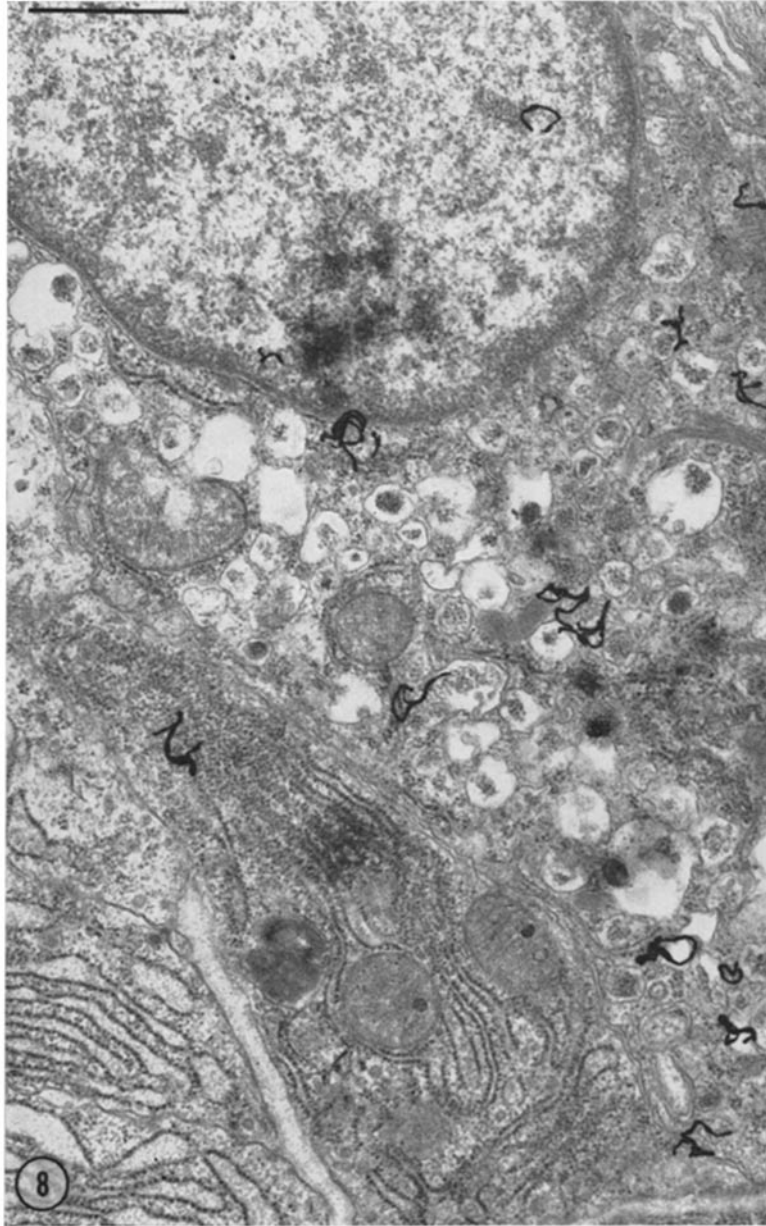


FIGURE 8 This moderately labeled cell, located among chief cells in a gastric gland in the corpus, resembles the type 4 or enterocatecholamine cell (6). It contains granules of various sizes, the larger ones of which contain small amounts of eccentric granular substance, so that they often appear empty on section. Approximately  $\times 21,000$ .

and coworkers (19) and Håkanson and coworkers (4) also noted this apparent paradox—that after exogenous 5-HTP administration newly synthesized 5-HT was accumulated primarily within

cells which had contained no or little endogenous 5-HT. Håkanson et al (4), however, considered these cells to be a separate type of gastric endocrine cell and called them “enterochromaffin-like”

