STUDIES ON SMALL INTESTINAL CRYPT EPITHELIUM

I. The Fine Structure of the Crypt Epithelium of the Proximal Small Intestine of Fasting Humans

JERRY S. TRIER, M.D.

From the Division of Gastroenterology, Department of Medicine, University of Washington, Seattle. Dr. Trier's present address is the Department of Medicine, Veterans Administration Hospital, University of Wisconsin, Madison

ABSTRACT

Small intestinal crypt epithelium obtained from normal fasting humans by peroral biopsy of the mucosa was studied with the electron microscope. Paneth cells were identified at the base of the crypts by their elaborate highly organized endoplasmic reticulum, large secretory granules, and small lysosome-like dense bodies within the cytoplasm. Undifferentiated cells were characterized by smaller cytoplasmic membrane-bounded granules which were presumed to be secretory in nature, a less elaborate endoplasmic reticulum, many unattached ribosomes and, in some cells, the presence of glycogen. Some undifferentiated cells at the base of the crypts contained lobulated nuclei and striking paranuclear accumulations of mitochondria. Membrane-bounded cytoplasmic fragments, probably originating from undifferentiated and Paneth cells, were frequently apparent within crypt lumina. Of the goblet cells, some were seen actively secreting mucus. In these, apical mucus appeared to exude into the crypt lumen between gaps in the microvilli. The membrane formerly surrounding the apical mucus appeared to fuse with and become part of the plasma membrane of the cell, suggesting a merocrine secretory mechanism. Enterochromaffin cells were identified by their location between the basal regions of other crypt cells and by their unique intracytoplasmic granules.

INTRODUCTION

Attention has been focused on the villous epithelium in most studies of fine structure of the small intestine. Only scattered reports have appeared describing the fine structure of the crypt epithelium in mammalian laboratory animals. Enterochromaffin cells from the guinea pig (1) and rat (2) and Paneth cells from the bat (3) have been described briefly. More thorough reports of Paneth and goblet cell structure in mice (4, 5) are available. A number of brief descriptions of the fine structure of mouse undifferentiated crypt cells have also been recorded (6, 7). Electron microscopic studies of small intestinal crypts of man are even less common. The only descriptions of normal human crypt fine structure which have appeared in the literature are brief reports that serve only to introduce studies of a pathologic and experimental nature (8, 9).

To obtain adequate samples of small intestinal mucosa from laboratory animals generally requires anesthesia followed by surgical removal of intestinal tissue or sacrifice of the small animal. It is therefore difficult to do serial intestinal biopsies over a prolonged period of time in the same experi-

mental animal under physiologic conditions. In contrast, the recent perfection of peroral intestinal biopsy techniques (10, 11) allows rapid, repeated and safe sampling of the small intestinal mucosa from unanesthetized ambulatory human subjects. Previous studies (8, 9, 12, 13) have shown that these biopsy samples are suitable for electron microscopic as well as light microscopic study if they are immediately fixed and properly processed. Human volunteers are therefore superior to laboratory animals as subjects for the many experiments which require study of multiple samples of intestinal mucosa obtained by biopsy sequentially from the same individuals over a prolonged period of time.

This paper describes the fine structure of the human small intestinal crypt epithelium obtained from fasting normal volunteers. These observations on the normal humans will serve as a base line for future reports of experimental studies currently in progress which are designed to clarify some aspects of the functions of the various crypt epithelial cells.

MATERIALS AND METHODS

The subjects for this study were four healthy young adults without known gastrointestinal disease. In addition, one patient was studied who had previously undergone gastric resection for a duodenal ulcer and whose proximal jejunal mucosa was histologically normal. Volunteers fasted for 8 to 16 hours prior to study. Samples of mucosa were obtained by peroral biopsy of the distal duodenum or the proximal jejunum, under fluoroscopic control, with either a pull-wire activated (10) or a hydraulically activated (11) biopsy tube. Both tubes consistently provided comparable full-thickness samples of mucosa 4 to 8 mm in diameter which appeared untraumatized and were judged suitable for electron microscopic study.

The whole specimens were placed in either Dalton's chrome–osmium tetroxide (14) or in 3.3 per cent *s*-collidine buffered osmium tetroxide fixative (15) within 1 minute after separation from their blood supply. Four minutes later the intact specimens were removed and cut into 1 mm-thick slices with a sharp razor blade (13) and returned to the fixing solution for 1 to 2 hours. The chrome–osmium tetroxide fixed samples were then placed in 10 per cent neutral isotonic formol for another hour. After fixation by either method, the tissue slices were rapidly dehydrated in graded strengths of ethyl alcohol and embedded in epoxy resin by the method described by Luft (16).

To facilitate orientation of the tissue and to select known areas of epithelium for electron microscopic study, sections of the entire tissue block were cut 1 to 2 μ thick, mounted on glass slides, stained with toluidine blue (17) and examined by light microscopy. Well preserved crypts were identified and the tissue blocks were suitably trimmed for thin sectioning. Thin sections were cut on Porter-Blum or LKB microtomes with diamond knives. Sections were mounted on carbon-coated copper mesh grids and were stained with lead salts, using Millonig's (18) or Karnovsky's (19) techniques. Specimens were examined with an RCA EMU 2C electron microscope.

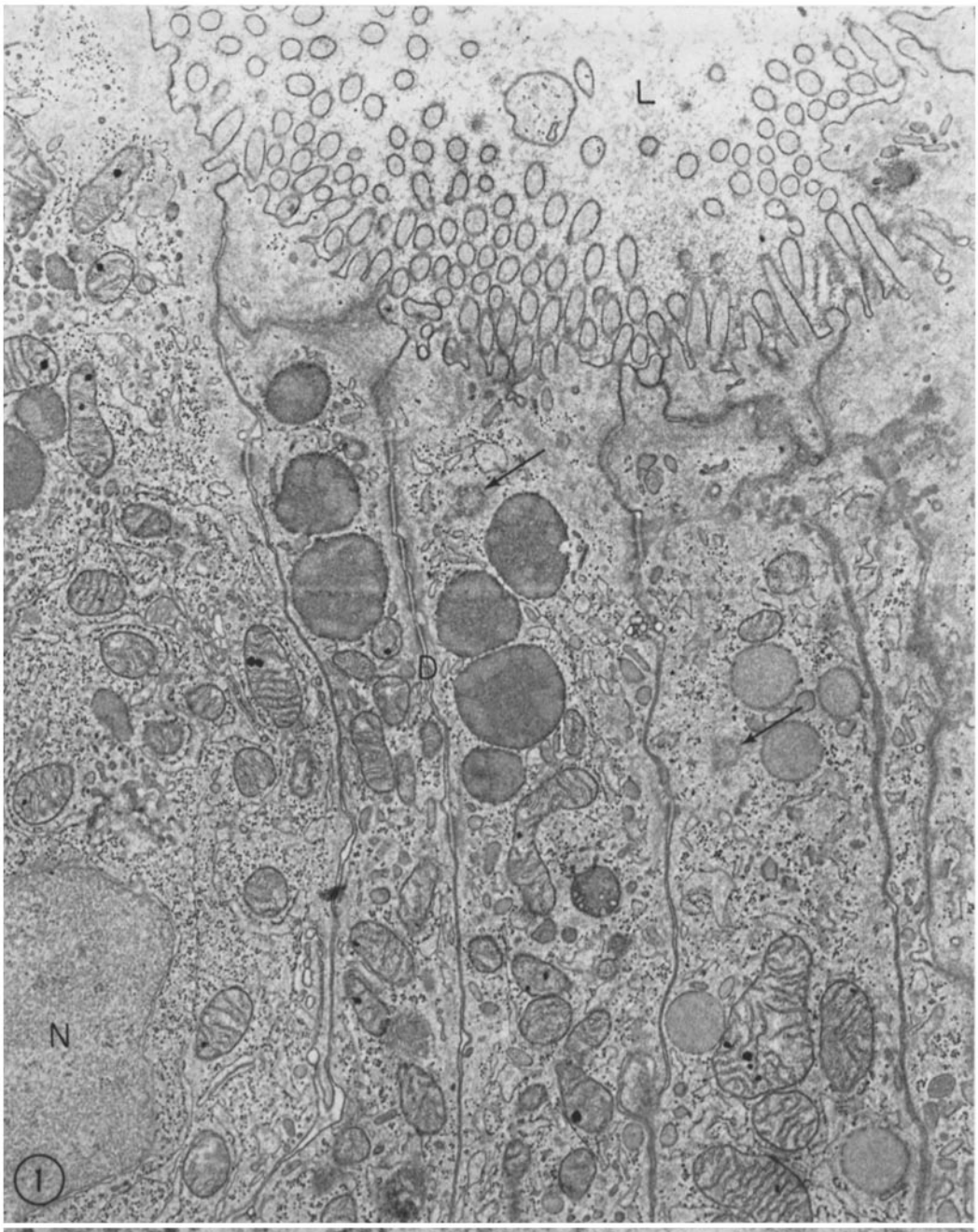
RESULTS

The crypt epithelium is composed of a single layer of columnar cells whose apical free surfaces form the crypt lumen. A thin, continuous basement membrane is closely applied to the base of the crypt epithelial cells, separating them from the surrounding lamina propria.

As previously described by light microscopists, the human intestinal crypt epithelium is composed of four distinct types of epithelial cells (20). These cell types, the undifferentiated, goblet, Paneth, and enterochromaffin cells, can be readily distinguished by electron microscopy.

FIGURE 1 Upper portion of several undifferentiated cells. The crypt lumen (*L*) is in the upper portion of the micrograph. Many membrane-bounded granules of different sizes presumably represent secretory material. Desmosomes (*D*) are seen along the lateral plasma membranes. Mitochondria, ribosomes, and elements of the endoplasmic reticulum are distributed throughout the cytoplasm. Centrioles (arrows) are apparent in two of the cells. A portion of the nucleus (*N*) of one of the cells is evident. Chrome–osmium tetroxide fixation. Approximately × 12,500.

FIGURE 2 A chain of five desmosomes along the lateral plasma membranes of two adjacent undifferentiated cells. At this magnification some of the cytoplasmic filaments are seen intimately associated with the desmosomes. *s*-Collidine–osmium tetroxide fixation. Approximately × 81,000.


L
D
N
1
2

UNDIFFERENTIATED CELLS: The undifferentiated cells are the most abundant epithelial cell type in our material. They are columnar unless actively undergoing mitosis, in which case they have a more spherical shape. The nuclei are generally elliptical in sections and are located in the basal half of the cells. Nucleoli are prominent and the nuclear surface may be somewhat irregular.

The luminal surface of the undifferentiated cell has many microvilli (Figs. 1, 4, and 11). These, however, are shorter, wider and less numerous than those on the absorptive cells of the villus (21). Cytoplasmic bridges occasionally interconnect adjacent microvilli (Fig. 3). Short, thin filaments regularly extend perpendicularly into the crypt lumen from the extracellular surface of the microvilli (Figs. 1, 3, 4, and 9). In addition, fine intracellular filaments can be identified regularly in the central core of the microvilli. In the crypt cells, however, these do not terminate or interdigitate in a well developed terminal web as they do in the villous epithelium (9, 22). Instead they course in bunches into the apical cytoplasm of the cell where they may be joined by filaments from neighboring microvilli. These aggregates of filaments then penetrate to a depth of approximately 1 to 3 μ into the apical cytoplasm and there appear to end abruptly, occasionally just above a centriole or, if located laterally, near a desmosome (Figs. 4 and 9).

