THE FINE STRUCTURE OF THE GASTRIC MUCOSA IN THE BAT

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ABSTRACT

A description of the cytology of the gastric mucosa is presented based upon an electron microscopic investigation of the bat stomach. The fine structure of the various cell types in this species is fundamentally similar to that of the corresponding cell types of other mammals, but the relative cell numbers and distribution are somewhat different. (*a*). *The surface mucous cells* are identified by their superficial location and by the character of their dense secretory granules. (*b*). *The mucous neck cells* are distinguished by a characteristically different appearance and distribution of their mucous granules, and by their varied shape and their location between parietal cells. (*c*). *The parietal cells* are very large and have unusually prominent secretory canaliculi and an extraordinary number of large mitochondria. (*d*). *The chief cells* are found at the base of the gastric glands and are similar in their fine structure to other zymogenic cells. They contain many large zymogen granules and have an extensively developed granular endoplasmic reticulum. The latter is sometimes aggregated in unusual, hexagonally packed straight tubules, each with twelve longitudinal rows of ribosomes uniformly spaced around its circumference and with the rows of ribosomes in precise register with those of adjoining tubules. (*e*). *Argentaffin cells* lodged between other cell types vary sufficiently in the structure of their mitochondria and the character of their specific granules to suggest that they are of more than one kind. The majority are at the base of the epithelium but some extend to the lumen and bear microvilli on their free surface.

INTRODUCTION

This study undertakes a description of the fine structural characteristics of the various cell types of the bat stomach in the hope that a detailed cytological analysis of this species may lead to a better general understanding of the structure of the mammalian gastric mucosa. The present observations will provide the morphological base line for future experimental work on the fine structural alterations associated with changes in the physiological state of the mammalian stomach.

Although the bat can scarcely be considered a representative mammal, this species has proved to be unusually favorable for several reasons. Good cytological preservation can be obtained more reproducibly in the bat than in some other species examined. The small size of the stomach and the thinness of its mucosa facilitate fixation and the distribution of cell types enables the investigator to find examples of all types within a block of relatively small size. Unlike the laboratory rodents, bats are not continuous feeders and thus offer a better opportunity to study this tissue in various states of digestive activity ranging from that of the recently fed active animal to the long fasted hibernator. Preliminary examination of several other species including man, beaver, rabbit, pig, guinea pig, cat, dog, and monkey, and a

perusal of published accounts of the rat (18) and the mouse (12, 13) provided assurance that the fine structure of the various cell types in the gastric mucosa is fundamentally similar in a wide range of animal species. The principal differences among species appear to be in the relative numbers of the cell types and in their regional distribution rather than in their submicroscopic structure. Thus the description of the cell types in the bat stomach presented here can be considered to apply with only very minor modifications to the corresponding cell types in other mammalian species.

MATERIAL AND METHODS

Adult little brown bats, *Myotis lucifugus lucifugus*, were collected throughout the year in New Jersey and Massachusetts. The animals were killed by cervical dislocation while hibernating or after arousal. The abdomen was opened and cold fixative was injected into the lumen of the stomach *in situ*. The whole organ was then removed and placed into a vial containing additional cold fixative. After 15 minutes the stomach was cut into regional segments, then into 1 mm blocks, and fixation continued for about 1 hour. In other instances stomachs were rapidly removed from the animal and portions were cut with a razor blade into small blocks in a drop of cold fixative on a wax plate and then placed in a vial containing about 1 ml of cold fixative. The two methods gave equally good results with this material. The fixatives used consisted of 1 per cent osmium tetroxide buffered with Veronal acetate or 1.33 per cent osmium tetroxide buffered with *s*-collidine (1). The Veronal- and collidine-buffered fixatives contained 0.25 M sucrose. All fixatives were buffered between 7.1 and 7.5 pH units. After fixation the tissues were rapidly dehydrated in a graded series of cold (0° to −10°C) ethanol and, when absolute alcohol was reached, the fixing vials were brought slowly to room temperature in a beaker of cold water. Dehydration was completed with additional changes of absolute ethanol at room temperature over a period of 1 to 2 hours.

Methacrylate embeddings were made according to the procedure described in an earlier paper (15) and Epon embeddings by the method described by Luft (22). Sections showing pale yellow to gold interference colors were picked up on carbon- and celloidin-coated grids, or on uncoated grids and stained with either uranyl acetate or lead hydroxide according to Watson (35, 36) or with the cacodylate-lead stain developed by Karnovsky (16). Electron micrographs were made with an RCA EMU 3B, 3D, or 3F, or with a Siemens Elmiskop Ib electron microscope.

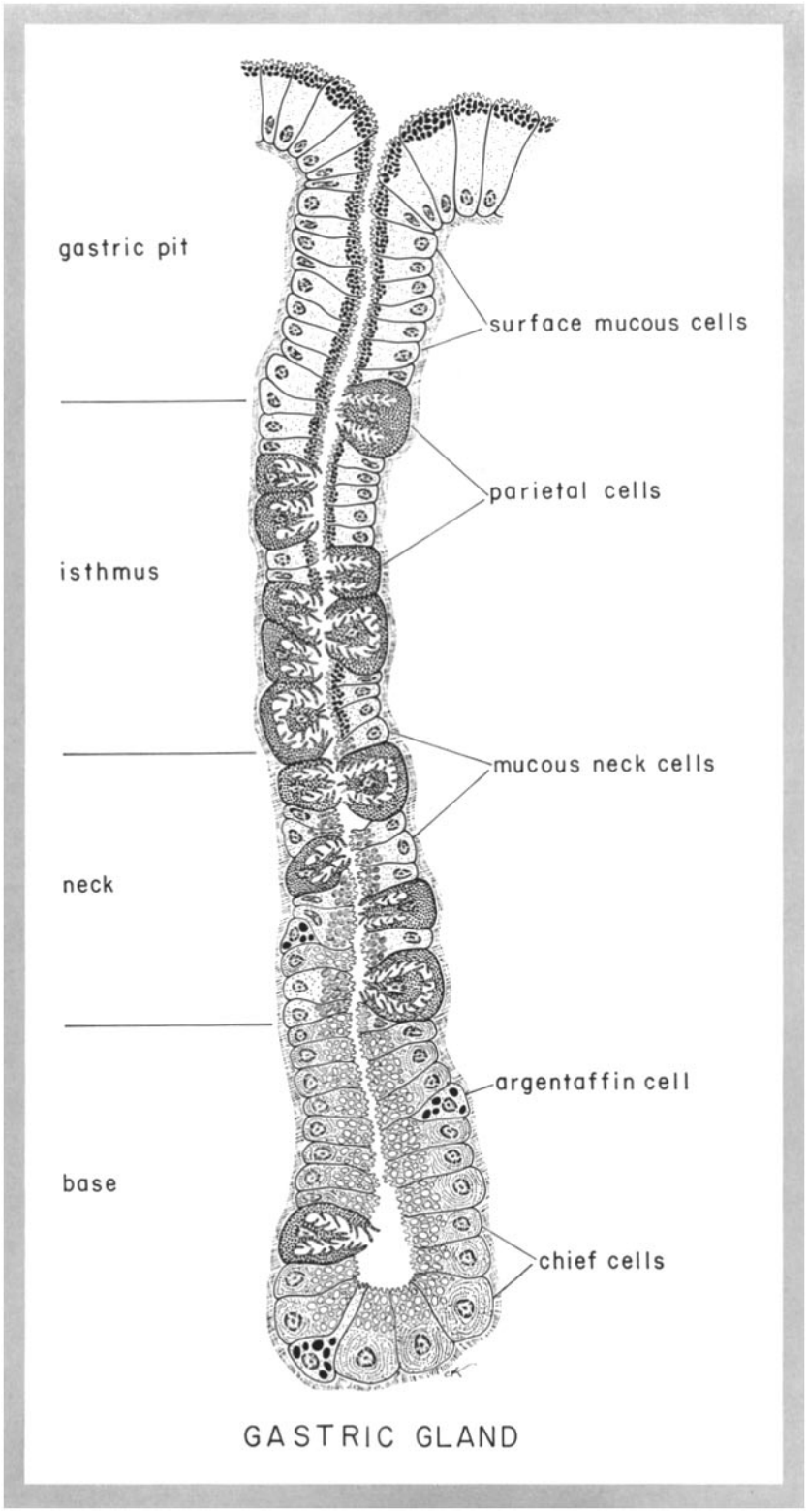
Preparations for light microscopy were made after formalin, Zenker's, Bouin's, or acrolein fixation and paraffin embedding. Sections were stained with toluidine blue, hematoxylin and eosin, Cason's trichrome stain, periodic acid–Schiff reaction (PAS) or Gomori's methenamine silver (2). The most useful preparations for light microscopy were found to be thin sections of large pieces of stomach fixed in osmium tetroxide and embedded in Epon as for electron microscopy. Pieces of tissues as large as a half of the bat stomach could be sectioned with an ultramicrotome at 0.5 to 2 μ using a glass knife. These plastic sections affixed to glass slides were then stained with methylene blue and Azure II as suggested by Richardson *et al.* (27) or with toluidine blue according to the method described by Trump *et al.* (33). A mixture which stains structures more differentially was obtained by using four parts of 1 per cent toluidine blue in 1 per cent Borax and one part of 1 per cent pyronine. This mixture stains the osmium-fixed, plastic-embedded tissue sections in 1 to 2 hours at room temperature or in a few seconds if heated on a hot plate. No special pretreatment of the sections is required, but after washing off the excess stain with water a few seconds' dip in absolute ethanol results in better differential staining. The section is then air-dried and mounted under a coverslip with Permount. This simple method usually gives extremely good cytological definition but unfortunately the degree of staining achieved is somewhat variable from tissue to tissue and for sections of different thickness.

GENERAL ORGANIZATION OF THE GASTRIC MUCOSA

The general shape of the bat stomach resembles that of the human and the same terms will be used here to designate its various regions, namely, the cardia, fundus, corpus, and pylorus. The gross dimensions of the empty bat stomach are about 7 mm in its greatest length from the fundus to the pyloris and about 4 mm in width across the

FIGURE 1

A diagram of a simple tubular gastric gland from the fundus or corpus region of a bat stomach. The designations employed here are adapted after those used by Stevens and Leblond (32) for a similar diagram of the rat gastric gland.


gastric pit
surface mucous cells
parietal cells
isthmus
mucous neck cells
neck
argentaffin cell
base
chief cells
GASTRIC GLAND

widest part of the corpus. The stomach wall is about 300 to 600 μ in thickness, of which approximately half is the mucosa. All of the mucosa is glandular. The fundus and the corpus contain the gastric glands which are made up of all the various specialized cell types while the cardiac and pyloric glands contain only mucus-secreting cells and a few *argentaffin* cells.

The mucosa of the empty stomach is thrown up into several longitudinal folds or plicae which are effaced upon filling the organ. The mucosa of the distended stomach is of smooth contour. The thickness of the mucosa shows some regional differences, the gastric glands being somewhat shorter in the fundic region and longer in the pyloric area. The cardiac glands in the bat stomach are limited to a very small zone surrounding the esophageal orifice. The area occupied by pyloric glands is more extensive and constitutes a segment extending about 2 mm cranial to the gastroduodenal junction. The remainder of the gastric mucosa contains glands with a mixed cell population but the proportions of the cell types vary somewhat. The *chief* or *zymogenic* cells are most abundant in the fundus and gradually diminish in number farther distal, becoming infrequent or absent in the lower portion of the corpus. The *parietal* or *oxyntic* cells are present in both the fundus and corpus and are most numerous just cranial to the pyloric region.

In the description of the cytology of the mucosa which is to follow, we will adhere to the terminology established by Stevens and Leblond (32) for the glands of the rat stomach. A typical gastric gland containing all the specialized cell types as they would appear in the upper part of the corpus is presented diagrammatically in Fig. 1. A representative vertical section of the entire thickness of the mucosa is shown in a low power photomicrograph in Fig. 2. Other specimens prepared by special techniques that reveal certain cell types to better advantage are presented in Figs. 3 to 6. At least five cell types are distinguishable: (1) the *surface mucous cell*, (2) the *parietal cell*, (3) the *mucous neck cell*, (4) the *chief cell*, and (5) the *argentaffin cell*. Each of these will be described under separate headings in the following section.

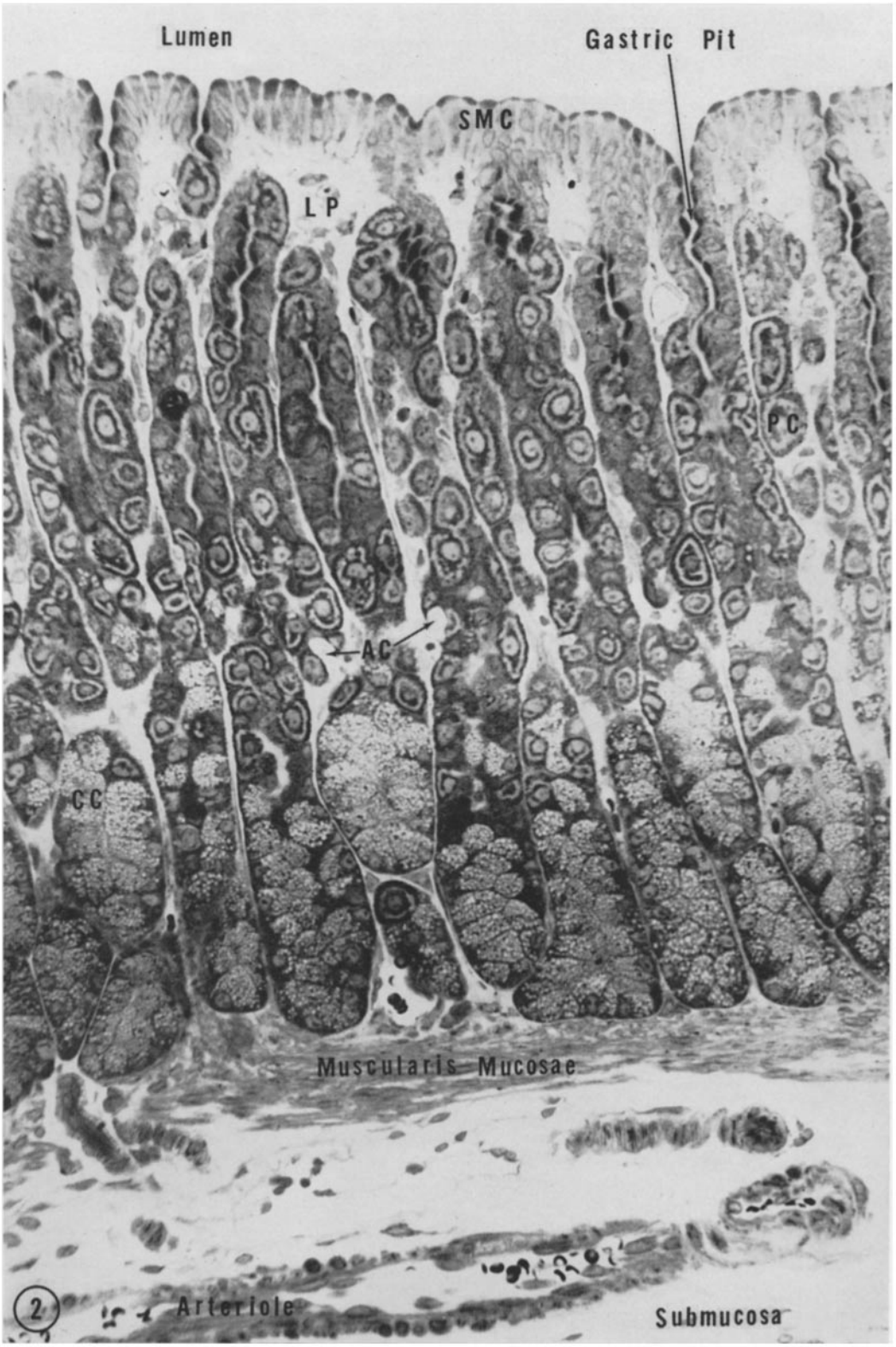
SPECIAL CYTOLOGY OF THE GASTRIC MUCOSA

The Surface Mucous Cell

A single layer of columnar surface mucous cells lines the gastric lumen and extends into the gastric pits. These cells are also found in the isthmus of the gastric glands where they intermingle with parietal cells. A schematic representation of the fine structure of this cell type is shown in Fig. 7 and illustrated in electron micrographs in Figs. 8 and 9. Surface mucous cells range from cuboidal to columnar in shape and have a few short microvilli on their free surface. The membrane limiting the microvilli has numerous, exceedingly fine, filamentous projections from its surface which impart a furry appearance to the surface. This appearance of the membrane is revealed more distinctly if sections are first stained with uranyl

FIGURE 2

A photomicrograph of an osmium tetroxide–fixed and Epon-embedded section of bat gastric mucosa stained with methylene blue and pyronine. Surface mucous cells (*SMC*) with their densely stained apical granules may be seen on the free surface and along the walls of the gastric pits. Large parietal cells (*PC*) with their clear unstained secretory canaliculi are found in the neck and isthmus regions. In many cells the secretory canaliculi appear to separate almost completely the outer rim of dense cytoplasm from the inner mass containing the nucleus. The intense staining of the cytoplasm is apparently due to the presence of numerous, closely packed mitochondria. Mucous neck cells are present between the parietal cells in the neck region, but they are not clearly demonstrated in this preparation. The bases of the gastric glands are occupied almost exclusively by chief cells (*CC*) containing large numbers of zymogen granules which are unstained. Argentaffin cells (*AC*) are not specifically stained in this preparation, but they may be recognized by their pale and sometimes granular cytoplasm and their location, intercalated between other cells near the basement membrane. In the submucosa, an arteriole and a lymphatic vessel may be seen beneath the muscularis mucosae. × 450.