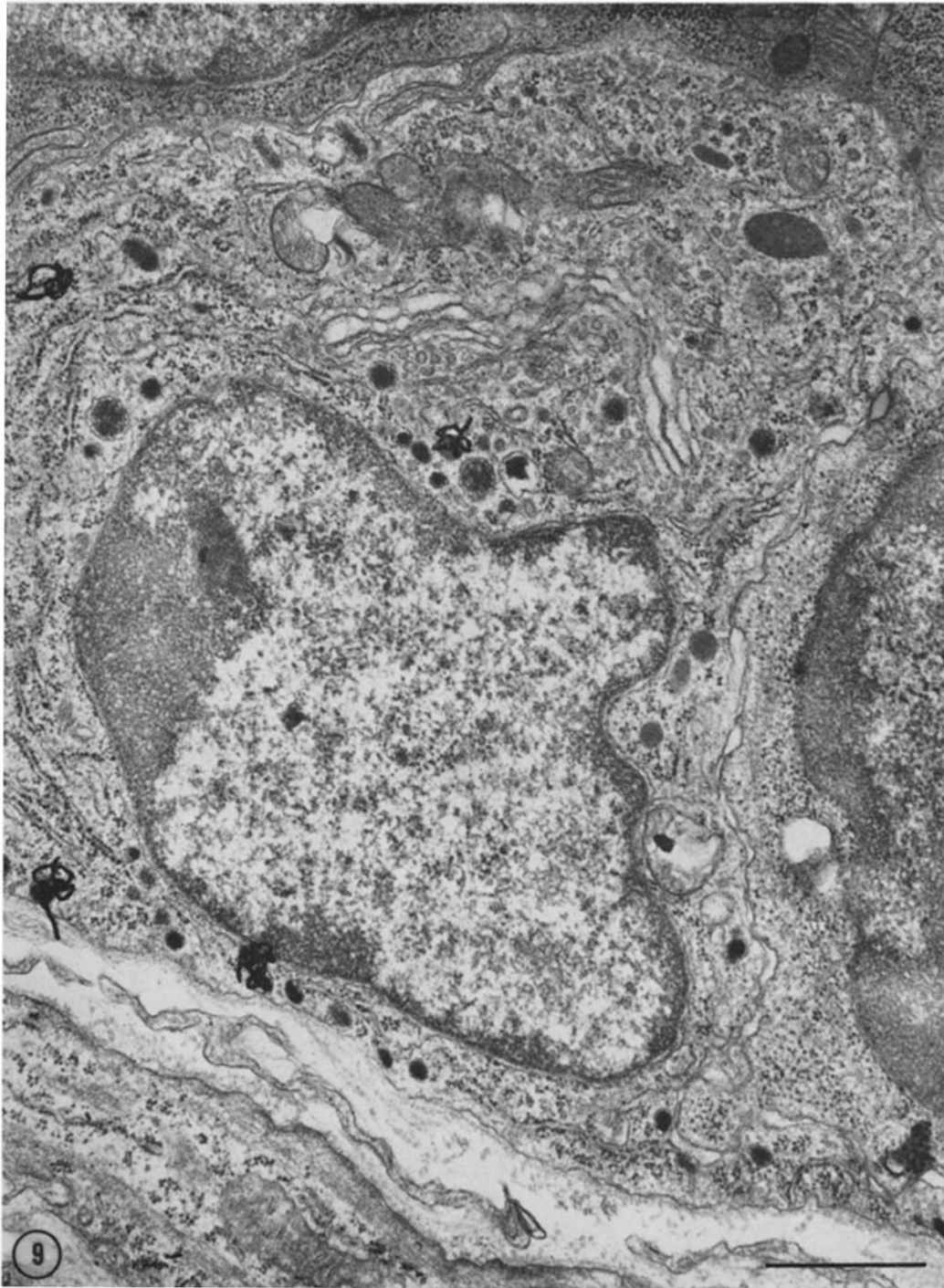


FIGURE 9 Endocrine cells with few granules were only weakly labeled and were usually difficult to classify on a morphologic basis. This one, with numerous profiles of ribosomes, rough endoplasmic reticulum, and Golgi complex, was located near the base of a pyloric gland. Approximately  $\times 23,000$ .