The lateral plasma membranes of the undifferentiated cells pursue a straight course with only occasional interdigitations with neighboring cells. At their apical limits one sees "tight junction" zones similar to those described by Farquhar and Palade in intestinal epithelium (23). Many desmosomes are present along the remainder of the lateral plasma membrane (Figs. 1, 2, and 4). Cross-sections reveal these to be similar in structure to those described by Karrer in human cervical epithelium (24), with five thin, dense strata separated by four lower density layers (Fig. 2). Fine intracytoplasmic filaments are intimately associated with the dense cytoplasmic attachment plaques at the periphery of the desmosomes. The desmosomes may occur singly or in chains of two to six or more separated only by short lengths of lateral plasma membrane (Figs. 1 and 2). The desmosomes and tight junctions anchoring Paneth cells, goblet cells, and enterochromaffin cells to one another and to adjacent undifferentiated cells appear identical in their structure with those seen

FIGURE 3 A portion of the apical surface of an undifferentiated cell. Four cytoplasmic bridges are seen connecting two adjacent microvilli. Fine filaments are apparent in the cores of the microvilli. *s*-Collidine–osmium tetroxide fixation. Approximately × 40,000.

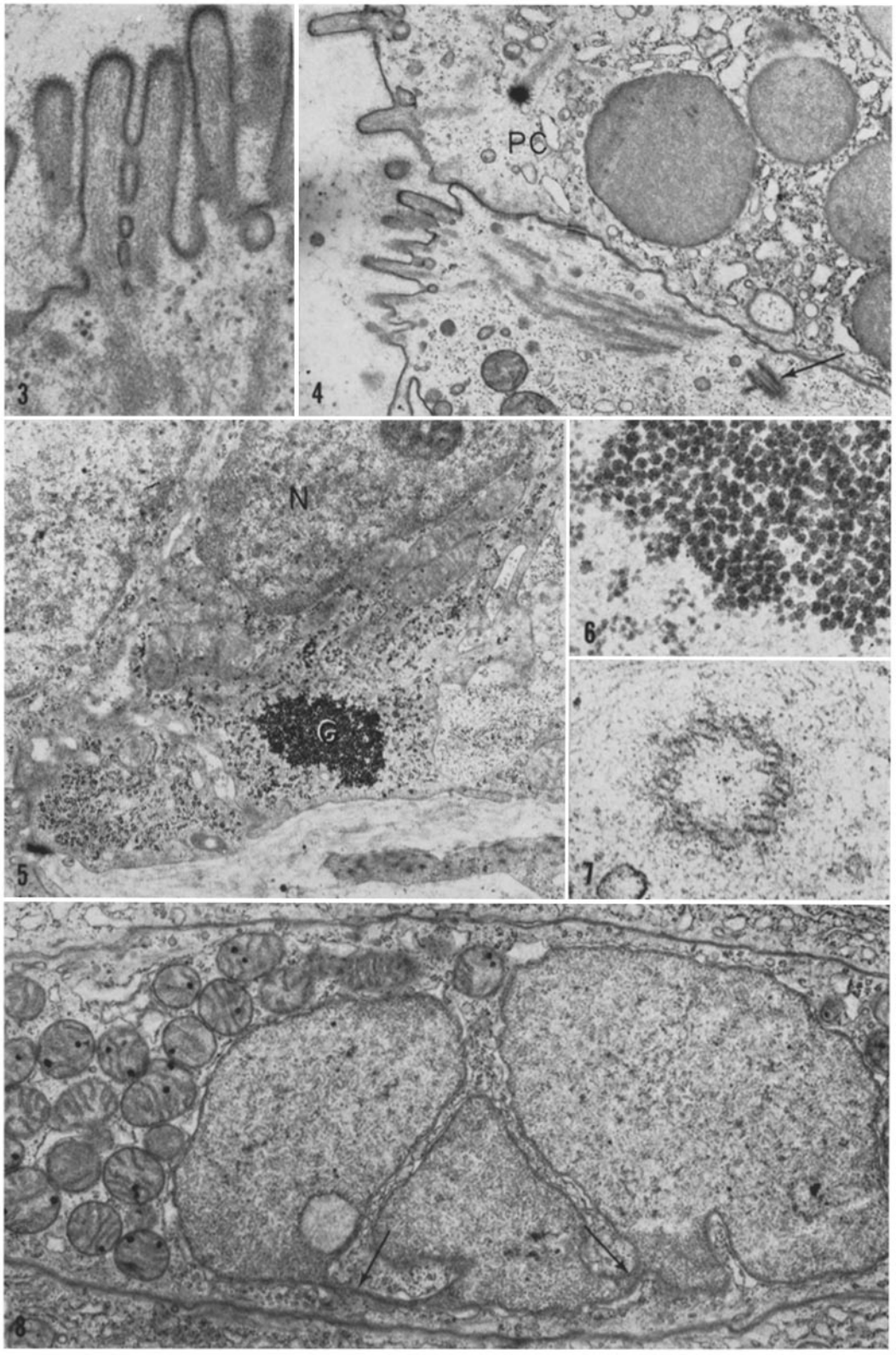
FIGURE 4 A portion of cytoplasm in the upper parts of an undifferentiated and an adjacent Paneth cell (*PC*). Intracellular filaments, presumably originating in the microvilli of the undifferentiated cell, course towards a tangentially sectioned centriole (arrow). *s*-Collidine–osmium tetroxide fixation. Approximately × 13,000.

FIGURE 5 Basal region of several undifferentiated cells. In one of the cells there is an accumulation of closely packed, dense granules (*G*) below the nucleus (*N*). These granules have a greater diameter than do ribosomes in the surrounding cytoplasm and presumably represent glycogen. Chrome–osmium tetroxide fixation. Approximately ×14,000.

FIGURE 6 Higher magnification of a portion of the glycogen accumulation and adjacent cytoplasm shown in Figure 5. Chrome–osmium tetroxide fixation. Approximately × 70,000.

FIGURE 7 A centriole in an undifferentiated cell, cut in cross-section. The centriole is composed of nine groups of circularly arranged, three-unit tubules. *s*-Collidine–osmium tetroxide fixation. Approximately × 70,000.

FIGURE 8 Nucleus and supranuclear cytoplasm of one of the peculiar undifferentiated cells, between two Paneth cells, at the base of a crypt. The polymorphic appearance of the nucleus is apparent. Attenuated bridges of nuclear material (arrows) connect lobules of the nucleus. A striking supranuclear accumulation of mitochondria is seen. *s*-Collidine–osmium tetroxide fixation. Approximately × 17,000.


3
4
PC
N
G
5
6
7
8

along the lateral membranes of adjacent undifferentiated cells (Figs 4, 9, 13, and 24).

Many irregularly shaped, fairly large mitochondria with numerous more or less transversely oriented cristae and many prominent intramitochondrial dense bodies are present in the undifferentiated cells. Short strands and vesicles of granular and smooth-surfaced endoplasmic reticulum as well as abundant free ribosomes are distributed throughout the cytoplasm. Moderate amounts of Golgi material are located about the upper pole of the nucleus and, occasionally, multivesicular bodies are apparent in the cytoplasm near the luminal surface of these cells.

Typical centrioles are frequently encountered in the apical third of the undifferentiated cell cytoplasm (Figs. 1 and 4). In cross-sections of these centrioles, nine groups of circularly arranged, three-unit tubules can be identified embedded in a moderately dense, homogeneous matrix (Fig. 7).

Many membrane-bounded granules of different sizes and shapes are seen in the upper half of the cell. The larger of these granules are up to 1.5 μ in diameter and usually appear as circular profiles (Fig. 1). These larger granules can be resolved with the light microscope and are of a deep magenta color after staining with the periodic acid–Schiff technique (9). The smaller granules often measure less than 0.1 μ in their largest diameter and may be circular, but frequently are oval, rod-shaped or even filamentous in form (Figs. 1, 4, and 15). These granules contain homogeneous material which may differ in electron opacity from granule to granule. Occasionally, individual granules contain materials of different densities and thus appear mottled (Fig. 1).

In addition, granules with an average diameter of 250 A which are not membrane-bounded are seen in some undifferentiated cells (Figs. 5 and 6). After lead staining, these granules are extremely dense and, under higher magnification, fine punctate dense spots are apparent in the matrix of the granule (Fig. 6). These granules have been found in both the basal and apical cytoplasm of the undifferentiated cells and it seems likely that they represent accumulations of glycogen.

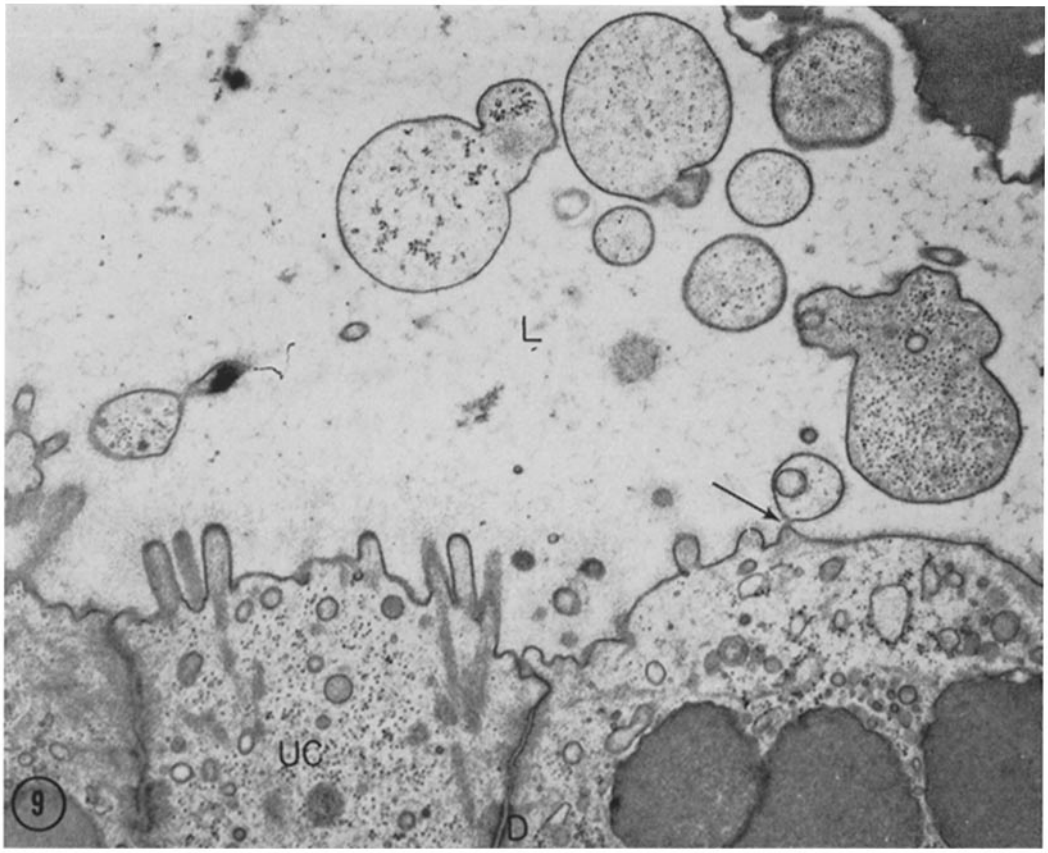
Membraned-bounded cytoplasmic fragments are often seen free in the crypt lumina (Figs. 1, 9, and 16). Some of these are located close to the apical surface of the undifferentiated and Paneth cells so that they may represent cytoplasmic extensions which communicate directly with the cell beyond the plane of section. Other cytoplasmic fragments, however, are located so far from the apical surface that there is little doubt that they are indeed lying free in the crypt lumina. These cytoplasmic fragments are completely enclosed by a typical triple-layered plasma membrane. They contain ribosomes, vesicles and the previously described small, membrane-bounded granules found in undifferentiated cells (Figs. 9 and 16). The appearance in some sections suggests that these fragments pinch off from the apical cytoplasm of the crypt cells (Fig. 9).