Lumen
Gastric Pit
SMC
LP
PC
AC
CC
Muscularis Mucosae
2
Arteriole
Submucosa

acetate and then with lead. These fine filaments evidently correspond to the "antennulae microvillares" described by Yamada (37) in gall bladder epithelium, and to the filamentous surface coat observed by Peachey (26) in the epithelium of the toad bladder.

The lateral plasmalemmata of the surface mucous cells may be straight near the apical border but are elaborately interdigitated toward the base. The membranes of adjoining cells are closely apposed and prominent terminal bars are always present near the luminal surface. Desmosomes are found at irregular intervals elsewhere along the lateral surfaces. The smooth cell membrane at the base of the cell rests on a thin, amorphous basement membrane that is reinforced with closely associated, thin collagen fibrils. A similar basement membrane underlies all gastric epithelial cells.

The nucleus is located toward the base of the cell and may be very irregular in shape. It contains a compact, centrally located nucleolus. In some preparations the material identified as chromatin is concentrated on the inner aspect of the nuclear envelope where it forms a dense band of variable thickness interrupted by small areas of rarification adjacent to the nuclear pores. These light areas of nucleoplasm draw attention to the presence of the pores, and suggest that they are channels for the exchange of material between the nucleus and the cytoplasm.

A sizable Golgi complex in the supranuclear region consists of several parallel arrays of flat cisternae and numberous small vesicles. Small vacuoles with a content similar in density and texture to the larger mucous droplets are also found in the region of the Golgi complex and apparently represent formative stages in the evolution of the secretory product.

The mitochondria of the surface mucous cells are not ordinarily found among the mucous droplets at the apex but appear to be randomly distributed elsewhere in the cytoplasm. They are similar to those of the other cell types of the gastric epithelium, possessing typical, transversely oriented cristae and dense intramitochondrial granules.

Ribonucleoprotein particles are present in the cytoplasmic matrix in considerable numbers but never in such high concentrations as in zymogenic

FIGURE 3

A photomicrograph of the bat gastric mucosa prepared as in Fig. 2, but stained with the PAS reaction. The intense staining of the mucous granules in the mucous neck cells (*MNC*) reveals their presence among the parietal cells (*PC*) in the neck region of the gastric gland. Their mucous granules extend into the basal cytoplasm whereas in the surface mucous cells (*SMC*) the granules are present only in the apical cytoplasm. Note that neither the parietal nor the chief cells (*CC*) stain with the PAS reaction. The muscularis mucosae (*MM*) is shown at the bottom of the illustration. × 600.

FIGURE 4

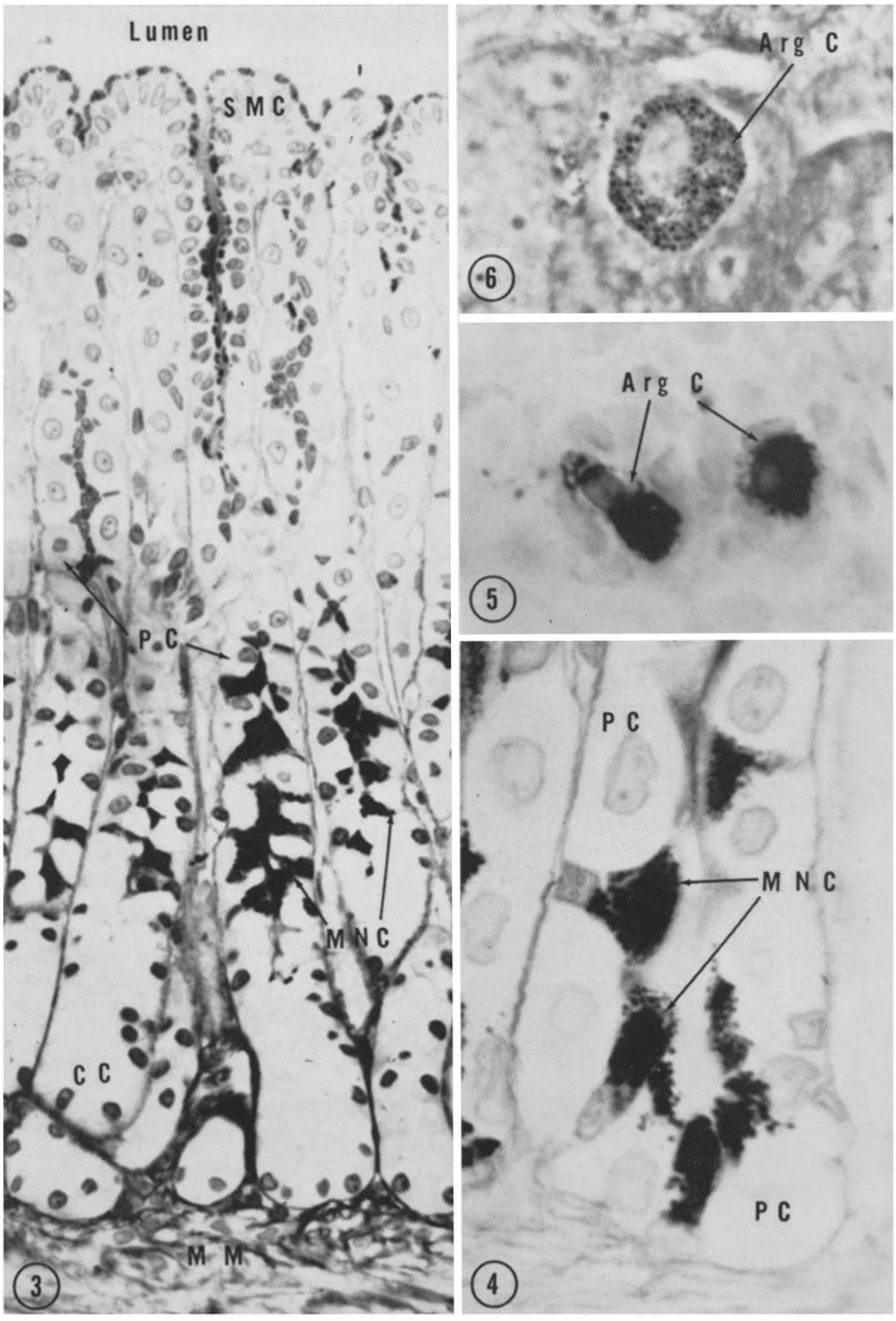
A photomicrograph from the same PAS-stained section as shown in Fig. 3, but from the mucosa of the stomach near the pyloric region where the base of the gastric glands is made up almost exclusively of parietal cells (*PC*) and mucous neck cells (*MNC*). × 1500.

FIGURE 5

A photomicrograph of the pyloric region of a stomach fixed in formalin, embedded in paraffin and stained with the methenamine silver reaction for argentaffin cells (*Arg C*). Intensely silvered granules fill much of the cytoplasm of the argentaffin cells, while other gastric epithelial cell types are almost entirely unstained. × 1500.

FIGURE 6

A phase contrast photomicrograph of an argentaffin cell (*Arg C*) in an osmium tetroxide–fixed and Epon–embedded preparation cut at about 2 μ and viewed without further staining. × 2000.


Lumen
SMC
P C
M N C
C C
M M
3
Arg C
6
Arg C
5
P C
M N C
P C
4

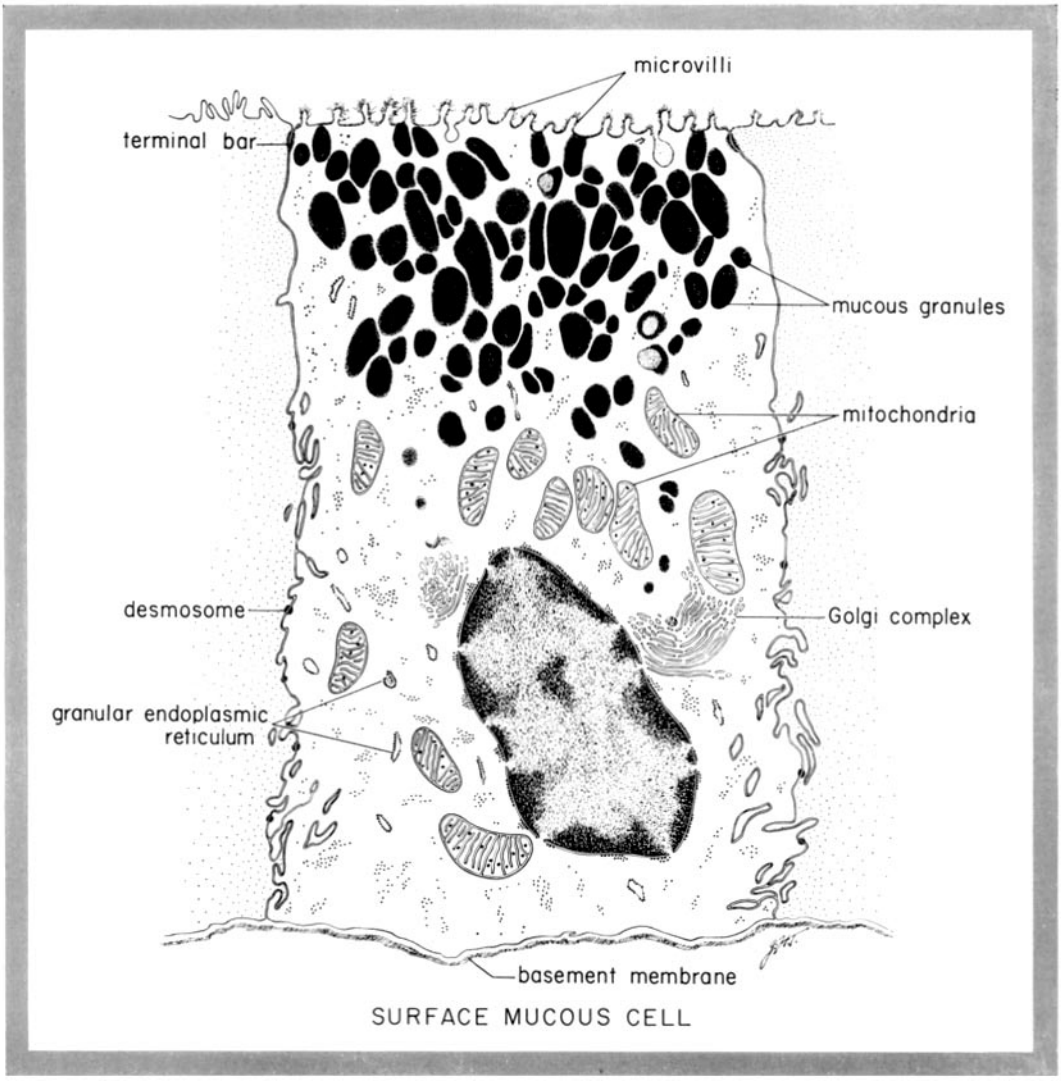
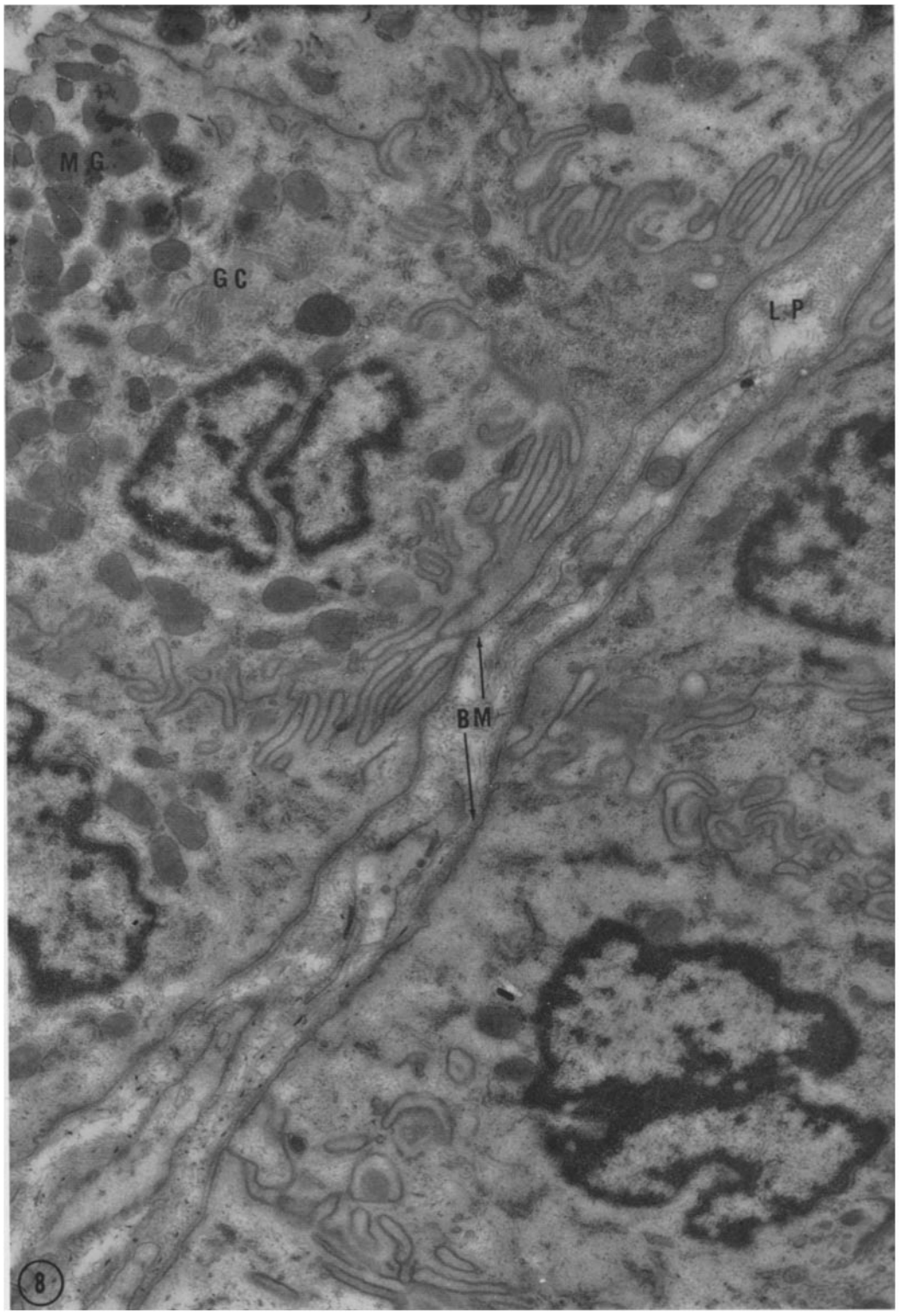


FIGURE 7

A diagram of a surface mucous cell from the bat stomach.

FIGURE 8

An electron micrograph of two layers of surface mucous cells separated by a thin lamina propria (*LP*). Part of the lumen of the gastric pit is shown at the upper left corner. The elaborate interdigitations of the lateral plasmalemmata towards the base of the cell are apparent. The basal cell membrane rests on a thin basement membrane (*BM*) which is closely associated with numerous collagen fibrils. Dense mucous granules (*MG*) fill the apical cytoplasm and a prominent Golgi complex (*GC*) occupies the supranuclear region. The nuclei are very irregular in shape. × 15,000.


M G
G C
L P
B M
8

cells. Although some granulated or rough surfaced endoplasmic reticulum is present, many of the ribosomes in the mucous cells are not associated with membranes. They are less numerous between the mucous granules at the apex than elsewhere in the cell and are absent from the microvilli and from a narrow ectoplasmic zone just beneath the surface plasmalemma.

The number of mucous granules in different cells varies considerably depending upon the regional location of the cells and the functional state of the stomach. After prolonged fasting, the cells lining the gastric lumen and the upper part of the gastric pits contain many more mucous droplets than those situated deeper in the gastric glands. The mucous granules or droplets are spherical, ovoid, or discoid in shape and range in size up to 0.6 μ. Most of them are of uniformly high density throughout but occasionally portions of a granule may be very light while other parts of the same granule are quite dense. In methacrylate-embedded material, the high density of the granule interferes with the clear delineation of the enclosing membrane, but Epon embedding often results in a decreased density of the mucus and distinct membranes limiting the granule are revealed. In some cells the granules immediately underlying the cell surface are in close apposition to the plasma membrane particularly at points between microvilli, suggesting that they may have a preferential attachment to these loci. Not infrequently one encounters, at these same locations, invaginations resembling pinocytotic vesicles. It does not seem unreasonable to assume that these are the limiting membranes of mucous vacuoles that have become continuous at their point of contact with the plasmalemma and have discharged their contents into the lumen.