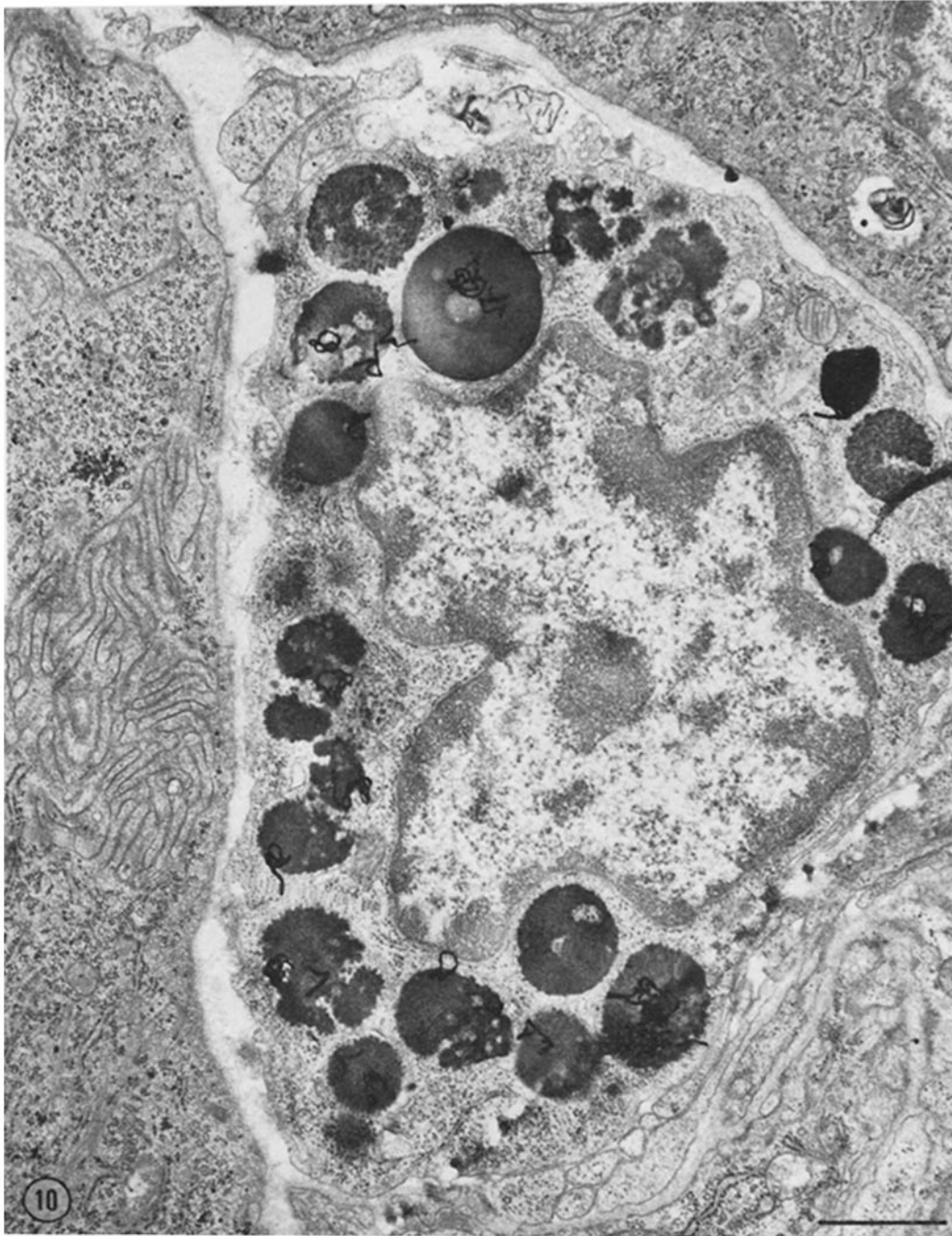


FIGURE 10 Mast cells were the only other type of mucosal cell which was labeled. Note the association of silver grains with granules of different morphologic appearance. Approximately  $\times 18,000$ .

cells. Singh (34) has recently produced strong evidence in the guinea pig duodenum that an argyrophil granule can be derived from an argentaffin granule by depleting the latter's 5-HT content. Thus, the present study and the previous experiments of Gershon and Ross (11, 12) and of Singh (34) strongly support the original suggestion of Vialli and Erspamer (see reference 37) that some argyrophil but nonargentaffin cells can be referred to as preargentaffin or preenterochromaffin—that only when they have accumulated sufficient amounts of an amine, such as 5-HT, will they be able to reduce silver and thereby give a positive argentaffin reaction (38). These studies, however, cannot exclude the possibility that some argyrophils never accumulate enough 5-HT to produce positive argentaffin and chromaffin reactions.