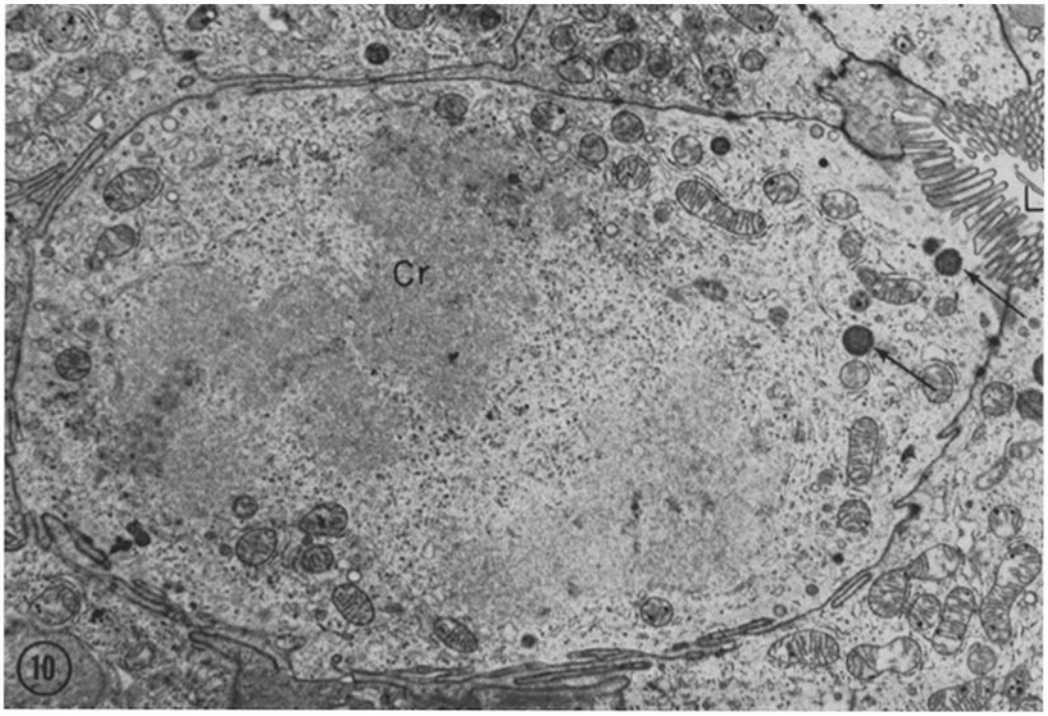
Undifferentiated cells are frequently seen in mitosis (Fig. 10). The dividing cells have a more spherical shape and the nucleus migrates toward the apical pole of the cell before dividing. Chromosome masses can be identified as clumps of fine, homogeneously granular material. It is of interest that the membrane-bounded granules are present in the apical cytoplasm even in the dividing undifferentiated cell (Fig. 10).

Cells resembling undifferentiated cells but having certain unique morphologic characteristics are seen in the base of the crypts adjacent to and interspersed among Paneth cells. The nuclei of these cells are lobulated so as to resemble the nuclei of polymorphonuclear leukocytes (Fig. 8). Thin bridges of nuclear material connect the lobules of

FIGURE 9 Apical cytoplasm of an undifferentiated cell (*UC*) flanked by two Paneth cells In the undifferentiated cell, intracellular filaments course from the cores of the microvill towards a desmosome (*D*). A number of membrane-bounded cytoplasmic fragments are seen in the crypt lumen (*L*). Such a fragment appears to be pinching off from the apical surface of one of the Paneth cells (arrow). *s*-Collidine–osmium tetroxide fixation. Approximately × 14,000.

FIGURE 10 An undifferentiated cell in mitosis. Chromosomal masses (*Cr*) are evident. It is of interest that granules (arrows), presumably representing secretory material, are present in the dividing cell. The crypt lumen (*L*) is seen in the upper right. *s*-Collidine–osmium tetroxide fixation. Approximately × 7,000.


L
UC
D
9


Cr
L
10

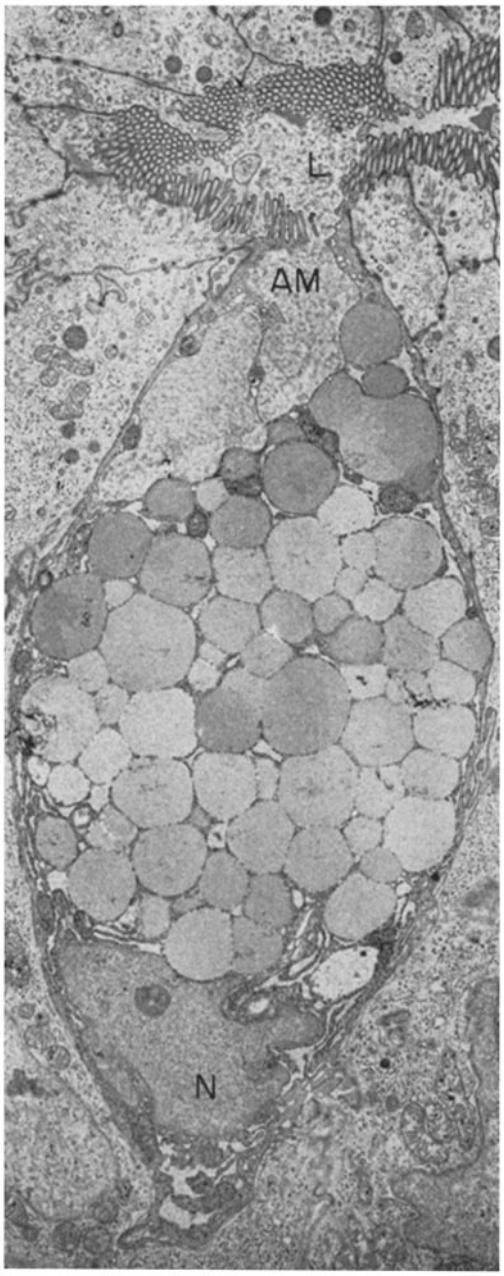


FIGURE 11 Longitudinally sectioned goblet cell filled with mucous granules. The difference in structure between the apical mucus (*AM*), which is being discharged into the crypt lumen (*L*), and most of the mucous granules in the remainder of the cytoplasm is apparent. The nucleus (*N*) is located at the base of the cell. *s*-Collidine–osmium tetroxide fixation. Approximately × 4,500.

the nucleus with one another. Striking paranuclear accumulations of mitochondria are another constant feature of these cells (Figs. 8 and 16). These mitochondria are similar in structure to those seen in the more characteristic undifferentiated cells. The cytoplasmic matrix in some of these cells is considerably less electron-opaque than that of other crypt cells (Fig. 16). In other respects, these cells resemble structurally the undifferentiated cells seen in the lateral wall of the crypts. They contain similar membrane-bounded granules, and other cytoplasmic organelles are similar in structure and distribution.

GOBLET CELLS: Goblet cells occur regularly in the lateral epithelial walls of the crypt. They are generally flanked by undifferentiated cells, though occasionally adjacent goblet cells or Paneth cells are found. As their name implies, their shape when distended with mucous granules resembles a brandy goblet. Those goblet cells which contain little mucus may resemble undifferentiated cells in their structural characteristics; however, they can usually be easily identified by the presence of a small number of mucous granules, a more elaborate ergastoplasm and Golgi complex and by their more electron opaque cytoplasm.

The nuclei of mucus-filled goblet cells are located in the basal portion of the cells and have a more or less semi-circular appearance when sectioned (Fig. 11). Microvilli are present on the apical surface. The cytoplasm between the nucleus and the apical border is distended with mucous granules. These granules are enveloped by a very fine membrane which frequently appears fragmented, perhaps due to artifact. The individual mucous granules within a cell may vary considerably in their electron opacity, and those closest to the supranuclear Golgi material often, but not always, are the least dense. The matrix of the mucous granules consists of homogeneously precipitated, fine particulate material.

Towards the apical surface of the cell, some of the mucous granules are larger. These larger granules have a less homogeneous appearance. Instead, their structure is characterized by more coarsely precipitated material embedded within an electron-transparent matrix (Fig. 11). In some sections this apical mucus appears to exude into the crypt lumen through gaps in the apical cell membrane between microvilli (Figs. 12 and 13). In this manner, the most apically located mucus