Mucous cells in mitosis are rarely encountered. When observed they are usually located among the parietal cells in the isthmus and neck regions. They are identifiable by the presence of typical mucous granules which persist even during cell division.

The Parietal Cell

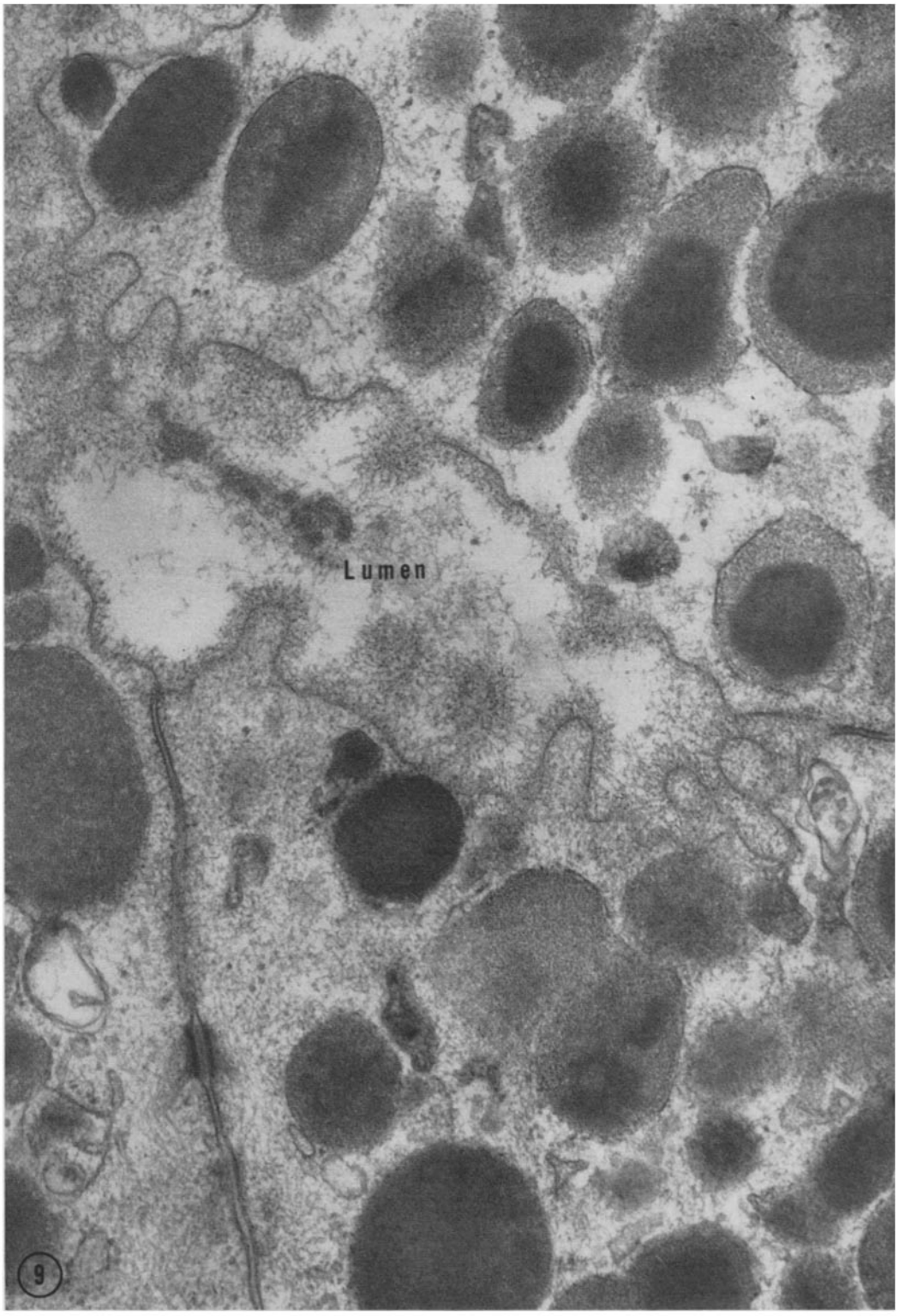
Parietal cells occur in all gastric glands but are most numerous in the region adjacent to the pylorus. In the typical gastric gland the parietal cells are found principally in the neck and isthmus region and only rarely among the chief cells in the deeper portions of the gland. However, in those glands in which zymogenic cells are absent, the parietal cells and mucous neck cells may occupy the basal part of the gland as well.

The parietal cell is shown diagrammatically in Fig. 10 and the electron micrographs in Figs. 11 to 15 illustrate the salient features of its fine structure. It is pyramidal or oval in outline and is the largest cell type in the mucosa. In histological sections examined with the light microscope, the cytoplasm is deeply stained with acid dyes and is intensely colored by specific stains for mitochondria. In the bat parietal cell there is a particularly prominent secretory canaliculus that usually remains unstained. This structure appears as a 1 to 2 μ clear channel that is continuous with the glandular lumen, but seems to penetrate into the cell and run a tortuous course within it. The canaliculus often completely encircles the nucleus so that the latter appears to be located in a central island of cytoplasm isolated from the peripheral cytoplasm by the canaliculus (Figs. 11 to 13).

Electron micrographs reveal that the well developed secretory canaliculus is lined by numerous, irregularly oriented microvilli and is limited by a membrane which is continuous with the plasmalemma at the apex of the cell. Thus, even though the irregular course of the canaliculus lies within the general limits of the cell, its lumen is entirely extracellular. The microvilli are longer and more numerous in the parietal cell of the bat than in many other species. They often seem to branch near their base and occasionally several

FIGURE 9

Apical parts of three surface mucous cells bordering the lumen of a gastric pit. The microvilli on the free surface have a furry coating of fine filamentous substance which in this preparation is stained with uranyl acetate and lead. In an Epon-embedded tissue section, such as this preparation, some mucous granules appear uniformly dense while others have varying degrees of lower peripheral density. A limiting membrane which often appears discontinuous is evident on the surface of some of the granules. × 74,000.


Lumen
9

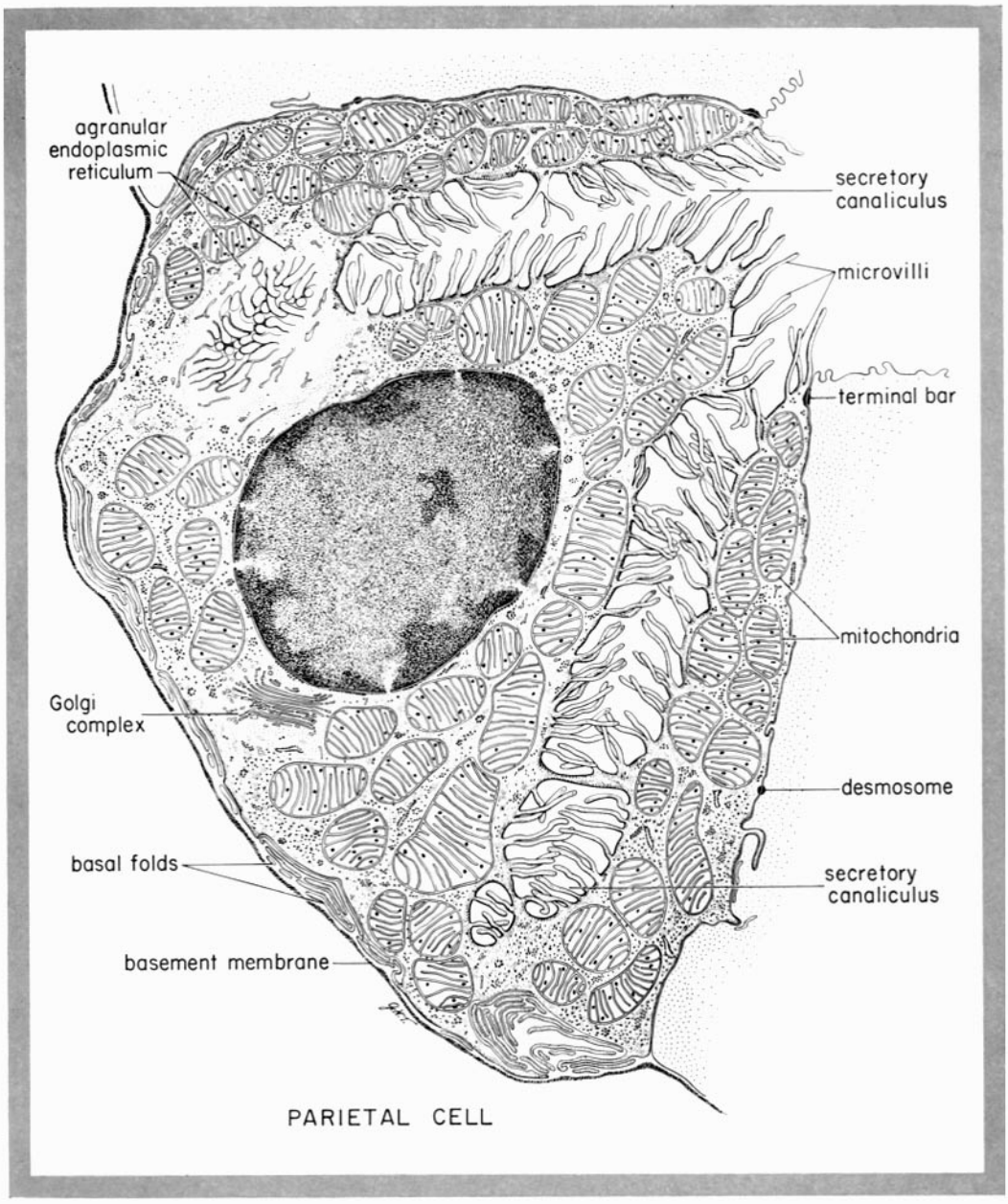


FIGURE 10

A diagram of a parietal cell from the bat gastric mucosa.

microvilli appear to arise from a single broad stem. The degree of patency of the lumen of the canaliculus varies markedly from cell to cell. In many cells the microvilli are very closely intermeshed and so completely fill the lumen of the canaliculus that the labyrinthine, free space between adjacent or opposing microvilli is no greater than 200 A. In other cells the microvilli are less closely packed and the canaliculus is relatively open with a free lumen a micron or so in diameter. While most cells have secretory canaliculi that are either open or closed, a few

have part of the channel patent and the remainder are largely obliterated by close packing of the microvilli. The functional significance of these variations in patency of the secretory canaliculus is not yet clear. The frequency with which electron micrographs of thin sections show the secretory canal as a complete ring around a central island of cytoplasm containing the nucleus suggests that this structure is not tubular. If it had the form of a meandering tubule it would usually appear discontinuous in thin sections. Instead it seems more likely that it is a continuous, curved sinus, concentric with the nucleus but traversed at various points by strands of cytoplasm. In sections the cytoplasmic cross-connections may be relatively broad when the canaliculus is collapsed but when it is patent they are often reduced to very slender strands scarcely thicker than a microvillus.

The cytoplasm of the parietal cell is crowded with an extraordinary number of mitochondria. Some of these are arranged in a single layer around the nucleus and the remainder are closely packed in the cytoplasm peripheral to the canaliculus. The mitochondria are considerably larger than those in other gastric epithelial cells and have a more extensive internal membrane structure. The cristae are abundant and closely spaced and the matrix contains numerous dense mitochondrial granules. In favorable planes of section many of the cristae are seen to traverse the full width of the mitochondrion. Occasionally there is a slight constriction along the length of a mitochondrial profile and at this point a pair of membranes resembling a crista bisects the mitochondrion, producing an image like images in liver cell mitochondria that have been interpreted by Fawcett (6) as stages in division or coalescence of mitochondria.

The cytoplasmic matrix contains occasional small vesicles and some tubular structures interpreted as part of a smooth surfaced endoplasmic reticulum. They are not found in great abundance in the bat parietal cell. They are usually located just beneath the microvilli bordering the secretory canaliculus. The remaining cytoplasm in the narrow interstices between the densely packed mitochondria elsewhere in the cell contains numerous ribosomes, some of which are attached to short tubular or cisternal elements of the endoplasmic reticulum. Large clear vesicles or vacuoles have been encountered only infrequently in specimens judged to be adequately preserved.

The Golgi complex is poorly developed and may consist of several small aggregations of parallel cisternae and vesicles at different sites in the peripheral cytoplasm, sometimes between the nucleus and the cell base. The cytoplasm contains, in addition, small lipid droplets and dense lysosome-like bodies. Multivesicular bodies are encountered only infrequently. Particles tentatively identified by Sedar (29) as glycogen in the parietal cells of dogs and frogs have not been observed regularly in the bat parietal cell.

The lateral plasmalemma of the parietal cell is smooth contoured and attached to adjacent cells by terminal bars at the apical border and by occasional desmosomes elsewhere on the surface of contact. The cell membrane at the base of the cell may be smooth or may form narrow folds or villi that are irregularly oriented and compressed between the cell body and the basement membrane. These basal plications or villi are about the same width as the microvilli lining the secretory canaliculus. In some regions where these elaborations of the cell base are quite extensive, the layer of cytoplasm intervening between the extracellular clefts in the basal labyrinth and the lumen of the secretory canaliculus is very thin (Figs. 14 and 15).

The Mucous Neck Cell

The mucous neck cell is distinguished from the other epithelial cells by its location in the gastric gland and by its characteristic, specific granules. The mucous neck cells occur singly or in small clusters among the most deeply located parietal cells in the neck region and occasionally between chief cells. They can easily be distinguished from the chief cells after staining with the PAS reaction (Fig. 3). The zymogen granules of the latter are not PAS-positive whereas the granules of the mucous neck cells are strongly stained. They can also be distinguished from the surface mucous cells on the basis of their granules which are larger, spherical and often are found in the perinuclear or even in the basal cytoplasm, whereas the granules of the surface mucous cells are smaller, more variable in shape, and always limited to the apical region of the cell.

The distribution just described for the mucous neck cell applies to the gastric glands of the fundus and corpus. In the pyloric and cardiac regions, on the other hand, surface mucous cells extend deep into the glands, occupying most of their length,

but at their base these glands consist almost exclusively of mucous neck cells.

The shape of the mucous neck cell is generally columnar but marked variations are encountered. Some are flask-shaped with a narrow apex and a broad base resting on the basement membrane. Others have a wide luminal border and a narrow base. They are apparently easily deformed and the shape of any particular cell seems to be determined by the contour of the adjacent parietal or chief cells. A diagram representing the form and fine structure of a mucous neck cell is shown in Fig. 16 and the principal features of the diagram can be verified in the electron micrograph in Fig. 17.

The free surface of the mucous neck cell has a sparse covering of short microvilli that resemble those of the surface mucous cells but differ in that the fine filamentous projections that impart a furry appearance to the microvilli of the surface mucous cells are less well developed in this cell type. The lateral margin of the cells are, for the most part, relatively straight but there are occasional shallow interdigitations with adjacent cells. Typical terminal bars and desmosomes are present in the same distribution as for the other cell types already described, and there is nothing distinctive about the smooth basal surface of the cell.

The characteristically spherical, membrane-limited secretion granules of the mucous neck cells often occupy most of the apical cytoplasm, and though smaller (0.5 μ or less) they resemble the pale zymogen granules of the chief cells. After osmium tetroxide fixation the granules of the mucous neck cells are consistently less dense than those of the surface mucous cell. Some of the granules have localized marginal areas of dense material. This may be due to partial extraction of some component of the mucous content as postulated for the chief cell zymogen granule but the mucous neck cell granules are much more consistent in their appearance.

The cytoplasm contains a moderate number of mitochondria which are located primarily in the perinuclear cytoplasm. Scattered profiles of granular endoplasmic reticulum are present, particularly toward the cell base, and there is an abundance of free ribosomal particles in the cytoplasmic matrix. A typical Golgi complex of small size is located in the supranuclear cytoplasm and associated with it are vacuoles of moderate size having a content that resembles that of the fully formed mucous granules. The nucleus is often irregular in outline with deep infoldings of its surface. Occasional mitotic figures have been observed in cells resembling mucous neck cells but it has not been possible clearly to establish their identity.