The suggestion of our present and earlier (12) studies that the fine structure of gastric endocrine cells cannot be correlated with their content of 5-HT and, thereby, with their argentaffin, chromaffin, or fluorescent (39) reactivity differs from the position of other investigators who have suggested a specific fine structure for the enterochromaffin cell (3, 5–8). These investigators have generally proposed the type 1 cell (Figs. 2, 3) as representing the true enterochromaffin cell, a cell with electron-dense, pleomorphic granules (3, 5–8). In the present study, however, the amount of newly synthesized 5-HT accumulated by such cells was extremely variable and comparable to that accumulated by other morphologic types. Some of these cells were heavily labeled while others appeared unlabeled. If only these cells represented the true argentaffin cells, then they should have accumulated little of the newly synthesized 5-HT (4, 12, 19), while other morphologic types, thought to represent nonargentaffin argyrophils (3, 5–8), should have accumulated more (4, 12, 19).

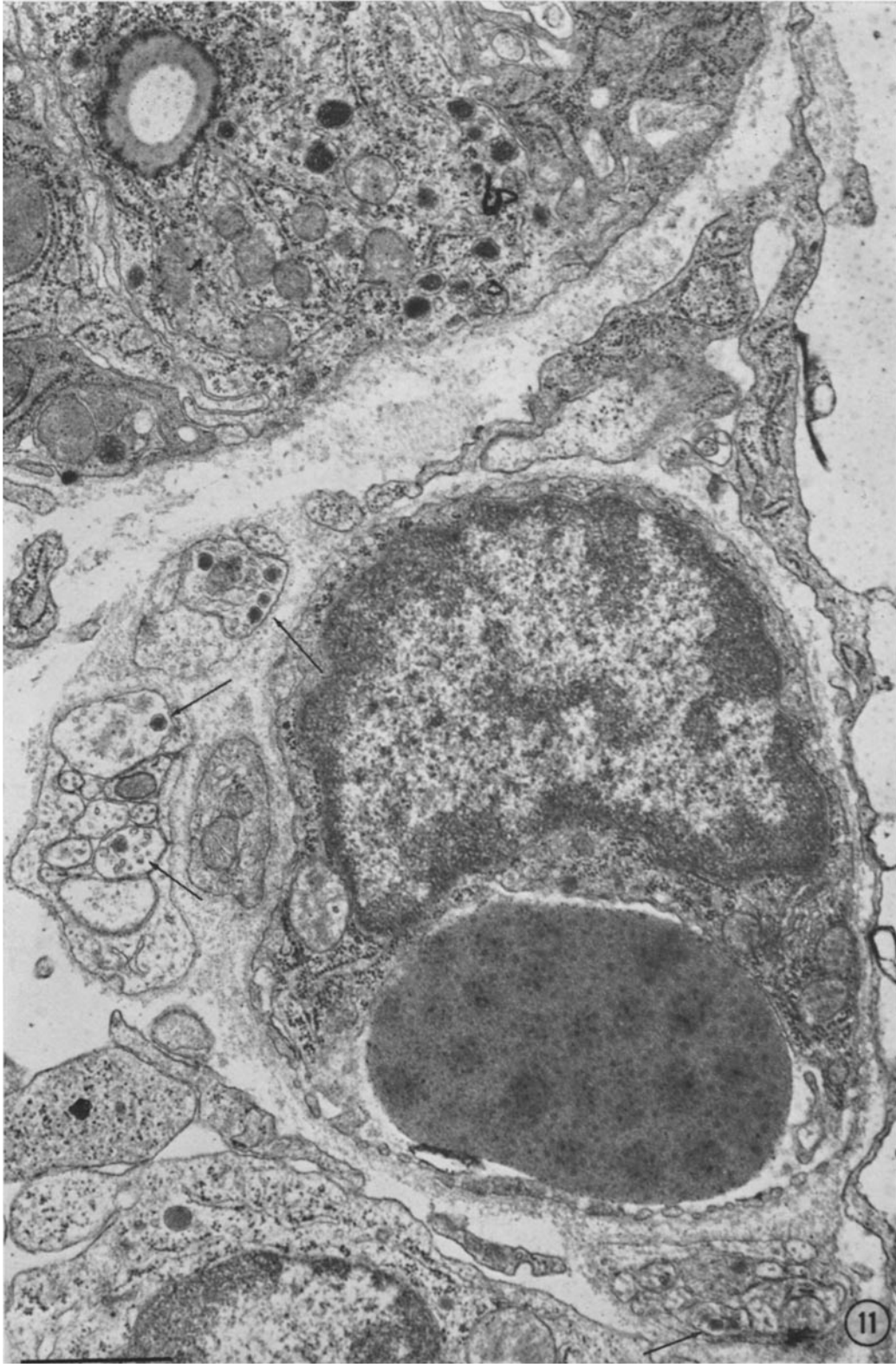
Although the fine structure of mouse gastric endocrine cells could not be correlated with their ability to synthesize 5-HT, it is still possible that differences in their fine structure may reflect differ-