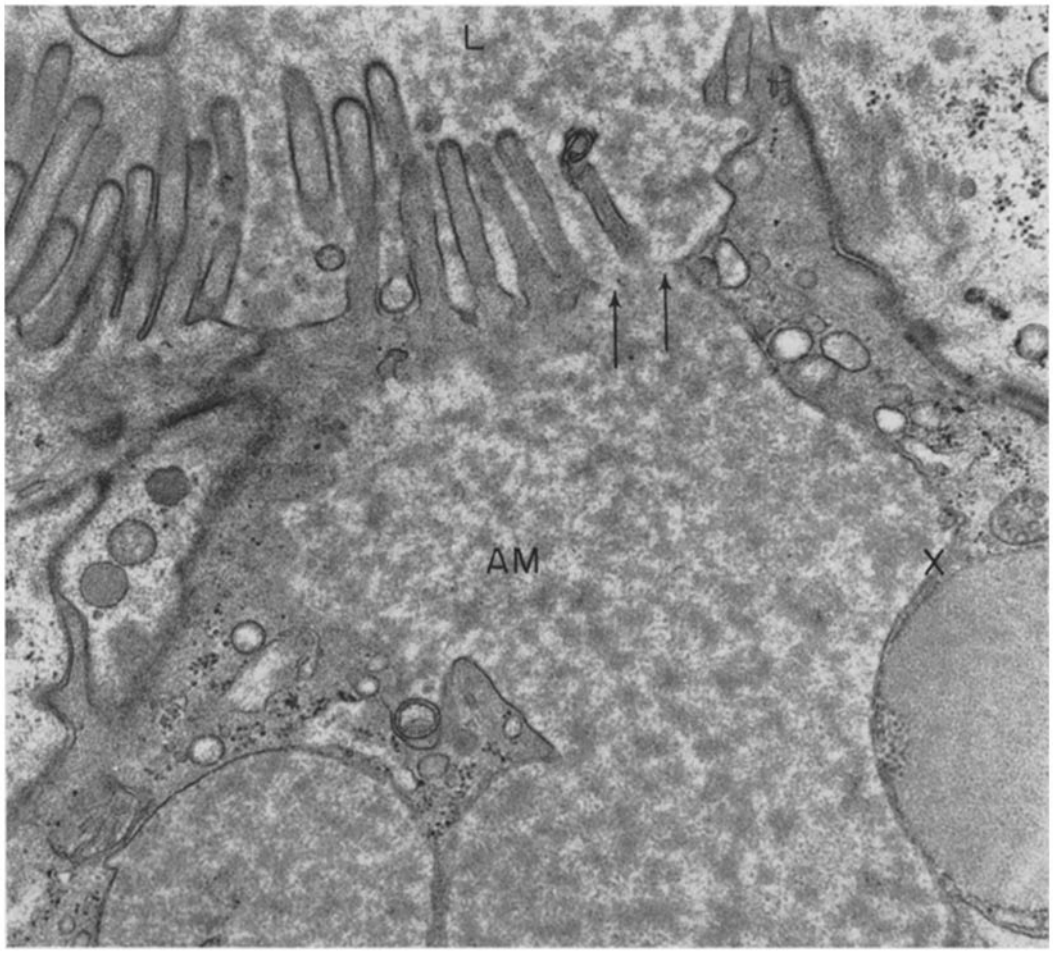


FIGURE 12 Higher magnification of the cytoplasm of the apical portion of the goblet cell seen in Fig. 11. Gaps (arrows) in the cell membrane, through which apical mucus (*AM*) is being delivered into the crypt lumen (*L*), are apparent between microvilli. The membrane (*X*) which formerly surrounded the apical mucus now separates the cytoplasm below the apical mucus from the crypt lumen and can be clearly seen on the right of the micrograph. It is less apparent on the left where it is tangentially sectioned. *s*-Collidine–osmium tetroxide fixation. Approximately × 25,000.

may communicate directly with the crypt lumen, while the cytoplasm below is separated from the lumen by the membrane formerly surrounding the apical mucus (Figs. 11–13 and 15). This membrane appears to fuse with and become part of the plasma membrane of the cell. In some cells, the region just below the microvilli which communicates with the crypt lumen appears virtually empty (Fig. 15). The mucus once located in this area has presumably emptied into the crypt lumen. In cells such as this, the true apical cell membrane is clearly the membrane which partially encloses this relatively empty space (Fig. 15). Intact membrane-bounded mucous granules were not seen free in the crypt lumina in our material.

Cytoplasmic organelles, including mitochondria, ergastoplasm, and free ribosomes, are located at the lateral and inferior margins of the cell and between mucous granules. The Golgi material is generally located just above the nucleus and, more rarely, between supranuclear mucous granules.

Occasionally, Golgi cisternae appear to empty into mucous granules (Fig. 14). However, the limiting membrane of the mucous granule is delicate and from our data one cannot be certain

of the structural continuity of cisternal and granular contents.

PANETH CELLS: The Paneth cells are the major cell type in the base of the crypts and may extend up the lateral wall a short distance. Occasionally, one or more Paneth cells can be seen considerably higher up in the crypt. Paneth cells appear columnar in shape and are wider at their base than at their apical surface (Fig. 16). The nuclei which appear more or less circular when sectioned are located in the basal portion of the cells.

Microvilli, though present along the apical surface of the Paneth cells, are considerably less numerous and more irregular in height than those of the undifferentiated cells (Fig. 9). As in undifferentiated cells, fine filaments course several microns into the apical cytoplasm from the microvillous cores. Mitochondria are scattered throughout the Paneth cell cytoplasm and are similar in structure to those seen in other crypt cell types. Centrioles, identical in structure with those in undifferentiated cells, are frequently encountered in Paneth cells. They are not confined to the centrosomal regions but are often seen in the apical third of the cytoplasm as well.

Many large, membrane-bounded secretory granules are seen in the cytoplasm between the nucleus and the apical surface. The matrix of the Paneth cell granules is composed of closely packed, fine particles with a width of 50 to 200 A (Fig. 18). These particles are less closely packed in those granules which are located within the supranuclear Golgi material than in the granules located towards the free surface of the cell. Consequently, many of the granules in and near the Golgi material appear less dense (Figs. 16 and 17). In some of the granules located in Golgi material, the fine particles within the matrix appear coarsely clumped (Fig. 20).

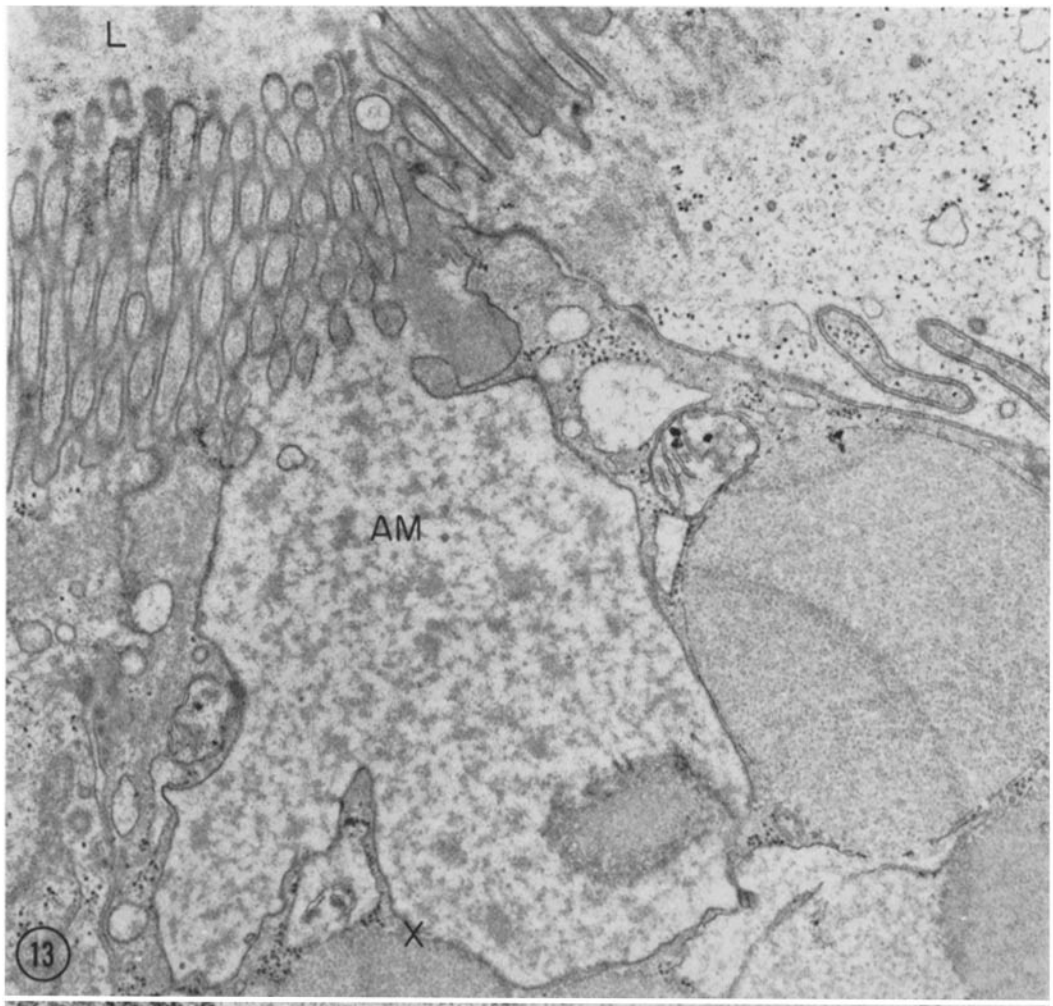
The Golgi material of the Paneth cells is extensive and elaborate and is reminiscent of that seen in pancreatic acinar cells (Figs. 17, 20, and 21). Many flattened Golgi cisternae are seen and these generally appear empty or filled with irregular clumps of finely granular material. Numerous small, membrane-bounded vesicles about 40 to 150 mμ in diameter are present in the Golgi region (Figs. 17 and 20). These contain fine particulate material and are frequently seen in large numbers next to pale secretory granules. Certain sections suggest that the membranes surrounding some of these small vesicles may fuse with the membranes surrounding the secretory granules and that the contents of the vesicle are incorporated into the granule (Figs. 17 and 20). The other alternative, namely, that these small vesicles pinch off from the secretory granule cannot be ruled out, of course.

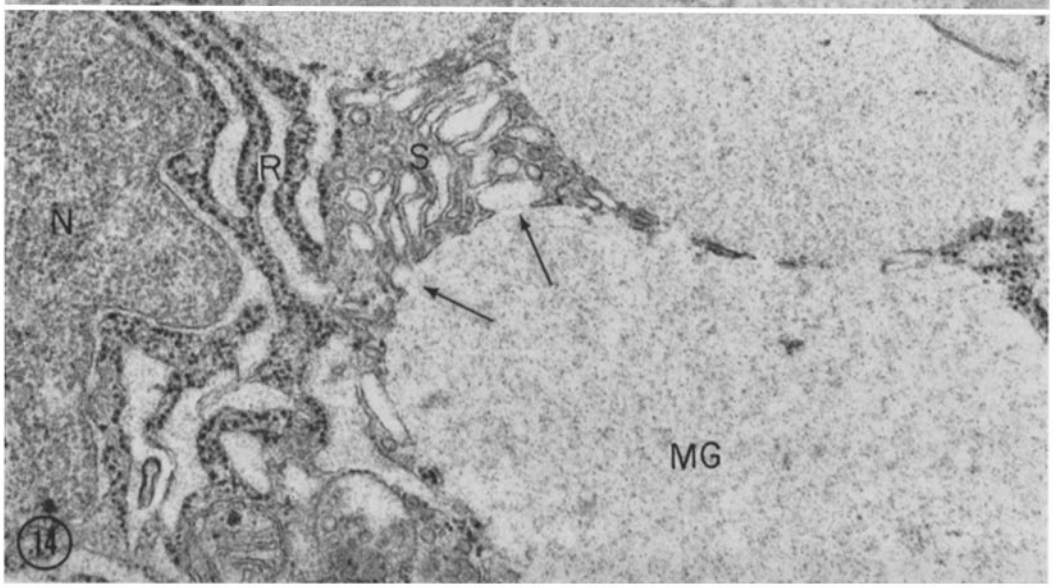
An elaborate, highly developed, ribosome-studded endoplasmic reticulum fills the Paneth cell cytoplasm not occupied by other organelles. Its organization is similar to that of the granular reticulum of the pancreas (25, 26). It is most extensive in the basal portion of the cell but it is also apparent in other regions, being consistently absent only from the extreme apical rim of cytoplasm beneath the microvilli (Figs. 4, 9, and 16). Many of the cisternae are elongate and flattened but others appear circular when sectioned. All contain sparse amounts of finely granular material. In the zone at the periphery of the Golgi region, many cisternae are seen in which the external surfaces facing the granular endoplasmic reticulum are studded with ribosomes while the external surfaces of the same cisternae facing Golgi material are devoid of granules (Fig. 21). This suggests a close structural interrelationship between the granular endoplasmic reticulum and the agranular membranous material of the Golgi re-tion.