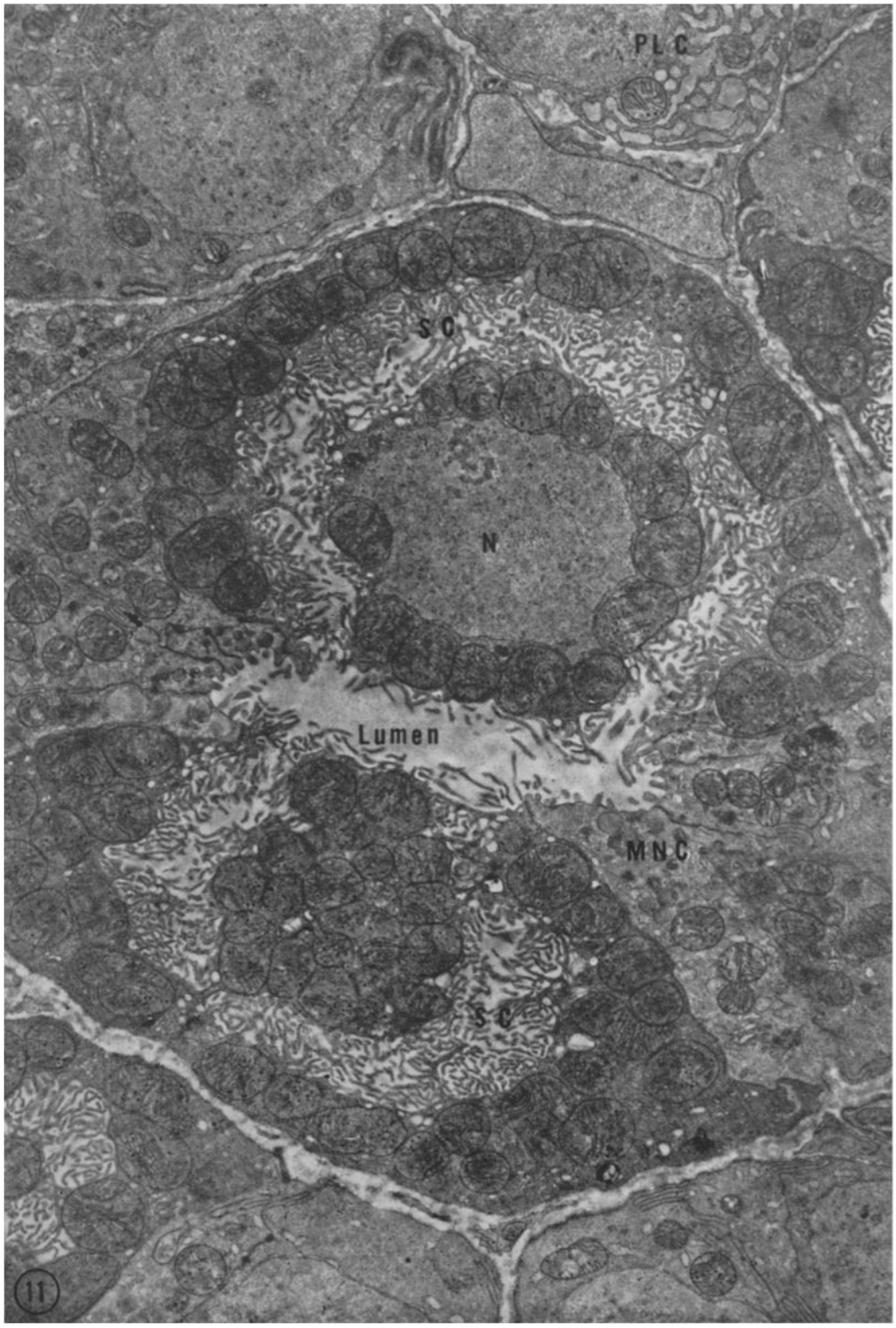
The Chief Cell

The chief cells of the gastric mucosa are similar in their light microscopic appearance to other zymogenic cells such as the pancreatic acinar cells. Their basal cytoplasm is highly basophilic and there is an accumulation of zymogenic granules at their apex. They are found in greatest number in the fundus and upper portion of the corpus. There, they are the predominant cell type, occupying the entire deep portion of the glands and intermingling with the parietal and mucous neck cells in the neck region. During

FIGURE 11

A low power electron micrograph of a transverse section through a gastric gland including two large parietal cells and several small mucous neck cells (*MNC*). The secretory canaliculi (*SC*) of the parietal cells are extensive surface invaginations which seem to isolate the central mass containing the nucleus (*N*) from the peripheral rim of mitochondria-rich cytoplasm. Close examination reveals thin cytoplasmic strands which bridge the lumen of the canaliculi. The sparse cytoplasmic matrix contains a dense packing of fine granules which at higher magnifications are recognizable as ribosomes. Several small Golgi complexes are present but are not clearly recognizable at this magnification.

The remaining cells bordering the gland lumen are sections through several mucous neck cells. Note that the total area occupied by the seven mucous neck cells is less than that taken up by the parietal cells. The upper and lower margins of the illustration show the parts of four other gastric glands. The lamina propria contains connective tissue elements including part of a plasma cell (*PLC*). × 6,500.


PLC
SC
N
Lumen
MNC
SC
11

fasting or hibernation, abundant zymogenic granules fill the entire cell apex and may extend down into the basal cytoplasm.

A diagram of the fine structure of the chief cell is shown in Fig. 18 and representative electron micrographs are presented in Figs. 19 to 21. The apical border of the cell has a covering of microvilli that are moderately long in some preparations and may have a fine, filamentous, extraneous coating similar to that found on the mucous neck cells. The lateral borders are relatively straight and display prominent terminal bars near the lumen and occasional desmosomes at intervals along the rest of the cell boundary. The base of the cell has a smooth contour and rests on a thin, continous basement membrane.

The nucleus of the chief cell may be somewhat irregular in outline and has a prominent centrally located nucleolus but no other distinctive features. The outer layer of the nuclear envelope is always studded with numerous ribosomes, whereas in other cell types of the gastric mucosa these are less abundant.

The apical cytoplasm contains numerous round or oval zymogen granules that measure up to 2 μ in diameter. Each is enclosed by a smooth surfaced membrane. Their contents vary in density from one preparation to another and even in different areas of the same specimen. In most of our preparations they have a very low density but occasionally, for reasons that are not clear, they may be quite dense (Fig. 19). This puzzling variability in the appearance of the secretory granules is tentatively attributed to different degrees of extraction during preparation of the tissue for microscopy. In stomachs of previously fasted animals examined shortly after a brief period of refeeding, the zymogen granules are greatly reduced in number. Empty-appearing vacuoles are found at the cell surface with their membrane continuous with the plasmalemma in such a manner as to suggest that they are zymogen granules which have been fixed in the process of discharging their contents into the lumen (Fig. 21). These images would indicate that these cells, like the pancreatic acinar cells, release their zymogen granules by fusion of their limiting membrane with the plasmalemma and the subsequent incorporation of this membrane into the cell surface.

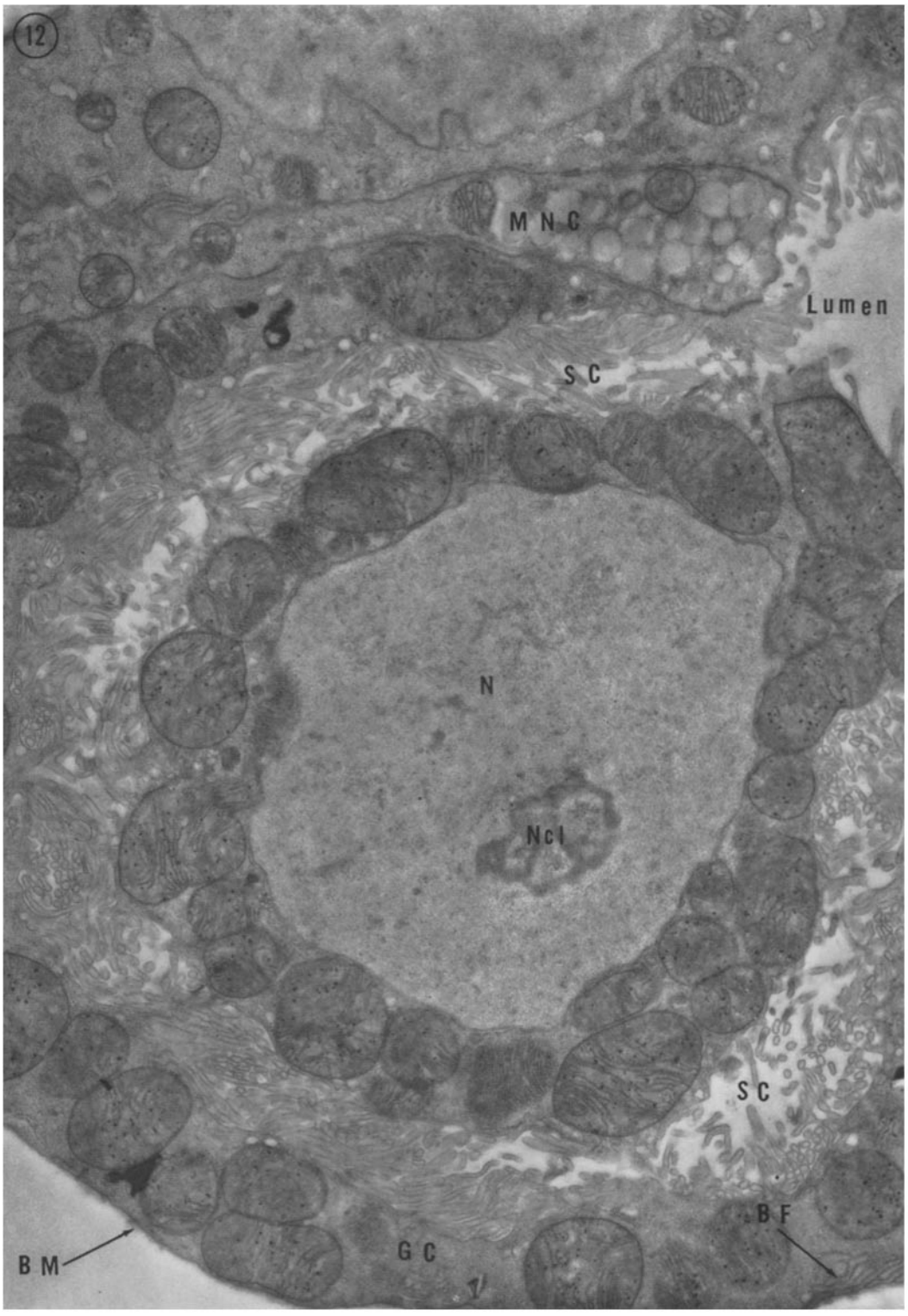
The mitochondria of the chief cell are neither so numerous nor so large as those in the parietal cell and are generally similar in structure to mitochondria in mucous cells. They are lodged between the cisternae of the granular endoplasmic reticulum at the cell base and are found only rarely in the granule-laden apical cytoplasm.

The ergastoplasm or rough surfaced endoplasmic reticulum is very much more abundant in the chief cell than in any other cell type in the gastric mucosa, and there are great numbers of free ribosomes throughout the cytoplasmic matrix. The cisternal and tubular elements of the reticulum occupy most of the basal cytoplasm. In the resting cell they tend to be oriented more or less parallel to the basal or lateral cell surfaces; but in certain functional states, particularly when the cell has recently been depleted of zymogen granules, the cisternae may become reorganized into concentric arrays.

In some instances there are local differentiations of the reticulum of a kind which has not been observed heretofore in any other cell type. In these, some 10 to 75 straight tubular elements are oriented parallel to one another and closely packed in hexagonal array. These elements are about 1200 A in diameter, which is considerably larger than is usual for the reticulum. The ribosomes associated with these tubules, instead of being randomly scattered or aggregated in beaded

FIGURE 12

A section through a parietal cell which includes the nucleus and part of the peripheral cytoplasm. The area illustrated here shows the nucleus (*N*) with its nucleolus (*Ncl*) surrounded by a layer of large mitochondria. The central island containing the nucleus is almost isolated from the outer part of the cell by the secretory canaliculus (*SC*). The microvilli lining the canaliculus occlude the lumen to varying degrees. The sparse cytoplasmic matrix at the base of the cell contains a small Golgi complex (*GC*). A few basal folds (*BF*) are shown at the lower right margin and a basement membrane (*BM*) underlies the generally smooth basal cell membrane. Parts of two mucous neck cells (*MNC*) and a parietal cell are shown near the upper margin. × 11,000.


12
M N C
Lumen
S C
N
Ncl
S C
B F
B M
G C

strands or rosettes, are arranged in straight rows. Furthermore, they are paired and uniformly spaced so that in transverse sections of these specializations there are six pairs of ribosomes around the circumference of the circular profile of each tubule. Each pair of ribosomes is almost in contact with a pair of ribosomes on one of the other six surroudning tubules. A very precise and distinctive pattern is thus formed (See Fig. 20 and inset). The functional significance of these intriguing specializations of the endoplasmic reticulum and the nature of the forces responsible for the establishment of their high degree of order remain obscure. This arrangement was encountered in a number of the chief cells from three bats fixed during winter hibernation in their natural habitat. To date, none of the more than 55 bats sacrificed while under various physiological states in the laboratory have been noted to have this configuration of the chief cell granular reticulum.

The Argentaffin Cell

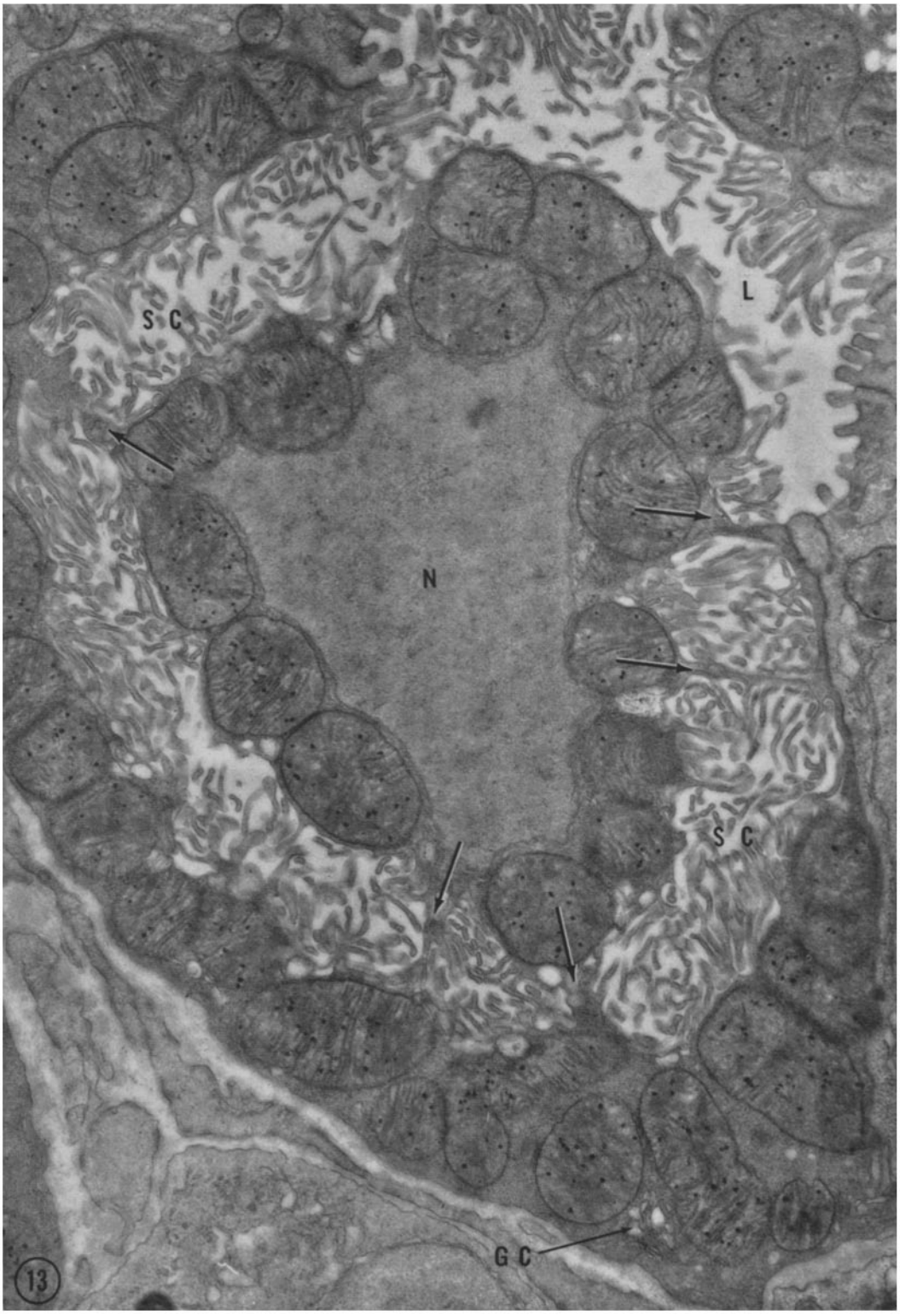
Of the five cell types in the gastric mucosa, the argentaffin or enterochromaffin cells are the smallest and least numerous. They are found only in the gastric glands and in the basal part of the cardiac and pyloric glands where they occur as isolated cells sequestered among the other epithelial cells. They have not been observed in the surface epithelium or the gastric pits of the mucosa in the bat stomach. Argentaffin cells are quite readily distinguishable from the other cell types by the selective staining of their granules with certain silver staining methods (Fig. 5). They are also easily identified with the phase contrast microscope in unstained, thick plastic sections of osmium-fixed material (Fig. 6). Several variations of this cell type are recognizable with the light microscope. Some have numerous dense granules of appreciable size, while others contain only fine, dust-like particles and still others appear to contain no granular inclusions at all. All of these forms are very different from the other epithelial elements, but the significance of the cytological variations within this cell type is not clear. In this study no attempt has been made to distinguish between the argentaffin and the argyrophile cell, and the common term argentaffin cell will be employed to describe all cells of this category.