ences in their metabolism of other amines and peptides, such as gastrin, catecholamine, and possibly glucagon, as previously suggested (3, 5–8, 26). Thus, although the gastric endocrine cells have generally been thought to represent either one cell type or two or more types, it is also possible that they represent only one cell type in respect to serotonin metabolism, but that they differ in their metabolism of other amines and peptides. In recent abstracts Ito and coworkers (40) have suggested the existence of at least two types of mouse gastric endocrine cells on the basis of their fine structure and their rates of 5-HTP incorporation *in vitro*; and Forssmann and coworkers (41) have suggested that their type I rat gastrointestinal endocrine cell, the "enteroserotonin cell," incorporates 5-HTP-<sup>3</sup>H at a different rate than the other types of endocrine cells.

The association of the silver grains with the granules in the present study supports the suggestion of previous investigators (17, 42) that the 5-HT in enterochromaffin cells is contained within the granules. Although the granules and their electron-opaque matrix seem to be formed within the Golgi complex, the site and manner of the 5-HT incorporation are probably different. The electron-opaque matrix within granules does not represent 5-HT; enterochromaffin cells depleted of their 5-HT still exhibit opaque granules (43). It is unlikely that the granules contain aromatic L-amino acid decarboxylase. Although this enzyme has been demonstrated throughout the glandular mucosa of the stomach (18), its subcellular compartmentalization has not been established. The enzyme has been localized to the soluble fraction of the cytoplasm in chromaffin cells of the adrenal medulla (44) and in adrenergic axons (see 45 for references), although a loose association with adrenergic granules has been claimed (46). If aromatic L-amino acid decarboxylase is a soluble cytoplasmic enzyme in gastric endocrine cells as well, then 5-HT is probably synthesized from 5-HTP in the cytoplasm and then transported into the granules where it is bound. In the present

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**FIGURE 11** The granulated vesicles (arrows) within mucosal axons were not labeled. Since these axons are thought to contain aromatic L-amino acid decarboxylase, the absence of labeling probably reflects an inability of these cells to take up exogenous 5-HTP or more likely to bind the synthesized 5-HT. These axons surround a fenestrated capillary. The endocrine cell above, which was located near the base of a pyloric gland and contains few granules, is weakly labeled. Approximately  $\times 23,000$ .



study, the labeled cells must have been able to take up exogenous 5-HTP, decarboxylate it to 5-HT, and then pump the newly synthesized 5-HT into the granules where it was bound. All morphologic types of gastric endocrine cells were apparently alike in these characteristics. As noted by others (38), no gastric endocrine cells in our studies were labeled by exogenous 5-HT-<sup>3</sup>H. Thus, their plasma membranes, unlike those of mast cells, appear to be much more permeable to exogenous 5-HTP than to 5-HT.

The significance of the occasional nuclear labeling observed in the present study (Fig. 4) is not clear. Although our wash-out studies (11, 12) demonstrated the retention of 5-HT within cells they cannot exclude the possibility of some artifactual intracellular movement during tissue preparation. However, it is also possible that some 5-HT synthesized within the cytoplasm may be able to penetrate the nuclear membrane and be bound by nuclear substance. It is interesting to note that Singh (34) has stained the chromatin of guinea pig duodenal argentaffin cells with the argentaffin reaction, and has, thereby, raised the possibility of a nuclear presence of 5-HT.

The gastrointestinal endocrine cells remain a fascinating but enigmatic group of cells. Despite the increasing attention that they have been receiving from numerous investigators, a full understanding of their ontogenesis, relationships, biochemistry, and physiology has yet to be achieved.

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