Small, irregularly shaped membrane-bounded

FIGURE 13 Apical portion of a crypt goblet cell. Again, apical mucus (*AM*) can be seen entering crypt lumen (*L*) through gaps in the cell membrane between microvilli. Here the membrane (*X*) that previously enclosed the apical mucous granule and now forms the true apical cell membrane can be seen throughout its course. *s*-Collidine–osmium tetroxide fixation. Approximately × 24,000.

FIGURE 14 Supranuclear region of a goblet cell. The nucleus (*N*) is to the left. Granular endoplasmic reticulum (*R*) is present, as is Golgi material (*S*). Some of the Golgi cisternae appear to communicate (arrows) with a mucous granule (*MG*). *s*-Collidine–osmium tetroxide fixation. Approximately × 31,500.


L
AM
X
13


R
S
N
MG
14

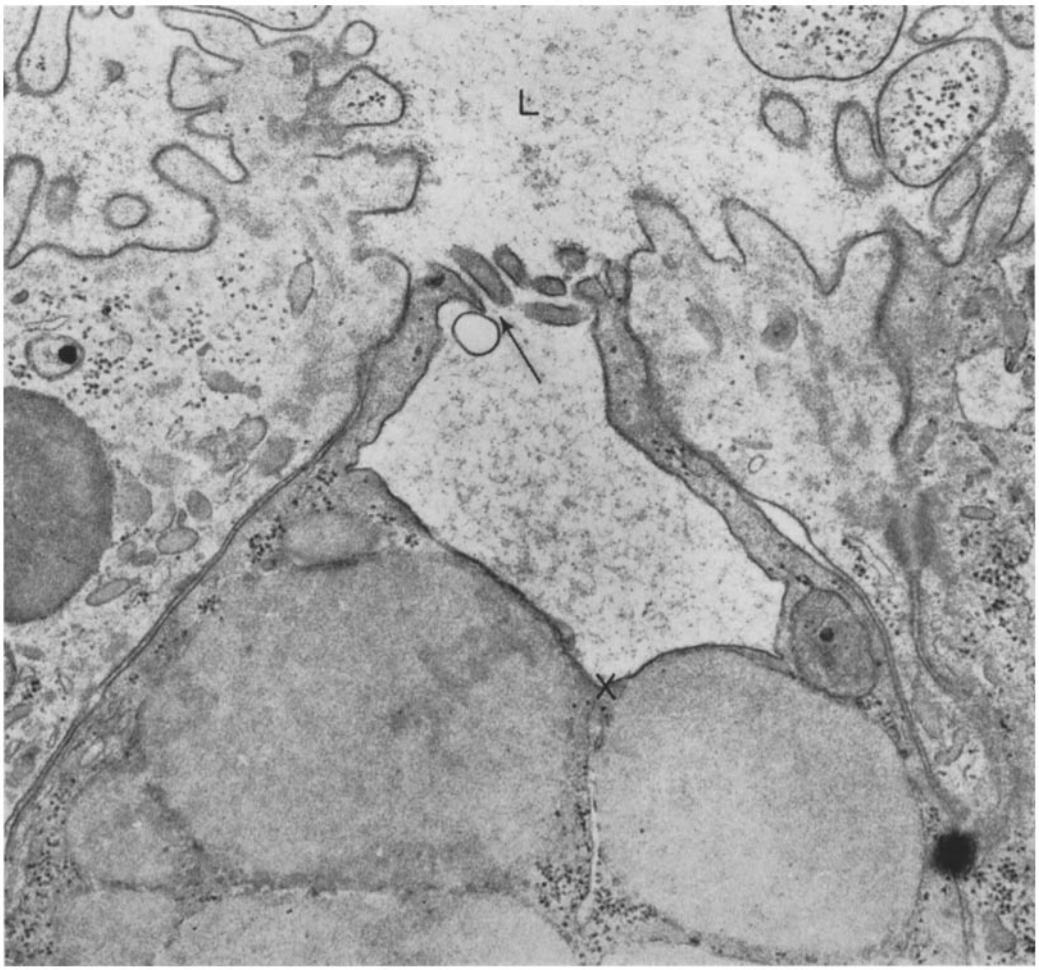
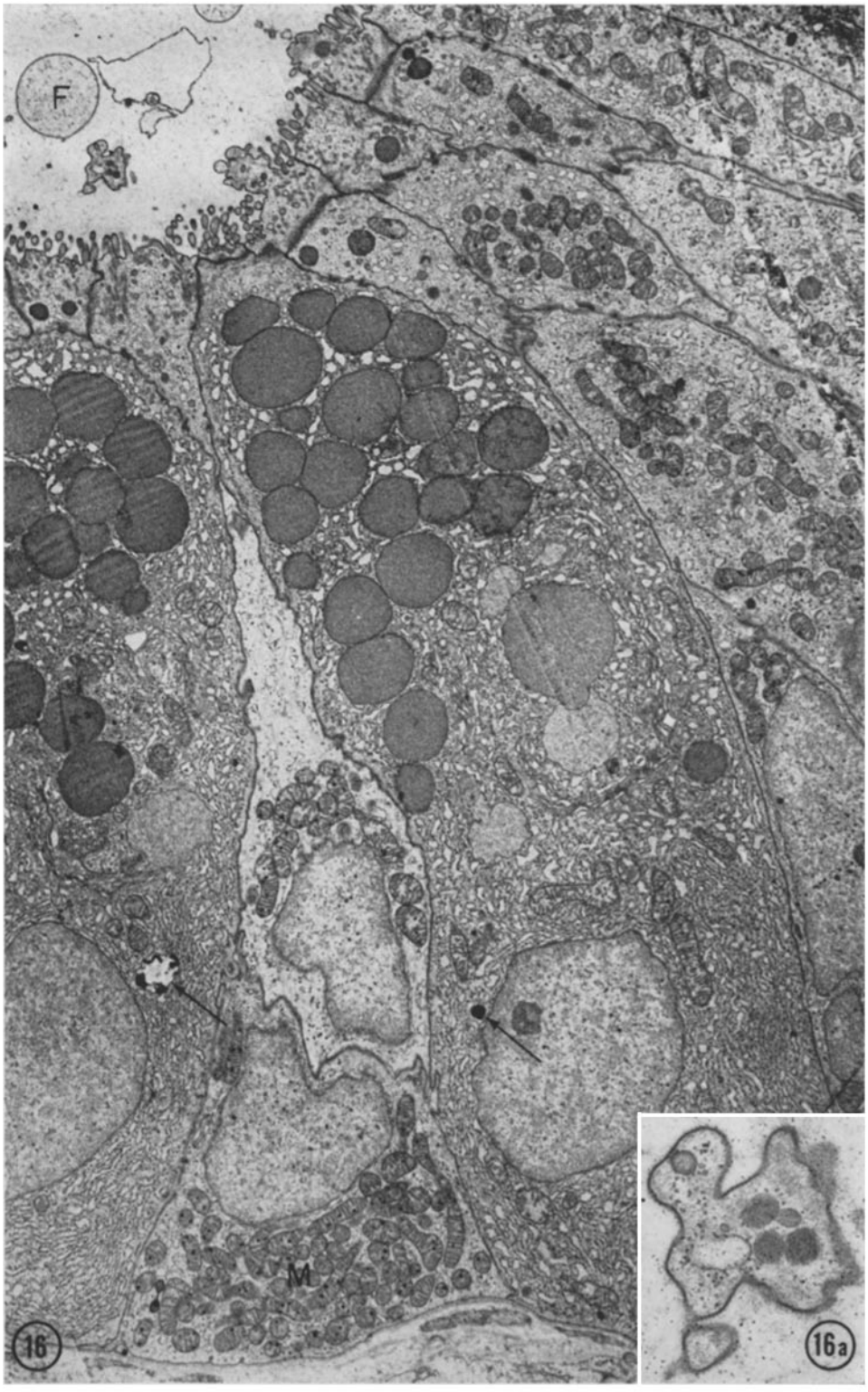


FIGURE 15 Apical portion of a crypt goblet cell. Here most or all of the apical mucus has already entered crypt lumen (*L*) through the gaps (arrows) between microvilli. A true apical cell membrane (*X*) is clearly demonstrated and is continuous with the lateral cell membrane. Chrome–osmium tetroxide fixation. Approximately × 24,000.

FIGURE 16 Base of a crypt. Two Paneth cells are seen flanking tangentially sectioned portions of two of the peculiar undifferentiated cells. The striking paranuclear accumulations of mitochondria (*M*) are readily seen in these unique cells. The cytoplasmic matrix of the upper cell is very pale. In the Paneth cells, the endoplasmic reticulum is extensive and well organized. Most of the Paneth granules located in the supranuclear region appear less dense than do those in the upper half of the cell. A few of the dense bodies (arrows) resembling lysosomes are present. Cytoplasmic fragments (*F*) are apparent in the crypt lumen. *s*-Collidine–osmium tetroxide fixation. Approximately × 5,000.

FIGURE 16 *a* Higher magnification of one of the cytoplasmic fragments seen in the crypt lumen in Fig. 16. The dense, spherical, membrane-bounded granules in the fragment resemble similar granules in the undifferentiated cells. Ribosomes and a vesicle of granular endoplasmic reticulum are apparent in the fragment. *s*-Collidine–osmium tetroxide fixation. Approximately × 22,000.


F
M
16
16a

structures, containing a variable amount of very electron-opaque substance, are frequent in Paneth cell cytoplasm (Figs. 16 and 20). At higher magnification it is apparent that the dense substance in some of the bodies is composed of many closely apposed dense membranes (Fig. 19). The dense bodies are most commonly seen in the perinuclear cytoplasm and are seen frequently in some Paneth cells but only rarely in others.

No evidence of actual secretion of Paneth cell granules into the crypt lumina was seen in our material obtained from fasting patients.

ENTEROCHROMAFFIN CELLS: The fine structure of the enterochromaffin cells of the human small intestinal crypt epithelium is similar to that of the enterochromaffin cells found in mouse gastric mucosa (27, 28). These cells generally appear triangular in shape when sectioned along their longitudinal axes (Figs. 23 and 24). Their basal surfaces are closely apposed to the basement membrane of the crypt epithelium while their other surfaces are closely applied to adjacent crypt cells. The enterochromaffin cells narrow as they approach crypt lumina and their lateral membranes meet at the top of the cells to form their apexes (Figs. 23 and 24). They did not extend to the crypt lumina in this material. The nucleus is located centrally within the cell and tends to be irregular in shape (Fig. 24). Nucleoli are present and the nuclear matrix is finely granular.