In electron micrographs the gastric argentaffin cells are pyramidal or ovoid with their broad end resting on the basement membrane. Most of them are lodged between the basal parts of other epithelial cells and do not have a free border on the lumen. However, occasional argentaffin cells have been observed to have a thin prolongation extending up to the lumen (Figs. 25, 26). A diagram of an argentaffin cell is shown in Fig. 22. Figs. 23 to 26 illustrate parts of argentaffin cells which exhibit some of the variations in appearance encountered. The type containing the most prominent and well formed granules is shown in Fig. 23. The numerous basally located granules are dense and spherical and measure up to 0.4 μ in diameter. Each is enveloped with a loose fitting membrane. Scattered elements of granular endoplasmic reticulum and some free ribosomes are present in the cytoplasmic matrix between the granules and elsewhere in the cytoplasm. The mitochondria in these cells are small and usually nearly spherical. In their general appearance and size they are not unlike those of the mucous cell. A few intramitochondrial granules are also present. The nucleus of these cells usually has deep infoldings of its envelope.

Another cell type occurs that somewhat resembles the typical argentaffin cell just described, but differs in at least three respects. The most obvious difference is in the appearance of the granules. These vary greatly in density and have a limiting membrane which is closely attached to the granule and often appears indistinct or dis-

FIGURE 13

An electron micrograph of a parietal cell bordering the lumen (*L*) of a gastric gland. The perikaryon is almost isolated from the peripheral cytoplasm by the patent secretory canaliculus (*SC*) except at the 5 sites indicated by arrows where narrow cytoplasmic strands bridge the canaliculus. The microvilli are irregularly oriented and some are branched. Mitochondria with numerous cristae and dense intramitochondrial granules almost completely fill the cytoplasm. A small Golgi complex (*GC*) is located near the base of the cell. *N*, nucleus. × 14,000.


S C
L
N
S C
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13

continuous. In this cell type, the granules are very tightly packed so that little intervening cytoplasm can be seen. The mitochondria are also unlike those of the previously described type of argentaffin cell. They are long and thin, lack intramitochondrial granules, and have sparse cristae that are either transversely or longitudinally oriented. The nuclei also differ in that they do not have the deep surface infoldings of the typical argentaffin cell, but numerous small irregularities that impart a wavy contour to the nuclear envelope.

Much less frequently encountered are cells that possess the fine structural features of argentaffin cells but are narrow and pyramidal in shape with a thin apical process extending to the free surface of the epithelium and bearing microvilli which have a filamentous surface coating. In favorable sections through the narrow apical region of the cell, centrioles or basal bodies have been observed in the cytoplasm just beneath the free surface and occasional cilia have also been encountered projecting into the lumen of the gastric gland. These cells contain mitochondria of the long, thin type and, in general, have cytological characteristics resembling those of argentaffin cells with granules of varying density. The apical cytoplasm contains numerous smooth surfaced tubules and vesicles of the endoplasmic reticulum. A typical Golgi complex of moderate proportions is located in the supranuclear region. In some cells (Fig. 23), vesicles and vacuoles containing material resembling formative stages of argentaffin granules are observed, but relatively few granules are found in these cells.

The lateral borders of these cells are relatively smooth and have typical terminal bars, but desmosomes are only very infrequently observed. Argentaffin cells seem to be consistently better preserved in epoxy resins. With methacrylate embedding, the appearance of the granules is inconsistent and the recognition of the different types of argentaffin cells is more difficult.

Occasionally cells have been observed that possess the fine structural characteristics of argentaffin cells but are entirely devoid of any specific granules. Such cells are very light in electron micrographs and have an abundant empty-appearing cytoplasmic matrix containing a moderate amount of granular endoplasmic reticulum.

DISCUSSION

Numerous early studies begining with the initial work of Heidenhain in 1870 (10) have established the identity of several morphological classes of cells in the mammalian gastric mucosa. But, in an organ possessing a complex epithelium consisting of an admixture of several kinds of cells, each specialized in its structure and function, the problem of investigating the physiological role of the individual cell types is complicated. Nevertheless, comparative considerations, clinical and histopathological correlations, and the cytochemical studies of Linderström-Lang (21) and others have made it possible to assign a function to the major cell types with some degree of confidence. How ever, the amount of information available on the physiology of the several cell types of the gastric mucosa varies greatly.

Certainly the most extensively studied and most unusually specialized cell in the gastric mucosa is the parietal cell to which the role of hydrochloric

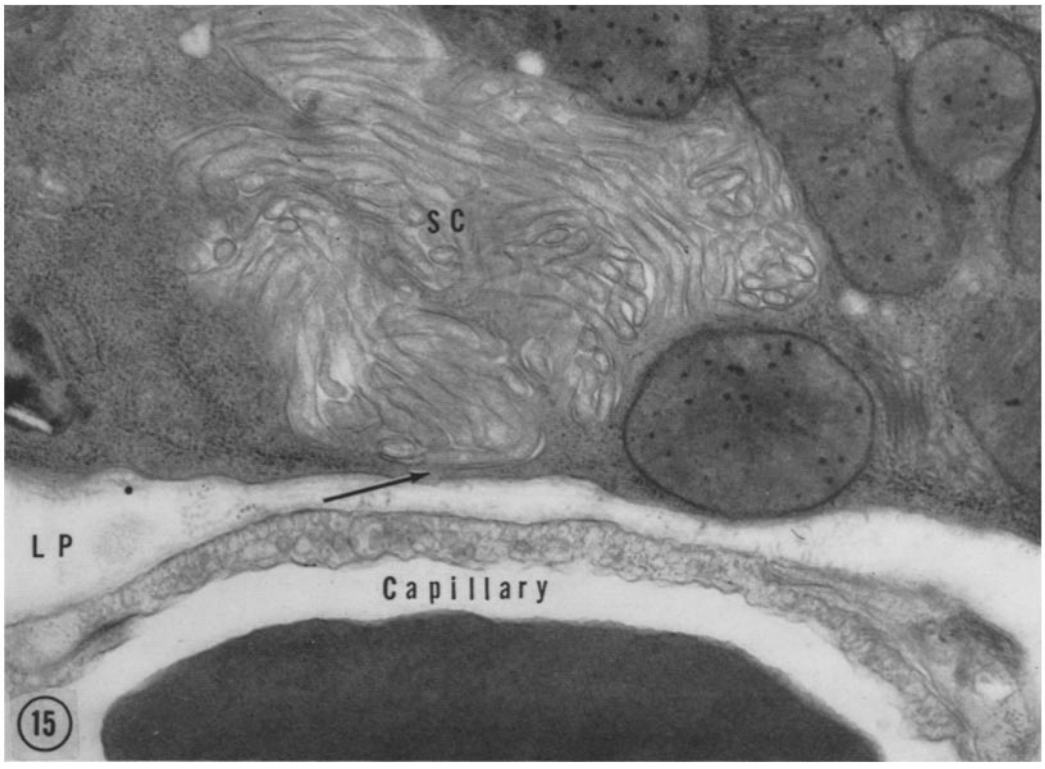
FIGURE 14

An electron micrograph of the basal parts of two parietal cells separated by a thin layer of the lamina propria. The lumen of the secretory canaliculi (*SC*) in these cells is patent and lined by numerous microvilli. Basal folds (*BF*) of the plasma membrane are tightly compressed between the cell and the basement membrane (*BM*). In certain regions (arrow) only a very thin layer of cytoplasm separates the lumen of the secretory canaliculus from the extracellular space. × 24,000.

FIGURE 15

The basal region of a parietal cell similar to that shown in Fig. 14. In this cell the lumen of the secretory canaliculus (*SC*) is almost occluded by closely packed microvilli. The limited space between the microvilli is separated from the exterior of the cell by a very thin layer of cytoplasm (arrow). A small capillary in the lamina propria (*LP*) is in close relation to the base of the parietal cell. × 24,000.


SC
BF
BM
BF
SC
14


SC
LP
Capillary
15

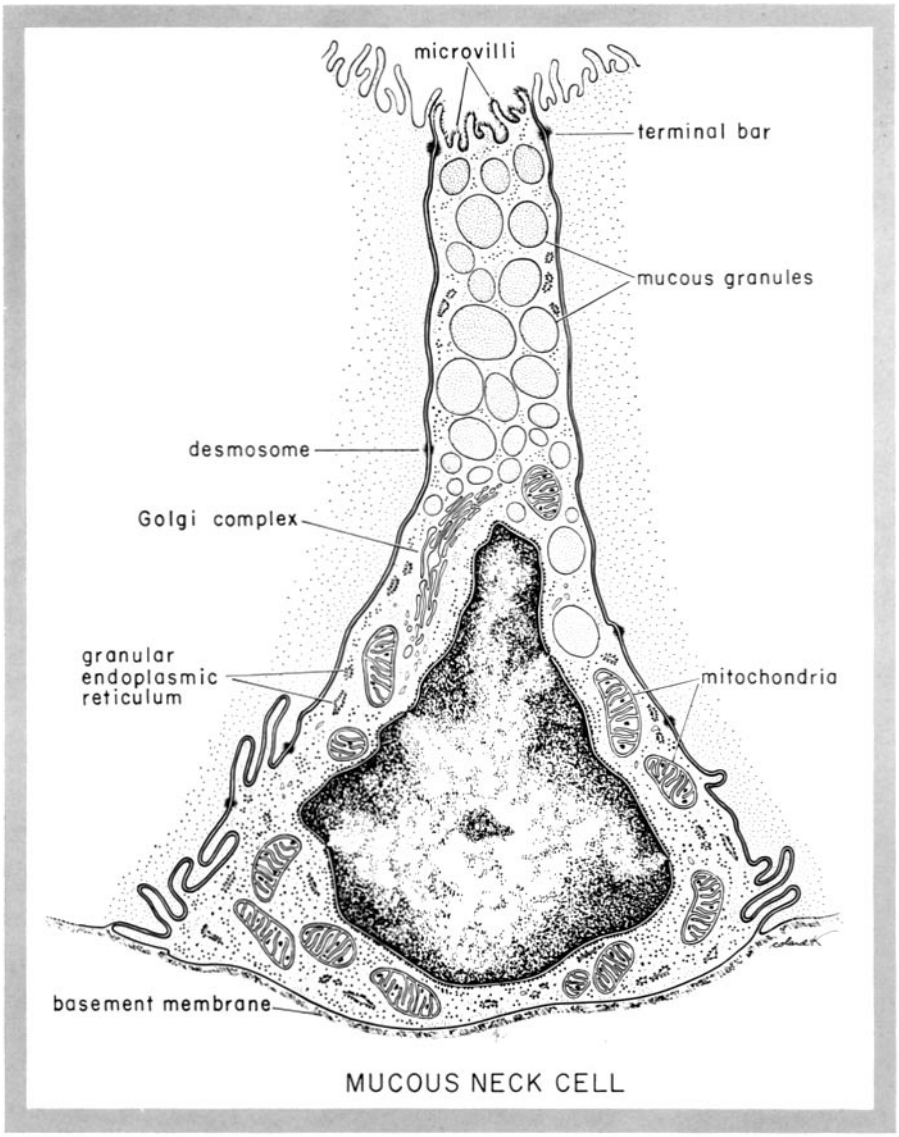


FIGURE 16

A diagram of a gastric mucous neck cell.

acid secretion is generally attributed (3). To date, however, it must be admitted that there is no unequivocal demonstration that the parietal cell does in fact possess that function. Investigators of its fine structure agree upon its most characteristic features, namely, its large size, its extraordinary number of mitochondria, and its unusual system of secretory canaliculi, but there has been some divergence of opinion as to the form of its endoplasmic reticulum. Some observers describe a very large number of separate vesicles and vacuoles which they regard as characteristic of this cell type (8, 12, 34). Others find, instead, a branching and anastomosing system of smooth surfaced tubules and cisternae (15) or a mixed population of vesicles and tubules (20, 29). The bat parietal cell is not particularly favorable for demonstrating the smooth reticulum, because it is

present in only limited amounts. Where it is found, it consists primarily of tubular elements rather than vesicles and vacuoles.

In a previously published survey of parietal cells from various vertebrate species, a plexiform, tubular configuration of the reticulum was the rule and the vesicular form described by other authors was interpreted as an artifact of specimen preparation (15). Recently Sedar (30) has also obtained the preservation of the smooth reticulum as a system of tubular networks rather than vesicles in the frog oxyntic cell after $KMnO_4$ fixation. On the other hand, Helander (12) interprets the presence of tubules in parietal cells as being due to preparatory maltreatment and that vacuoles are a truer representation of these structures in the living cell. Inasmuch as this organelle may well be directly involved in the secretory activity of the cell, it is of some importance to establish which form of the reticulum, particularly whether it is continuous or discontinuous, more closely approximates the system in the living cell.

There have been several investigations (9, 12, 20, 29, 31) of the fine structural changes in the parietal cell during acid secretion. In the stimulated dog stomach, Sedar and Friedman (31) have observed that, in specimens taken during active secretion, the canaliculi become more extensively developed, the vesicular elements of the smooth surfaced endoplasmic reticulum decrease in relative number, and the mitochondria are slightly swollen. In preliminary observations on bat material, the normal stimulus of feeding causes a marked change in the appearance of the parietal cell. There is a transient increase in the size and a change in the shape of the cell accompanied by a marked increase in the relative amount of free cytoplasm. The dense intramitochondrial granules seem to disappear. A detailed investigation of these changes is in progress.

The gastric chief cells are undoubtedly the source of gastric pepsin as postulated by earlier workers and demonstrated by Linderstöm-Lang (21) using microchemical techniques. The present observations of the bat chief cell indicate that it is similar to the corresponding cell type of other mammals and has the structural characteristics of protein-secreting cells. Although the manner in which the gastric zymogen is formed and secreted has not been studied in detail, the similarity of this cell to the pancreatic acinar cell suggests that its synthetic and secretory mechanism is much the same.

The variations in the density of the stored zymogen granules do not seem to be related to variations in their formative stage but seem, rather, to be due to the incomplete preservation of the contents by the fixative. The very dense granules have probably retained more of the material originally present than those of intermediate density, and the light granules have probably lost most of their contents by extraction during specimen preparation. Just why this occurs is not known, but the difference in the appearance of these granules as compared to those of the pancreas, which are often uniformly dense, may be related to chemical differences in composition of the secretory product. Close examination of the micrographs containing the denser granules reveals that these granules invariably have an intact limiting membrane while those with a less dense periphery or completely empty appearance often have ruptured sites in their limiting membrane. These breaks may have facilitated the extraction of the contents during the preparation of the tissue.

In the secretory cell of the frog gastric mucosa, Sedar (28) has distinguished four types of zymogen granules on the basis of differences in density. Kurosumi (17) has observed the lack of density in the zymogen granules of the rat chief cell and reported that it was more pronounced in the larger granules. He also noted the apparent loss of the limiting membrane of some of the zymogen granules. This latter feature was interpreted as being a preliminary step in the solubilization and diffusion of the zymogen through the intact plasma membrane. Our observations suggest a very different mode of release. In the bat material, we have noticed breaks in the limiting membrane which seem to be artifacts but we have never observed the disappearance of the limiting membrane of the zymogen granule. Instead, vacuolar profiles similar in size and appearance to the empty or extracted zymogen granules have been seen in continuity with the plasmalemma. Helander (12) has observed similar configurations in mouse chief cells. These images observed in the bat and mouse gastric zymogenic cells are very similar to images described by Palade (25) and Fawcett (7) for pancreatic acinar cells and interpreted as stages of secretion in bulk. Thus it appears that the gastric chief cells of the bat re-

semble other zymogenic cells in the mode of release of their secretory product.

The mucus-secreting cells of the gastric mucosa comprise two distinct cell types, the surface mucous cell and the mucous neck cell. The distinction between the two can be made by their differences in shape, distribution, and the density of the mucous granules. Except for the presence of secretion products, the fine structure of these cells does not reveal their respective functional roles. The paucity of the granular reticulum and the absence of a significant amount of smooth surfaced reticulum suggests that neither of these cytoplasmic components plays an important role in the formation of mucopolysaccharide secretions. The large Golgi complex seems to be the site where small mucous droplets are found and this may well be the site of their formation as previously suggested by Kurosumi (17).

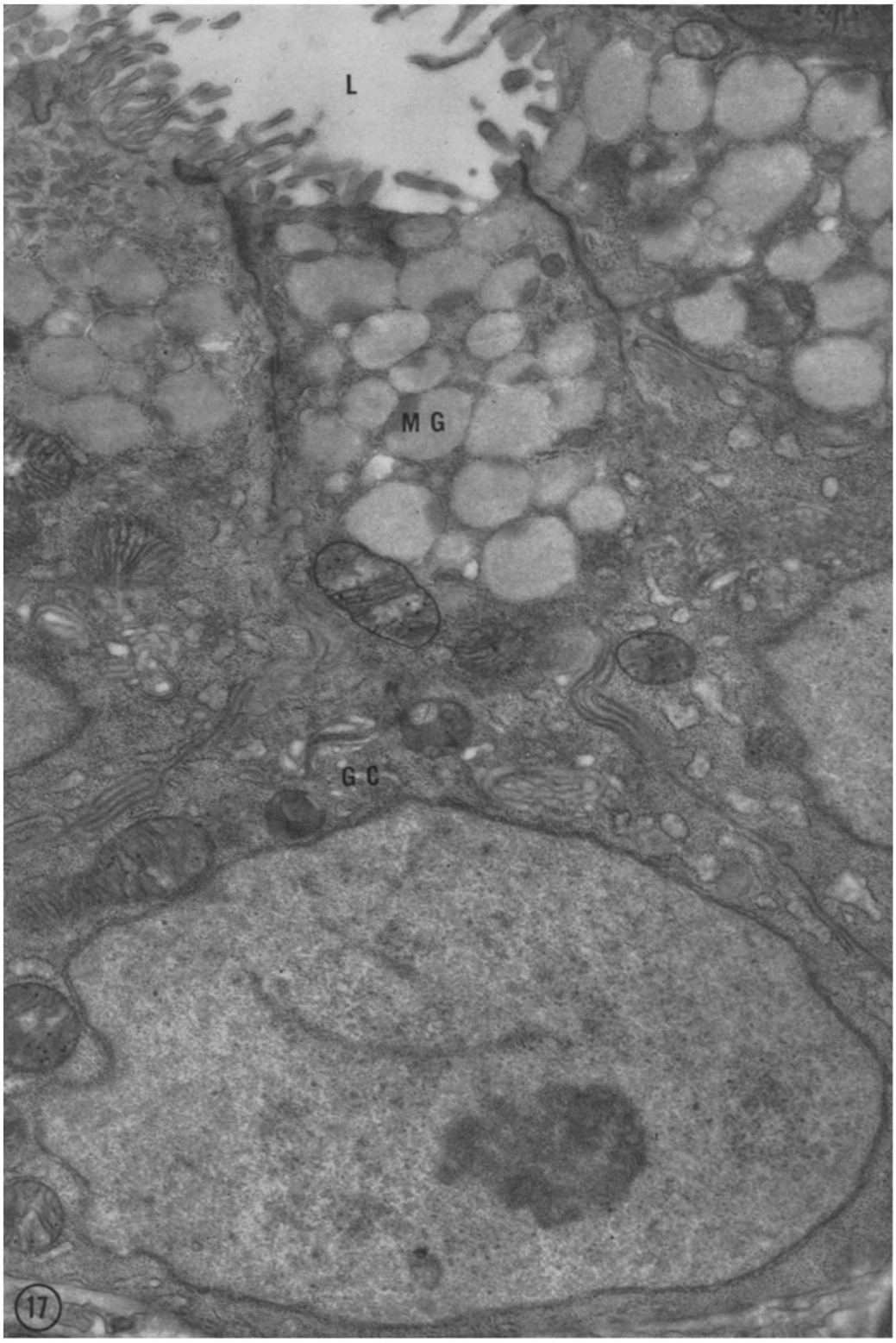
The stored mucous granules of the surface mucous cells examined after Epon embedding often reveal a distinct limiting membrane which is not apparent in methacrylate-embedded tissues. Some of the granules near the apical surface are elongate or discoid in shape and are in close contact with the cell membrane at sites between the bases of adjacent microvilli. Occasionally invaginations of the surface cell membranes are found in this same region, suggesting that these are empty residues of mucous granules that have discharged their contents into the lumen. While these structures suggest a mode of secretion similar to that of the zymogenic cells, the possibility that other processes are involved is not precluded. In a limited number of observations on actively secreting mucosa, images suggestive of apocrine secretion have also been noted. It is possible that, if the material in the mucous granule were solubilized while still in the apical cytoplasm, secretion by diffusion through the intact surface membranes may take place. If this does occur, the preservation of the filamentous strands of mucus may account for the furry coating on the surface mucous cell. It is also possible that this filamentous material is not related to mucus secretion but that it is an extraneous coating which is characteristic of certain cell membranes.

Peachey (26) has observed a similar fuzzy-appearing cell membrane bordering the lumen of the toad urinary bladder and interpreted the filamentous substance as a coating of secreted material. Other examples of a plasmalemma with this appearance include the secretory cell of the toad stomach (15), and a close examination of the published electron micrographs of Helander (12), who studied the mouse gastric mucosa, and of Sedar's micrographs of the dog stomach also shows filamentous projections from the microvilli of mucous cells. Fawcett (7) has also observed a surface membrane of similar character on the pancreatic acinar cells of the bat. Further study will undoubtedly reveal this type of plasma membrane on the luminal surface of many cell types having different functions.

In the gastric mucosa, both types of mucous cells and, to a lesser degree, the chief and certain argentaffin cells all have a similar appearing plasmalemma. The sole exception is the parietal cell. If this appearance is due to non-specific adherence of secreted material, as suggested by Peachey (26), it would seem reasonable to expect that some of the parietal cell microvilli would also be "coated." However, the parietal cell microvilli are always smooth surfaced even though they are often adjacent to and even intermingled with microvilli and mucous cells. It is not known whether the furry surface membrane is a permanent structure on the cell or whether its presence

FIGURE 17

A vertical section of a mucous neck cell depicting some of the features illustrated in the previous diagram. Parts of several other mucous neck cells are also present. The apical mucous granules (*MG*) are seen to be limited by a membrane and are consistently of lower density than those in the surface mucous cell. The regions of higher density in some of the mucous granules may be due to incomplete extraction of these areas during preparation. A well developed Golgi complex (*GC*) is present in the supranuclear region. The nucleus occupying most of the basal cytoplasm contains a prominent nucleolus and some coarse filamentous strands formed by granules not unlike those found in the nucleolus. A few mitochondria, elements of the granular endoplasmic reticulum, and numerous free ribosomes are found in the cytoplasmic matrix. *L*, lumen. × 15,000.


L
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17

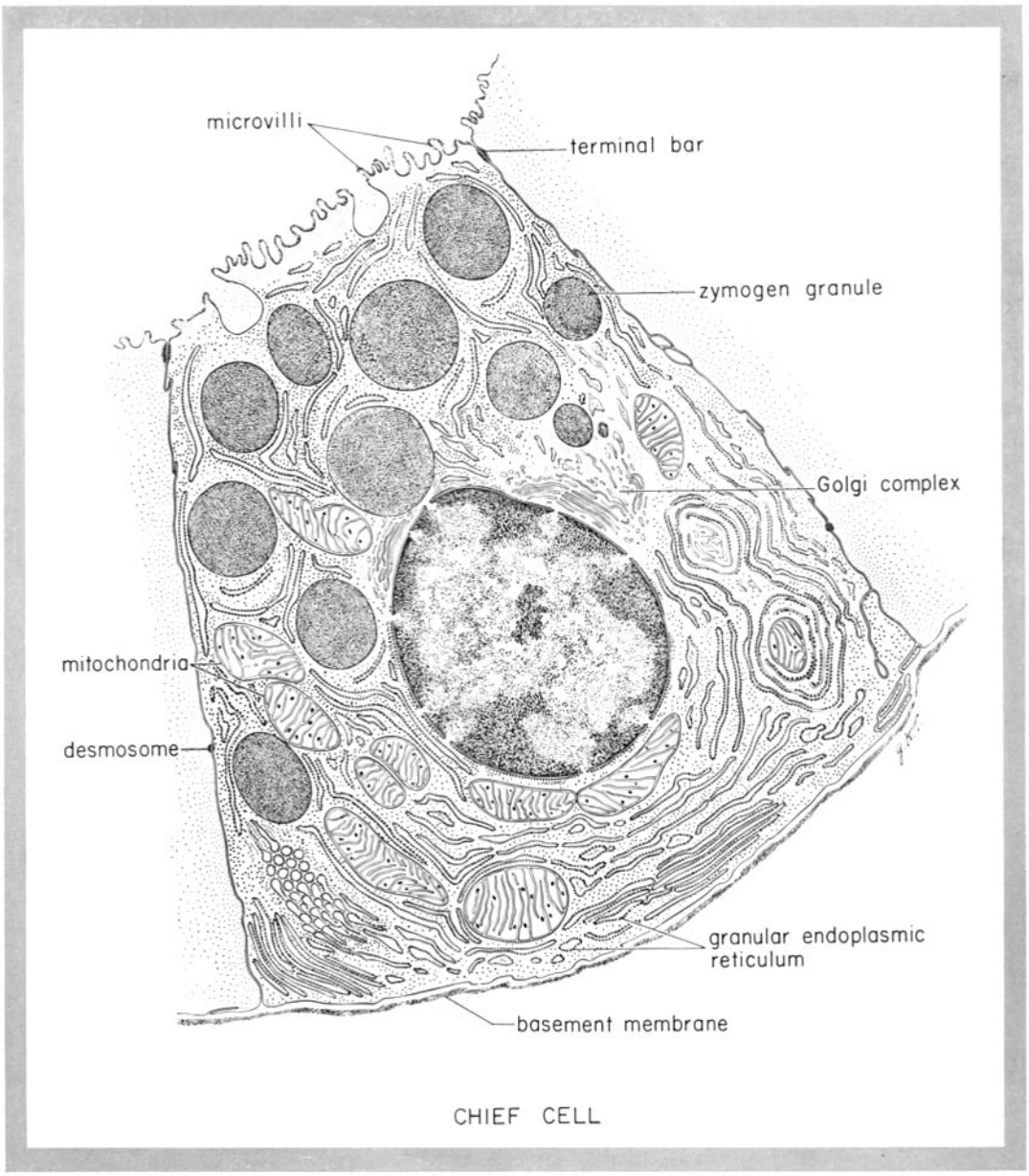

FIGURE 18

A diagram of a gastric chief cell.

is variable. Although the function of a furry cell membrane is not known, it is possible that it has a protective role. On the other hand, preliminary studies in which 0.1 N hydrochloric acid has been used as a bathing medium for the gastric mucosa reveal that the smooth surfaced plasma membrane of parietal cells are far more resistant to the disruptive effects of this acid treatment than the furry plasmalemma of the other cell types. These observations suggest that the parietal cell has an unusual capacity to maintain its integrity under these adverse conditions.

If the composition of the gastric juice is considered, some remarkable protective mechanism

must be present to prevent autodigestion of the stomach by its own secretions. Physiologists have long assumed the presence of a protective layer of mucus over the epithelial cells. However, in microscopic preparations such a layer of secreted mucus is often absent and, when present, seems to be only loosely applied to the surface. Furthermore, mucus is found only infrequently in the gastric pits or glands where the presence of a protective layer would seem to be most needed. Therefore, it seems likely that the mucus performs a lubricant function and acts as a barrier against abrasion rather than as a layer protecting the cell against autodigestion. The alternative remains, however, that the mucus in the lumen either is not preserved or is not rendered electron-opaque by our present preparation techniques.

The remaining cell type in the gastric mucosa, the argentaffin cell, has been studied with the electron microscope by several investigators (11, 17, 23). Its similarity to other endocrine cells such as the pancreatic islet cells (20) or to the chromaffin cell of the adrenal medulla (5) has been used as a basis for attributing to it a possible endocrine role. However, there is no evidence for such a function. The similarity of the gastric argentaffin cells to those of the carcinoid tumors which have a high content of 5-hydroxytryptamine has been used by Luse and Lacy (23) as an argument favoring their suggestion that the granules of the gastric argentaffin cell may be rich in serotonin.

We have described granulated cells of varying appearance under the heading "argentaffin cells," even though on morphological grounds they are sufficiently different that they could be regarded as two or even three distinct cell types. Light microscopists have long noted the multiplicity of appearances within this cell type and such descriptive names as basal clear cell, yellow cell, acidophile, basal granulated cell, have been used to describe it. The earlier literature has been comprehensively reviewed by Macklin and Macklin (24). More recently, light microscopists (4, 14) have distinguished two major classes of these cells by the differences in their affinity for silver. Only those cells with granules that reduce silver salts without special pretreatment are considered to be true *argentaffin* cells. Other cells having the same general characteristics but requiring an exposure to a reducing substance before they will stain with silver have been designated as the *argyrophile* cells. The distinction between the two is not well established, however, since all argentaffin cells are apparently argyrophilic but only certain of them react directly with silver in the so-called argentaffin reaction. We have not carried out a correlated light and electron microscopic study to establish the fine structural distinction between these cells having differences in silver staining, but have simply used here the term, *argentaffin* cell, to include all granulated cells that seemed to fall into this category.

The electron microscopic studies of mouse gastric mucosa by Helander (11, 12) and of the rat by Kurosumi *et al.* (17, 18) describe the argyrophile cells as having a uniform fine structural appearance in these species. Kurosumi states that the true argentaffin cells are absent in the rat stomach and that this species possesses only argyrophilic cells as defined by staining characteristics. It is not clear at this time whether the methods used in their study failed to reveal the differences found in the bat stomach or whether the variability described here is peculiar to this species.

The differences in appearance of the granules in various argentaffin cells could well be related to a functional cycle of this cell, and it is not difficult to arrange the various forms in a plausible sequence supporting this view. On the other hand, the striking differences in mitochondrial form among the varieties of argentaffin cells argue somewhat against this possibility. In most cells, cyclic functional changes do not significantly affect mitochondrial morphology.

Another difference among the argentaffin cells of the bat gastric mucosa is the occurrence, though infrequent, of cells that have a slender portion of the apical cytoplasm extending to the glandular lumen. This has not been observed in the rat or mouse (11, 17), but Luse and Lacy (23) have observed luminal borders on argentaffin cells of the human stomach. The significance of these luminal extensions of the argentaffin cell is not known, but it does open the possibility of their secreting or absorbing substances from the glandular lumen. These findings are not entirely unexpected, for light microscopists had reported such forms of the argentaffin cell. Although the infrequent occurrence of cells that reach the lumen may be due, in part, to the plane of section, it seems reasonable to assume that most of the argentaffin cells do not abut on the lumen.

The infrequent occurrence of desmosomes along

the argentaffin cell membrane and their absence in most of the sections suggests that these cell may be free to wander from place to place within the epithelium. On the other hand, the uniform oval or elongate shape does not suggest that it is an active ameboid cell. The presence of terminal bars would also seem to indicate that this cell is a relatively fixed integral part of the epithelium. The site where argentaffin granules seem to be formed is the Golgi complex. It thus appears that this organelle is directly involved in the formation of the argentaffin granule in a manner similar to the formation of the secretory granules in the mucous cells. Little is known of the way these cells release their products. The preferential localization of the granules near the base has long been regarded as an indication of secretion into the circulatory system through the basal cell membrane. No fine structural evidence for this activity has yet been observed with the electron microscope.

It is apparent that the present description of the bat gastric mucosa leaves a number of areas to be further clarified. Much more information is needed to correlate the fine structure of the cells with their specific functional roles. It is hoped that further studies of the gastric mucosa during and after various types of stimulation will reveal more precisely the actual mechanism involved in the function of the various cells. Correlated fine structural and physiological studies, such as those being carried out by Sedar and his collaborators, can add much to our understanding of the histophysiology of the stomach.