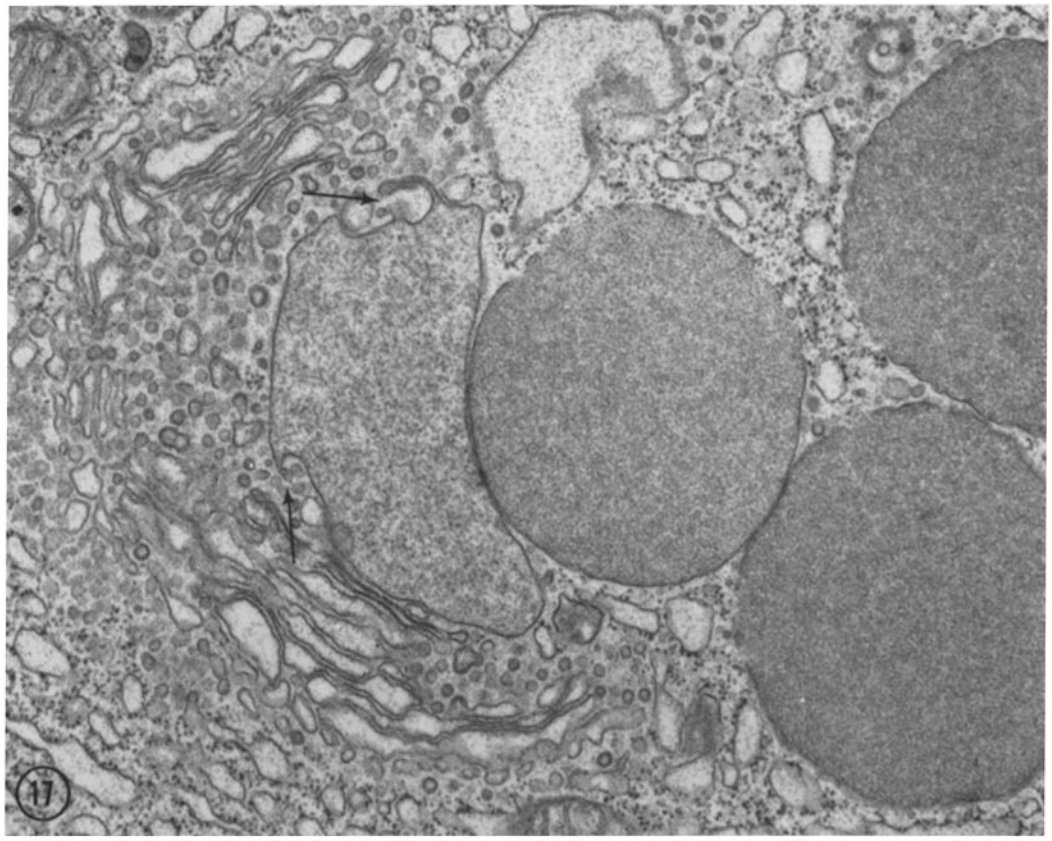
Enterochromaffin cells are easily recognized by their characteristic cytoplasmic granules. Most of the granules are located in the basal cytoplasm of the cell, but granules are often seen in the supranuclear region as well. The granules vary considerably in their density and shape (Figs. 22–24). At one extreme, there are granules which are so dense that they appear black when viewed with the electron microscope. Most of these tend to be circular in shape but some are elliptical and others triangular or almost square in tissue sections. Each granule is enclosed by a fine membrane but this can be seen only when a clear zone is interposed between the membrane and the dense core of the granule. At the other extreme, there are larger spherical structures containing sparse amounts of finely granular, precipitated material within a lighter matrix. Some of these more or less empty-appearing granules seem to be only partially surrounded by fine membranes. Between these two extremes, one sees many intermediate forms of granules of different densities (Figs. 22–24). In some enterochromaffin cells, the dense granules predominate (Fig. 22) while in others the empty-appearing structures are more prevalent (Figs. 23 and 24).

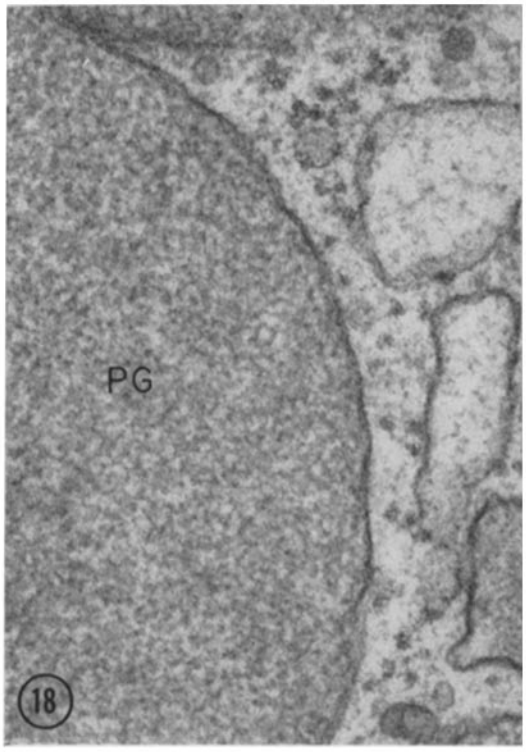
Strands and vesicles of granular and smooth-surfaced endoplasmic reticulum as well as free ribosomes are present in the enterochromaffin cell cytoplasm. These strands of granular endoplasmic reticulum are sometimes several microns in length and may be disposed in a lamellar fashion parallel to one another. A well organized Golgi complex is frequently seen in the upper half of the cell (Fig. 24). A variable number of mitochondria with the same structural features as those seen in undiffer-

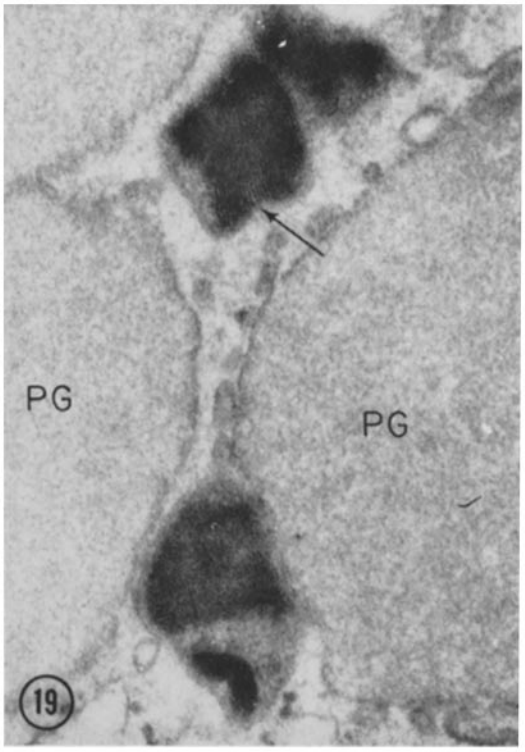
FIGURE 17 Supranuclear portion of a Paneth cell. The elaborate Golgi material is illustrated with its flattened cisternae and many small membrane-bounded vesicles. Some of these vesicles appear to fuse (arrows) with the membrane surrounding an irregularly shaped Paneth granule. The content of this granule appears less dense than the contents of the other Paneth granules in the micrograph. *s*-Collidine–osmium tetroxide fixation. Approximately × 23,500.

FIGURE 18 Higher magnification of a portion of the cytoplasm of the Paneth cell shown in Fig. 17. A distinct membrane is apparent separating a portion of a Paneth granule (*PG*) from adjacent cytoplasm. Many closely packed particles with a width of 50 to 200 A form the matrix of the granule. *s*-Collidine–osmium tetroxide fixation. Approximately × 87,000.

FIGURE 19 A portion of Paneth cell cytoplasm. Two irregularly shaped, membrane-bounded bodies are seen which contain dense material. The dense substance of the upper body appears composed of many closely apposed dense membranes (arrow). These lysosome-like dense bodies are located in the cytoplasm between Paneth granules (*PG*) in this micrograph. Chrome–osmium tetroxide fixation. Approximately × 90,000.




PG


PG
PG

entiated crypt cells are present in the enterochromaffin cells which contain many dense granules (Fig. 22). In the cells which contain primarily the larger empty-appearing granules, the mitochondria appear less abundant. Those present are smaller in size with more irregular, often scalloped surface membranes and cristae and with a denser intramitochondrial matrix (Figs. 23 and 24).

All the epithelial cells could readily be distinguished from each other either as undifferentiated (including the pale variety), goblet, Paneth, or enterochromaffin cells.

DISCUSSION

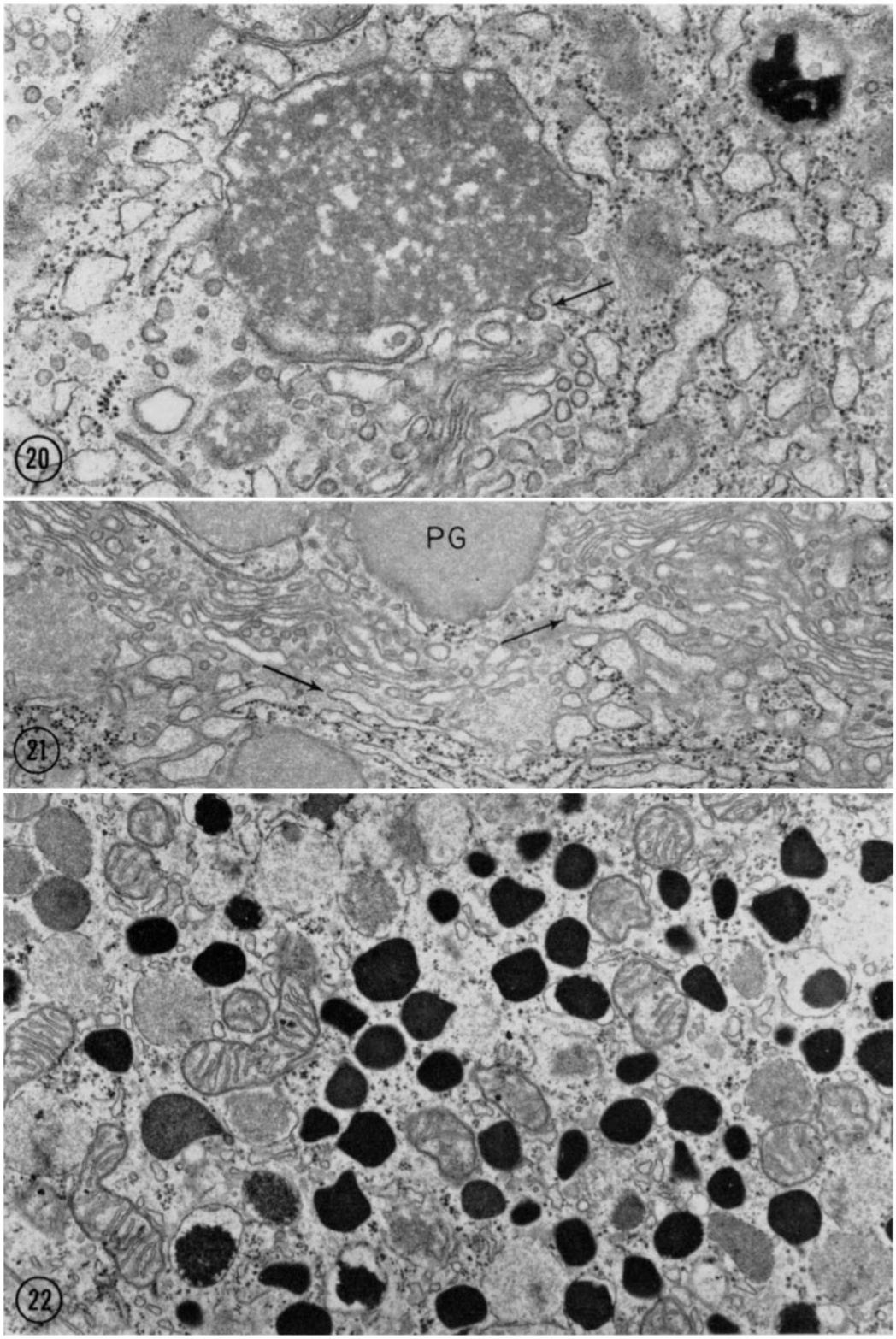
For years, many light microscopists have ascribed a secretory as well as a regenerative function to the undifferentiated crypt cells (20, 29). The nature of this postulated secretion and its role in the function of the small intestine has not been clarified satisfactorily. The abundance of membrane-bounded granules in the upper half of the cytoplasm of the undifferentiated crypt cells in our material helps support the concept that these cells do form a secretory product. The structural similarity between these granules and secretory granules from known secretory cells, as well as the constant periodic acid–Schiff positivity of at least those granules of sufficient size to be seen with the light microscope, suggests that they represent secretory material. However, from our data one cannot exclude the possibility that they represent accumulations of material absorbed from crypt lumina. At any rate, it is clear that these so called undifferentiated cells are apparently sufficiently differentiated either to secrete or absorb or perhaps to do both.

The small membrane-bounded fragments of undifferentiated and Paneth cell cytoplasm which seem to lie free in crypt lumina in our material (Figs. 9 and 16) probably correspond to similar structures reported by Clara in his studies of bird undifferentiated crypt cells (30) and by Feyrter in his work on human duodenal undifferentiated crypt cells (31). These investigators made their observations with the light microscope and suggested that the material which appeared to be budding off into the crypt lumina represented the secretory product of these cells. The nature of these fragments is difficult to determine by light microscopy even when 1 μ Epon sections are studied after being stained with toluidine blue. The electron micrographs, however, show clearly that these structures are discrete fragments of cytoplasm surrounded by a plasma membrane and that they contain ergastoplasm and vesicles, and only occasionally contain membrane-bounded granules suggestive of secretory material. It is indeed possible that this pinching off of cytoplasmic fragments may represent a specialized type of apocrine secretion in which cytoplasmic materials, in addition to secretory granules, are lost from the cells. It is of interest that Parks recently described similar apical budding of cytoplasmic fragments in his studies of parotid acinar cells in rodents (32). Further studies are needed to clarify the significance and functional role of this process, but there are indications that it may be more prevalent than is currently appreciated.

FIGURE 20 Supranuclear cytoplasm of a Paneth cell. In the center there is an irregularly shaped Paneth granule containing coarsely precipitated material. It is possible that the outpouching of the granule indicated by the arrow represents a Golgi vesicle which is being incorporated into the granule. A lysosome-like dense body is seen in the upper right. *s*-Collidine–osmium tetroxide fixation. Approximately × 33,000.

FIGURE 21 Paneth cell cytoplasm at the junction of the Golgi region and granular endoplasmic reticulum. Some Paneth granules (*PG*) are present. Individual membranes are seen (arrows) which seem to contribute to the structure of both the Golgi material and the granular endoplasmic reticulum, suggesting a close structural interrelationship between these membrane systems. Chrome–osmium tetroxide fixation. Approximately × 23,500.

FIGURE 22 A portion of the cytoplasm of an enterochromaffin cell. Many characteristic membrane-bounded granules of varying density are seen. In this cell, extremely dense granules predominate. Mitochondria, ribosomes and small elements of endoplasmic reticulum are also present. *s*-Collidine–osmium tetroxide fixation. Approximately × 19,000.


20
PG
21
22

The absence of a well developed terminal web in the crypt region may be related to the relative sparsity of microvilli on the apical surfaces of cells of the crypt epithelium compared to the villous epithelium in which a continuous web is generally present (9, 22). The role of the terminal web in villous epithelium is unknown, but it has been postulated that it serves to stiffen and stabilize the apical cell surface (33–35). Likewise, it is tempting to speculate that the groups of fine filaments originating in the cores of the microvilli and extending several microns into the cytoplasm of crypt epithelial cells and at times fusing laterally with desomosomes lend structural stability to the upper part of the cell.

The accumulations of particulate glycogen within undifferentiated cells of adult humans (Figs. 5 and 6) appear identical in fine structure with glycogen accumulations identified in other tissues (36). The presence of glycogen in our material comes as no surprise, for glycogen has been identified in the past in the cytoplasm of human intestinal epithelium by light microscopy (37).

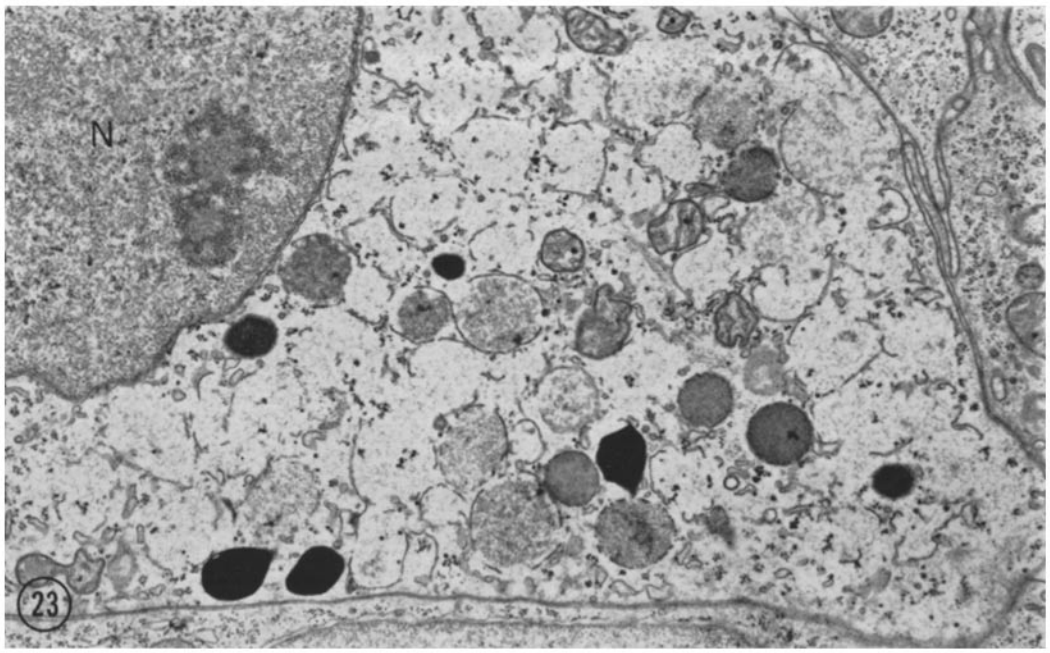
The peculiar undifferentiated cells located at the base of the crypts, and which are adjacent to the Paneth cells, have not been noted previously, to the author's knowledge. Though they are structurally similar in many ways to the undifferentiated cells lining the lateral crypt walls, their pale cytoplasm, polymorphic nuclei and striking paranuclear accumulations of mitochondria (Figs. 8 and 16) clearly distinguish them. Further studies will be required to elucidate the functional significance of these cells.

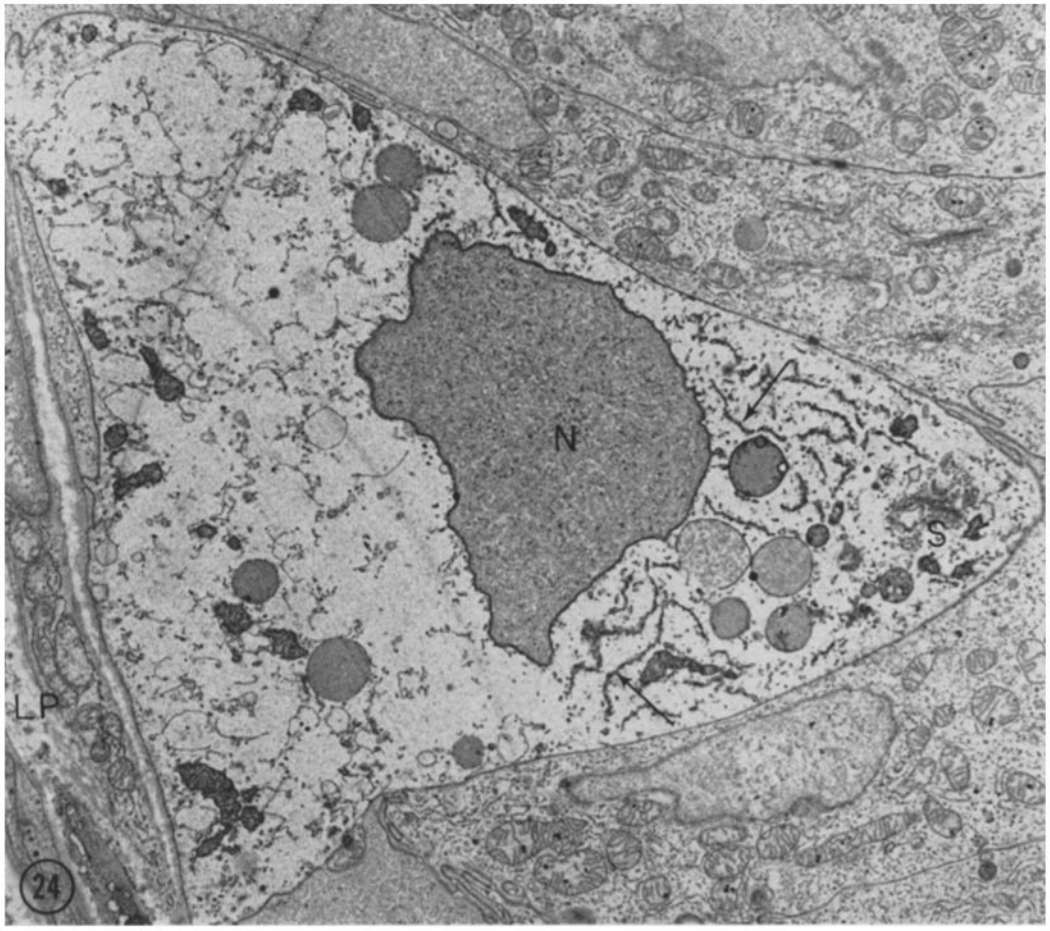
The mechanism by which the crypt goblet cells secrete their mucus is not at all clear from the available literature. Radioautographs, studied by Jennings and Florey, in which mucous granules of goblet cells from ileal crypts of small mammals were labeled with S^{35} showed initial incorporation of label into the supranuclear mucus followed by gradual migration of the labeled mucus to the apical cell surface during a 6 hour period (38). These findings suggested continuous synthesis of mucus in the supranuclear zone and gradual secretion of mucus from the apical surface of the goblet cells. Clara, from studies of bird intestine, proposed that some apical mucous granules appear to imbibe water, swell, and coalesce prior to secretion (39). He suggested that this dilution and swelling of mucous granules was then followed by rupture of the cuticle, with secretion of mucus into the intestinal lumen. In electron microscopic studies of rat jejunal epithelium, Palay described *en masse* secretion of the apical mucus of crypt and villous goblet cells which he considered apocrine in nature (5).