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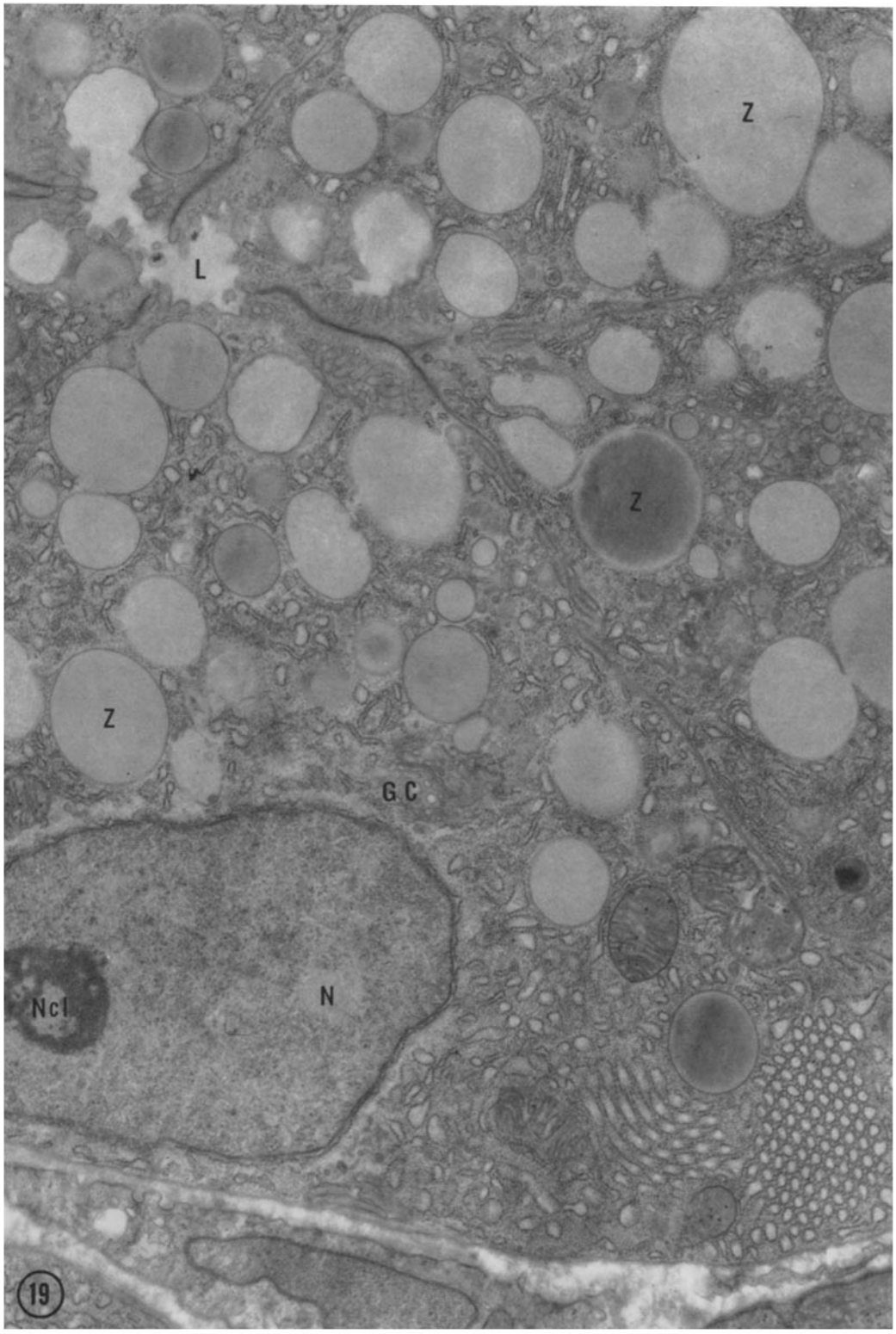
BIBLIOGRAPHY

1. BENNETT, H. S., and LUFT, J. H., *s*-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 113.
2. BURTNER, H. J., and LILLIE, R. D., A five hour variant of Gomori's methenamine silver method for argentaffin cells, *Stain Technol.*, 1949, **24**, 225.
3. DAVIES, R. E., The metabolism for the acid-secreting stomach, *Am. J. Digest. Dis.*, 1959, **4**, 173.
4. DAWSON, A. B., Argentophile and argentaffin cells in gastric mucosa of rat, *Anat. Rec.*, 1948, **100**, 319.
5. DEROBERTIS, E., and VAZFERREIRA, A., Electron microscope study of the excretion of cathecol-containing droplets in the adrenal medulla, *Exp. Cell Research*, 1957, **12**, 568.
6. FAWCETT, D. W., Observations on the cytology and electron microscopy of hepatic cells, *J. Nat. Cancer Inst.*, 1955, **15**, 1475.
7. FAWCETT, D. W., Physiological significant specializations of the cell surface, *Circulation*, 1962, in press.
8. HALLY, A. D., The fine structure of the gastric parietal cell of the mouse, *J. Anat.*, 1959, **93**, 217.
9. HALLY, A. D., Functional changes in the vacuole-containing bodies of the gastric parietal cell, *Nature*, 1959, **183**, 408.
10. HEIDENHAIN, R., Untersuchungen über den Bau der Labdrüsen, *Arch. mikr. Anat.*, 1870, **6**, 368.
11. HELANDER, H. F., A preliminary note on the ultrastructure of the argyrophile cells of the mouse gastric mucosa. *J. Ultrastruct. Research*, 1961, **5**, 257.

FIGURE 19

An electron micrograph of the apical portions of several chief cells arranged around the lumen of a gland (*L*). Included in one of these is a typical nucleus (*N*) and prominent nucleolus (*Nel*). The large zymogen granules (*Z*) in the apical cytoplasm vary in density. Some are uniformly dense while others have only a dense central zone and a pale or seemingly empty periphery. These differing appearances are probably due to incomplete preservation or to partial extraction during dehydration. The cytoplasmic matrix is crowded with both free and membrane-associated ribosomes. A small area at the lower right of the figure shows transverse and oblique sections of the tubular endoplasmic reticulum with attached granules. A Golgi complex (*GC*) is shown near the nucleus. × 13,000.


Z
L
Z
Z
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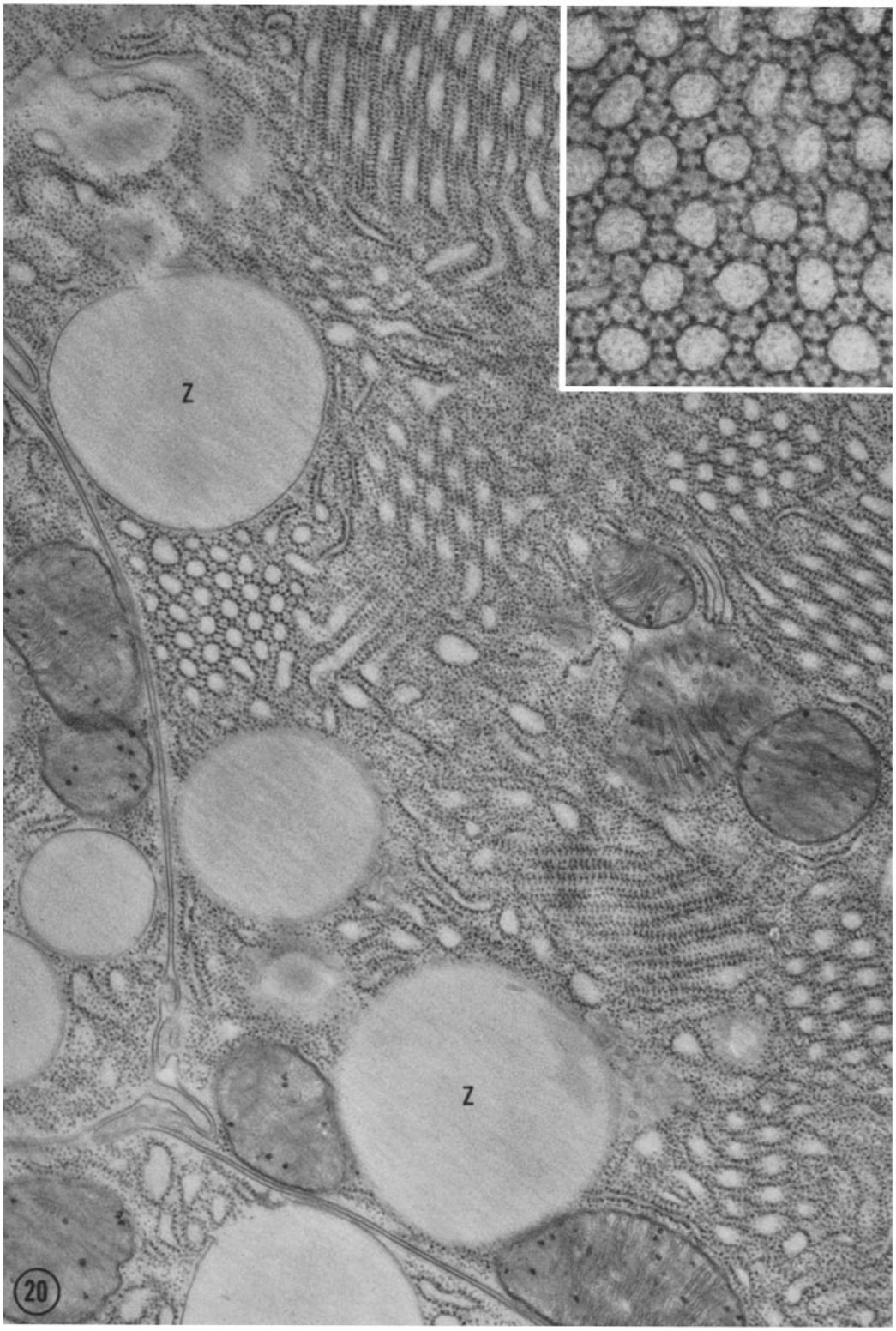
Ncl
N
19

12. HELANDER, H. F., Ultrastructure of fundus glands of the mouse gastric mucosa, *J. Ultrastruct. Research*, 1962, suppl. 4.
13. HELANDER, H. F., and ECKHOLM, R., Ultrastructure of epithelial cells in the fundus glands of the mouse gastric mucosa, *J. Ultrastruct. Research*, 1959, **3**, 74.
14. HELLWEG, G., Uber vorkommen und gegenseitiges verhalten der argentaffinen und argyrophilen zellen im menschlichen magen-darm-trakt, *Z. Zellforsch. u. Mikroskop. Anat.*, 1952, **36**, 546.
15. ITO, S., The endoplasmic reticulum of gastric parietal cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 333.
16. KARNOVSKY, M. J., Simple methods for "staining with lead" at high pH in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 729.
17. KUROSUMI, K., Electrom microscopic analysis of the secretion mechanism, *Internat. Rev. Cytol.*, 1961, **11**, 1.
18. KUROSUMI, K., SHIBASAKI, S., UCHIDA, G., and TANAKA, Y., Electron microscope studies on the gastric mucosa of normal rats, *Arch. Histol. Jap.*, 1958, **15**, 587.
19. LACY, P. E., Electrom microscopic identification of different cell types in the islet of Langerhans of the guinea pig, rat, rabbit, and dog, *Anat. Rec.*, 1957, **128**, 255.
20. LAWN, A. M., Observations on the fine structure of the gastric parietal cell of the rat, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 161.
21. LINDERSTRÖM-LANG, K., The distribution of enzymes in tissues and cells, *Harvey Lectures*, 1939, **34**, 214.
22. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
23. LUSE, S. A., and LACY, P. E., Electron microscopy of a malignant argentaffin tumor, *Cancer*, 1960, **13**, 334.
24. MACKLIN, C. C., and MACKLIN, M. T., The intestinal epithelium, *in* Special Cytology, (E. V. Cowdry, editor), New York, Paul Hoeber Inc., 2nd edition, 1932, 233.
25. PALADE, G. E., Functional changes in structure of cell components, *in* Subcellular Particles, (T. Hayashi, editor), New York, The Ronald Press Co., 1958, 64.
26. PEACHEY, L. D., and RASMUSSEN, H., Structure of the toad urinary bladder as related to its physiology, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 529.
27. RICHARDSON, K. C., JARETT, L., and FINKE, E. N., Embedding in epoxy resins for ultrathin sectioning in electron microscopy, *Stain Technol.*, 1960, **35**, 313.
28. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog (*Rana catesbiana*), *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 1.
29. SEDAR, A. W., The fine structure of the oxyntic cell in relation to functional activity of the stomach, *Ann. New York Acad. Sc.* 1962, **99**, 9.
30. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog, *Rana catesbiana*. III. Permanganate fixation of the endoplasmic reticulum. *J. Cell Biol.*, 1962, **14**, 152.
31. SEDAR, A. W., and FREIDMAN, M. H. F., Correlation of the fine structure of the gastric parietal cells (dog) with functional activity of the stomach, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 349.
32. STEVENS, C. E., and LEBLOND, C. P., Renewal of the mucosa cells in the gastric mucosa of the rat, *Anat. Rec.*, 1953, **115**, 231.
33. TRUMP, B., SMUCKLER, E. A., and BENDITT, E. P., A method for staining epoxy sections for light microscopy, *J. Ultrastruct. Research*, 1961, **5**, 343.
34. VIAL, J. D., and ORREGO, H., Electron microscope observations on the fine structure of parietal cells, *J. Biophysic and Biochem. Cytol.*, 1960, **7**, 367.
35. WATSON, M. L., Staining of tissue sections for

FIGURE 20

An electron micrograph showing parts of three chief cells from a hibernating bat. Much of the abundant granular reticulum is in the form of aggregations of hexagonally packed tubules of uniform diameter. Each tube has twelve evenly spaced rows of ribosomes on its outer surface. Transverse sections of these groups of tubules, presented at higher magnification in the inset, show each pair of ribosomes directly opposite a pair of ribosomes on each of the six adjacent tubes. In longitudinal sections tangential to the surface of the tubes, the ribosomes are seen to be arranged in straight rows. Although much of the reticulum in the cells shown here is aggregated in this unusual manner, some ordinary cisternae and numerous unattached ribosomes are present as well. *Z*, zymogen granules. × 26,000. Inset, × 67,000.

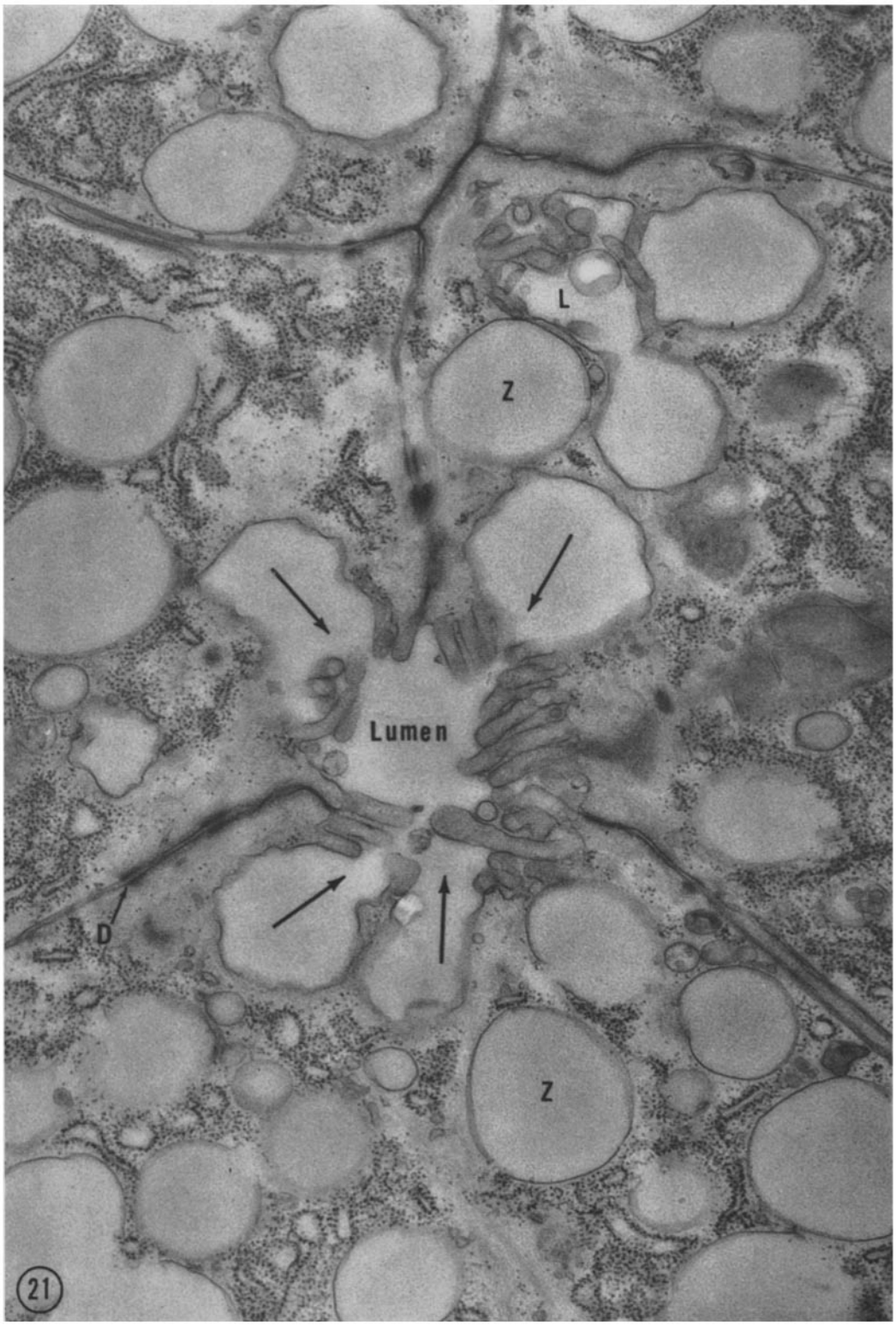

Z
Z
20

electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

36. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
37. YAMADA, E., The fine structure of the gall bladder epithelium of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 445.

FIGURE 21

A micrograph of the apical parts of several chief cells bordering the lumen of a gastric gland. Numerous large zymogen granules (*Z*) whose contents appear light are present. Four zymogen granules (at arrows) have their limiting membrane in continuity with the surface membrane. These are interpreted as representing a stage in the release of zymogen. The section also includes two more zymogen granules in continuity with another portion of the lumen (*L*). The cytoplasm between the granules is occupied by granular endoplasmic reticulum and free ribosomes. Well developed terminal bars and desmosomes (*D*) are visible on the lateral cell boundaries near the lumen. × 26,000.


L
Z
Lumen
D
Z
21

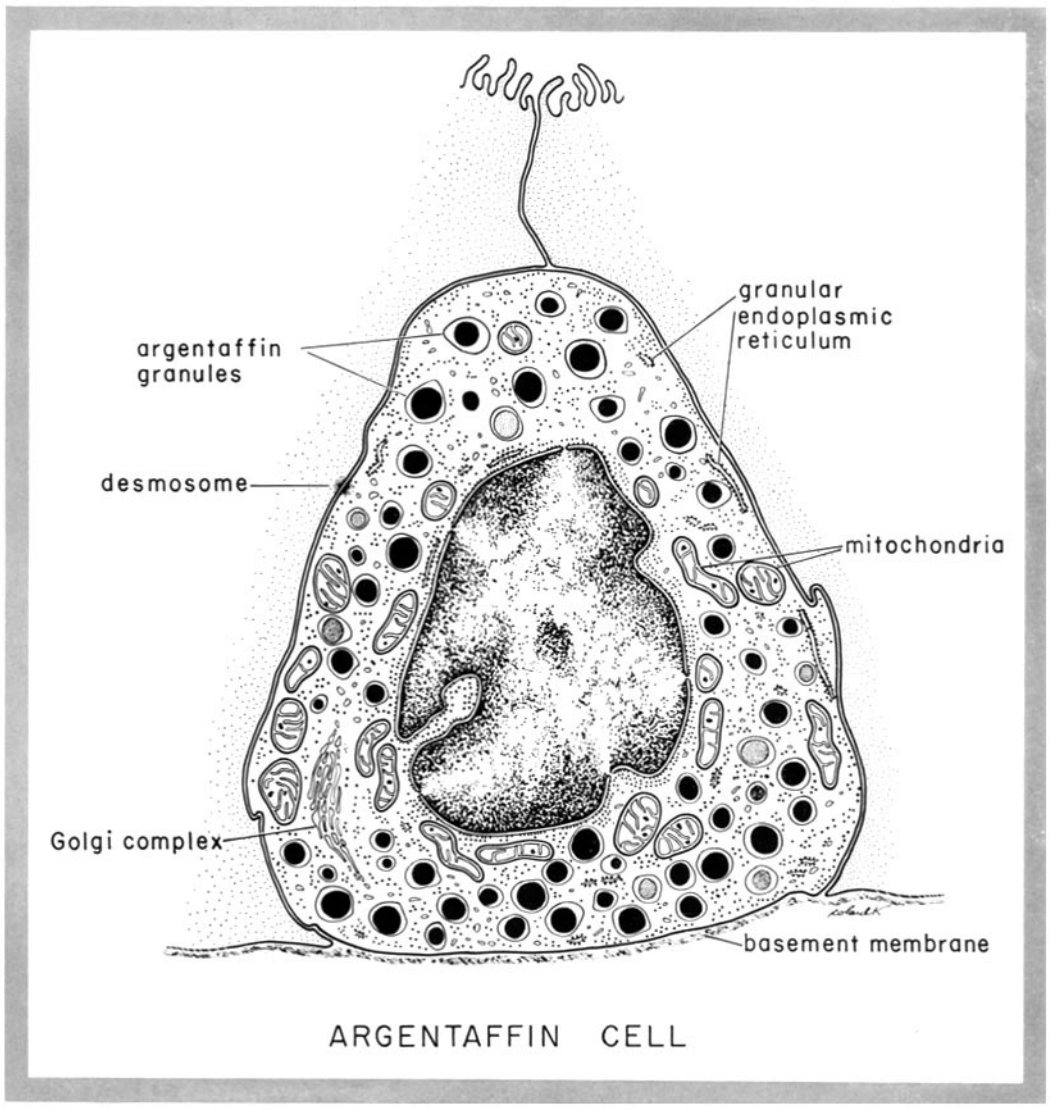
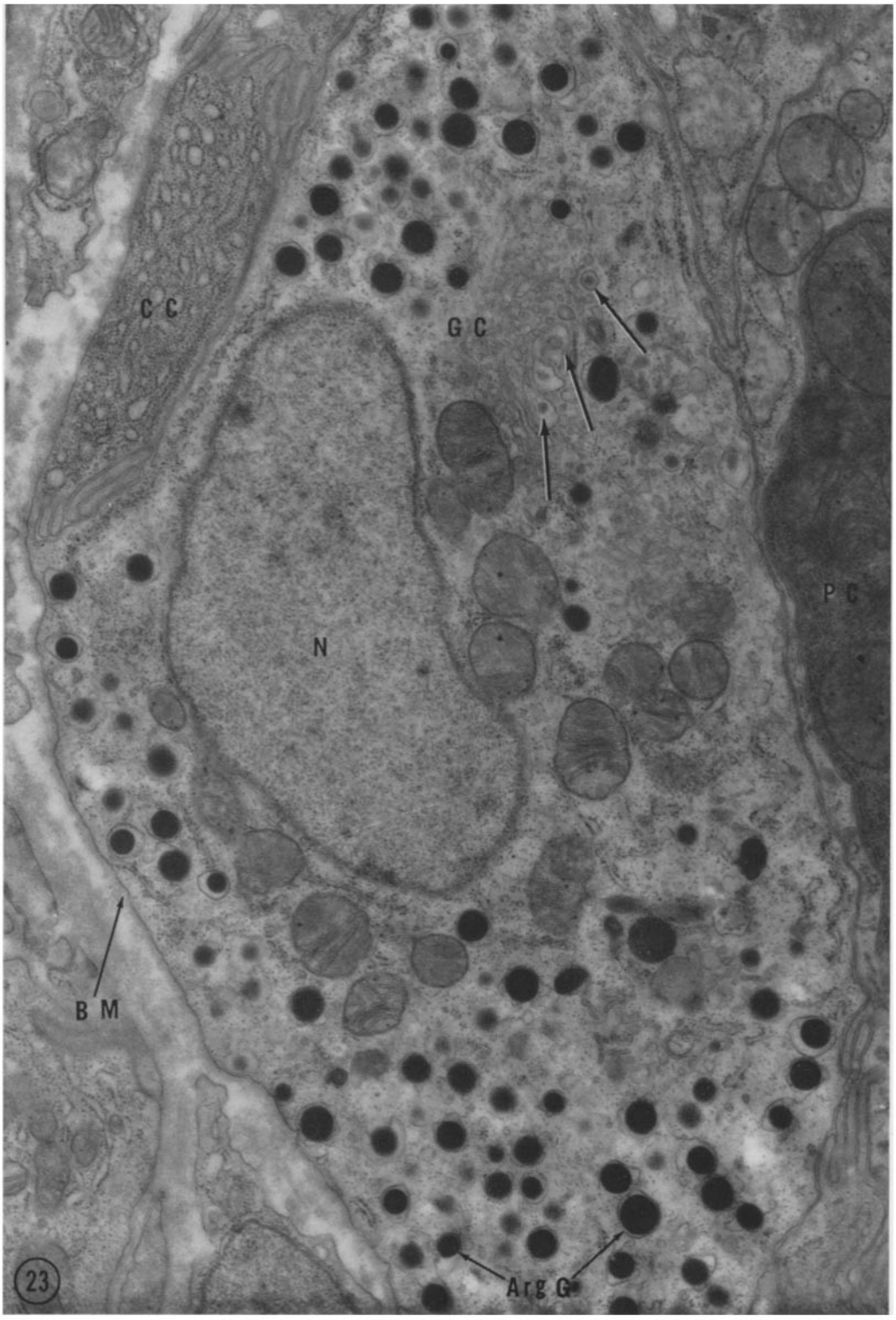


FIGURE 22

A diagram of a gastric argentaffin cell.

FIGURE 23

An electron micrograph of an argentaffin cell containing a uniform population of dense argentaffin granules (*Arg G*) each enclosed in a loose-fitting membrane. A broad expanse of the basal cell membrane is in contact with the basement membrane (*BM*) of the epithelium. The mitochondria are localized in the perinuclear region and resemble those found in mucous cells. There are a few cisternae of granular reticulum and free ribosomes in the cytoplasmic matrix. In the Golgi complex (*GC*) are several small vacuoles which contain a dense substance (arrows). These are interpreted as formative stages of argentaffin granules. Part of a chief cell (*CC*) and a parietal cell (*PC*) border the argentaffin cell. *N*, nucleus. × 16,000.


C C
G C
N
P C
B M
Arg G
23

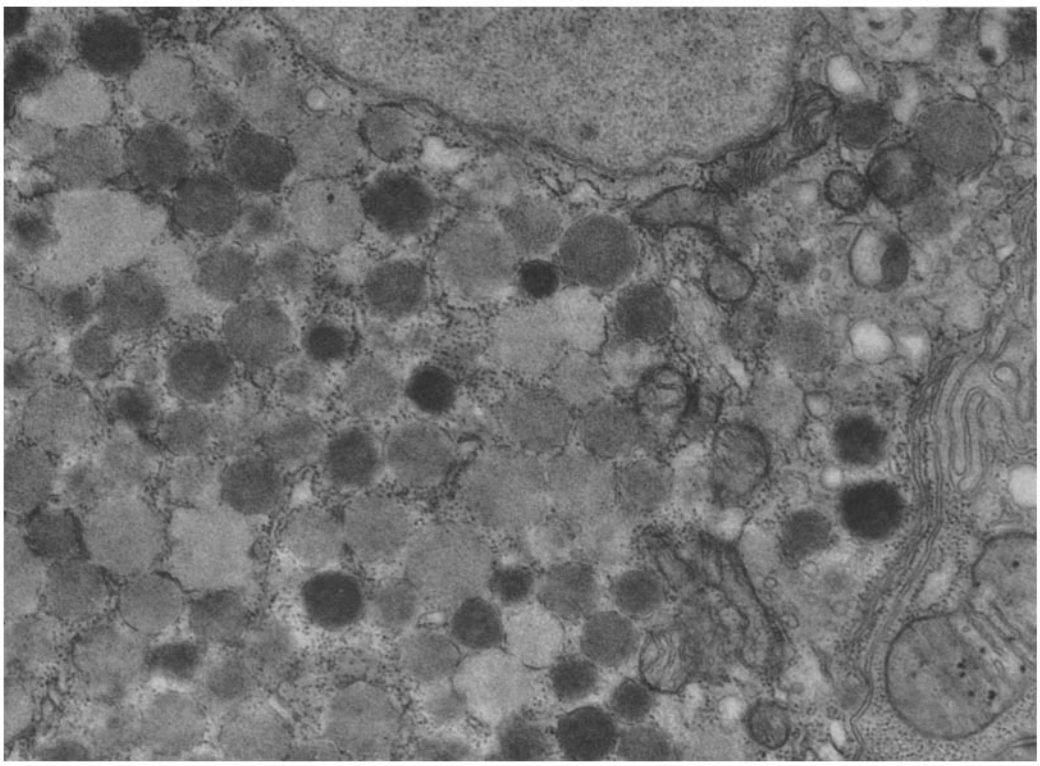


FIGURE 24

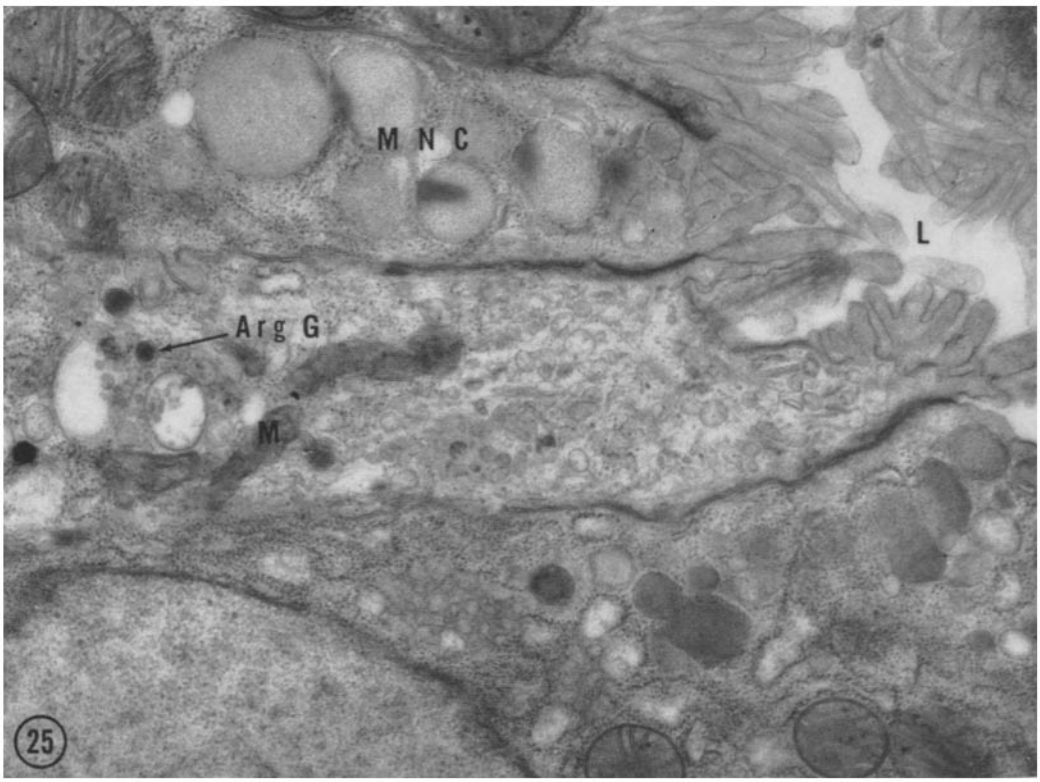
A part of an argentaffin cell having granules different in appearance from those illustrated in Fig. 23. These granules vary in their density, most of them being considerably lighter than those in the more typical argentaffin cell. The granules are enclosed by a closely adhering membrane which often appears discontinuous. The mitochondria are relatively small and have few cristae. Granular reticulum and free ribosomes are present in limited amounts between the argentaffin granules. A small part of a mucous neck cell is included at the right lower margin of the figure. × 22,000.

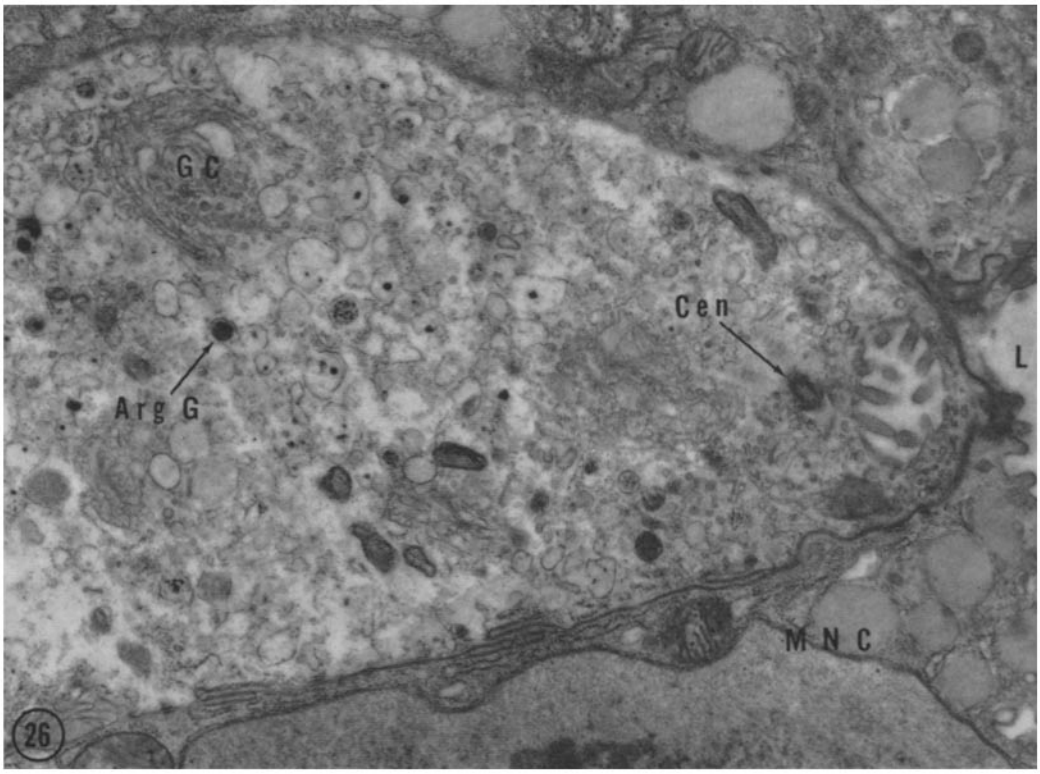
FIGURE 25

An electron micrograph showing the narrow apical end of an argentaffin cell extending to the lumen (*L*) between two mucous neck cells (*MNC*). As in other cell types of the glandular epithelium, the free surface of the argentaffin cell has a covering of microvilli. Terminal bars are prominent near the luminal surface of the plasma membranes, and a desmosome may be seen on the boundary between the argentaffin cell and the mucous cell above. Mitochondria (*M*) in this argentaffin cell are small and elongated, and have a poorly developed internal membrane system. A few argentaffin granules (*Arg G*) are present toward the base of the cell, while numerous smooth surfaced vesicles occupy much of the apical cytoplasm. × 20,000.

FIGURE 26

Part of an argentaffin cell whose apical end reaches to the lumen of the gastric gland (*L*). This plane of section passes through a depression of the free surface so as to give the erroneous impression of an intracellular vacuole lined with microvilli. Near the free surface is a centriole (*Cen*) in a position similar to that assumed by the basal body of a cilium. Lateral to the arrow indicating the centriole are two cross-striated structures suggestive of ciliary rootlets. The cytoplasm contains numerous small vacuoles, some enclosing dense material like that of argentaffin granules (*Arg G*) while the remainder either contain small dense granules or are empty. A rather well developed Golgi complex (*GC*) and some endoplasmic reticulum are also shown. *MNC*, mucous neck cell. × 12,000.


M N C
L
Arg G
M
25


G C
Cen
L
Arg G
M N C
26