Our findings suggest that when the mucous granules in crypt goblet cells reach the apical surface they appear to coalesce and swell (Figs. 11 to 13). The heterogeneous appearance of the mucus in the apical region compared to the homogeneous appearance of the more centrally located mucus may be brought about by imbibition of fluid by the apical mucous granules, as previously suggested by Clara (39). Then, as the apical mucus is secreted through gaps in the cell membrane between microvilli, the membrane formerly surrounding the mucus fuses with and becomes part of the apical cell membrane (Figs. 12, 13, and 15), much as it does during secretion in other merocrine glandular cells (5, 32). It is thus concluded that many of the goblet cells in the jejunal crypts of fasting humans secrete their mucus by the mecha-

FIGURE 23 A portion of an enterochromaffin cell. The nucleus (*N*) is in the upper left. This cell contains many almost empty-appearing granules which are only partially surrounded by membranes. In addition, a few granules of intermediate density as well as a few very dense granules are present. *s*-Collidine–osmium tetroxide fixation. Approximately × 19,000.

FIGURE 24 An enterochromaffin cell between undifferentiated cells. The nucleus (*N*) is centrally located and has an irregular surface. The basal portion of the cell is to the left and is closely applied to the basement membrane separating the lamina propria (*LP*) from the epithelium. In this section, no dense granules are seen in the enterochromaffin cell. A few discrete granules of intermediate density are evident. Most of the cytoplasm is filled with finely precipitated, less dense material throughout which are scattered a few fine membranes, mitochondria and small vesicles. Some strands of granular endoplasmic reticulum (arrows) and a small Golgi complex (*S*) can be identified in the upper portion of the cell. *s*-Collidine–osmium tetroxide fixation. Approximately × 6,000.


N
23


N
S
L.P.
24

nism previously ascribed to merocrine cells and consequently should be classified as merocrine glandular cells under these conditions. However, it is very likely that, with stimulation, these same goblet cells would respond differently and secrete their mucus in an apocrine fashion. Convincing micrographs of apocrine secretion of mucus by rat colonic goblet cells after direct stimulation of the colonic epithelium with the irritant, mustard oil, have been obtained by Florey (40).

This study adds some further morphologial details to what is already known about the Paneth cells (3, 4, 9, 20). Hally described a clear halo interposed between the substance of most Paneth cell granules and their surrounding membrane in the Paneth cells of mouse jejunal crypts (4). This halo is not apparent in any of our material; instead, the membrane surrounding the substance of the Paneth cell granule is applied directly to it. It is likely that, as suggested by Hally, this halo represented artifact (4).

The dense bodies in the Paneth cell cytoplasm seem similar in structure to those reported in gastric parietal cells (28, 41) and to the lysosomes of parenchymal liver cells (42). It is likely that the dense bodies seen in the Paneth cell cytoplasm indeed represent lysosomal structures although chemical confirmation by enzyme studies is needed to be certain.

There seems little doubt that the Paneth cells are glandular secretory cells. Their general structure and cytoplasmic organization strongly retemble those of cells of known high secretory posential (25, 26, 43, 44). Furthermore, light microscopists have shown that Paneth cells discharge their granules after food ingestion (45) and after pilocarpine administration (46). The present study provides no further information concerning the nature of the Paneth cell secretory product or its mechanism of secretion. However, in view of the striking structural similarity between Paneth cells and pancreatic acinar cells, it is tempting to speculate that the pathway of synthesis and secretion of the secretory product of Paneth cells is similar to that of pancreatic acinar cells as established by Siekevitz and Palade, using both biochemical and morphological techniques (47–51), and by Caro, utilizing electron microscopic radioautographic techniques following leucine-H^3 administration (52, 53). If so, then the secretory product is synthesized by the ergastoplasm, transported to the Golgi region, and there "packaged" into secretory granules which in turn migrate into the apical cytoplasm of the cell from which they are extruded. Unfortunately, in our material obtained from fasting subjects none of the Paneth cells were observed at a time when they were delivering their secretory product into the crypt lumina. It is hoped that future studies utilizing more dynamic techniques will help clarify the functions of this interesting and as yet poorly understood cell.

The variability in the morphology and the number of membrane-bounded granules apparent in the enterochromaffin cells in our material is of interest (Figs. 22, to 24). It seems likely that the cells with relatively few well formed granules have in part divested themselves of their secretory product and that the degree of granulation within these cells may well reflect the various stages in their functional cycle. It is not clear why the individual granules vary so much in density. It is conceivable that the larger, less dense granules may represent diluted or otherwise altered forms of the more dense granules. In our material enterochromaffin cells were not seen discharging intact granules or recognizable fragments of granules from their cytoplasm. Therefore, we can contribute no information regarding their secretory pathway.

The author gratefully acknowledges the helpful aid and advice given by Dr. Cyrus E. Rubin, Dr. Richard L. Wood, Dr. John H. Luft, and Miss Patricia Phelps during the course of this study. This study was performed during the tenure of Traineeship AT-563, National Institutes of Arthritis and Metabolic Disease, National Institutes of Health, and was supported by United States Public Health Service grant C-4320, National Cancer Institute, National Institutes of Health, and United States Public Health Service grant HE-02698.

Received for publication, February 6, 1963.

BIBLIOGRAPHY

1. Christie, A. C., A study of the Kultschitzky (Argentaffin) cell with the electron microscope after fixation by osmium tetroxide, *Quart. J. Micr. Sc.*, 1955, **96**, 295.

2. Taylor, J. J., and Hayes, E. R., An electron microscopic study of the ultrastructure of the argentaffin cell, *J. Appl. Physics*, 1959, **30**, 2033.

3. BLOOM, W., and FAWCETT, D. W., A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1962, 449.
4. HALLY, A. D., The fine structure of the Paneth cell, *J. Anat.*, 1958, **92,** 268.
5. PALAY, S. L., Frontiers of Cytology, New Haven, Yale University Press, 1958, 305.
6. RUSKA, H., Die Zellstrukturen des Dündarmepithels in ihrer Abhängigkeit von der physikalisch-chemischen Beschaffenheit des Darminhalts. I. Wasser und Natriumchlorid, *Z. Zellforsch. u. Mikr. Anat.*, 1960, **52,** 748.
7. DALTON, A. J., Electron microscopy of epithelial cells of the gastrointestinal tract and pancreas, *Am. J. Anat.*, 1951, **89,** 109.
8. PADYKULA, H. A., STRAUSS, E. W., LADMAN, A. J., and GARDNER, F. A., A morphologic and histochemical analysis of the human jejunal epithelium in non-tropical sprue, *Gastroenterology*, 1961, **40,** 735.
9. TRIER, J. S., Morphologic alterations induced by methotrexate in the mucosa of human proximal intestine. II. Electron microscopic observations, *Gastroenterology*, 1962, **43,** 407.
10. BRANDBORG, L. L., RUBIN, C. E., and QUINTON, W. E., A multipurpose instrument for suction biopsy of the esophagus, stomach, small bowel and colon, *Gastroenterology*, 1959, **37,** 1.
11. FLICK, A. L., QUINTON, W. E., and RUBIN, C. E., A peroral hydraulic biopsy tube for multiple sampling at any level of the gastrointestinal tract, *Gastroenterology*, 1961, **40,** 120.
12. TRIER, J. S., PHELPS, P. C., and RUBIN, C. E., Electron microscopy of mucosa of small intestine, *J. Am. Med. Assn.*, 1963, **183,** 768.
13. PHELPS, P. C., and RUBIN, C. E., Electron microscopic techniques for studying fat absorption in man, *Gastroenterology*, 1962, **42,** 765.
14. DALTON, A. J., A chrome-osmium fixative for electron microscopy, *Anat. Rec.*, 1955, **121,** 281.
15. BENNETT, H. S., and LUFT, J. H., *s*-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 113.
16. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
17. TRUMP, B. F., SMUCKLER, E. A., and BENDITT, E. P., A method for staining epoxy sections for light microscopy, *J. Ultrastruct. Research*, 1961, **5,** 343.
18. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.
19. KARNOVSKY, M. J., Simple methods for "staining with lead" at high pH in electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 729.
20. PATZELT, V., Der Darm in Handbuch der mikroskopischen Anatomie des Menschen, (W. von Möllendorff, editor), Berlin, Springer, 1936, **5/3,** 1.
21. BROWN, A. L., Microvilli of the human jejunal epithelial cell, *J. Cell Biol.*, 1962, **12,** 623.
22. ZETTERQVIST, H., The ultrastructural organization of the columnar absorbing cells of the mouse jejunum, Stockholm, Karolinska Institutet, Aktiebolaget Godvil, 1956.
23. FARQUHAR, M. G., and PALADE, G. E., Junctional complexes in various epithelia, *J. Cell Biol.*, 1963, **17,** 375.
24. KARRER, H. E., Cell interconnections in normal human cervical epithelium, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 181.
25. WEISS, J. M., The ergastoplasm, *J. Exp. Med.*, 1953, **98,** 607.
26. SJÖSTRAND, F. S., and HANZON, V., Membrane structures of cytoplasm and mitochondria in exocrine cells of mouse pancreas as revealed by high resolution electron microscopy, *Exp. Cell Research*, 1954, **7,** 393.
27. HELANDER, H. F., A preliminary note on the ultrastructure of the argyrophile cells of the mouse gastric mucosa, *J. Ultrastruct. Research*, 1961, **5,** 257.
28. HELANDER, H. F., Ultrastructure of fundus gland of the mouse gastric mucosa, *J. Ultrastruct. Research*, 1962, suppl. 4, 1.
29. MACKLIN, C. C., and MACKLIN, M. T., The intestinal epithelium *in* Special Cytology, (E. V. Cowdry, editor), New York, Paul B. Hoeber, 1932, 233.
30. CLARA, M., Beiträge zur Kenntnis des Vogeldarmes. II. Die Hauptzellen des Darmepithels, *Z. Mikr. Anat. Forsch.*, 1926, **6,** 1.
31. FEYRTER, F., cited by Patzelt *in* Handbuch der mikroskopischen Anatomie des Menschen, (W. von Möllendorff, editor), Berlin, Springer, 1936, **5/3,** 82.
32. PARKS, H. F., Morphological study of the extrusion of secretory materials by the partoid glands of mouse and rat, *J. Ultrastruct. Research*, 1962, **6,** 449.
33. SAUER, F. C., Some factors in the morphogenesis of vertebrate embryonic epithelia, *J. Morphol.*, 1937, **61,** 563.
34. PUCHTLER, H., and LEBLOND, C. P., Histochemical analysis of cell membranes and associated structures as seen in the intestinal epithelium, *Am. J. Anat.*, 1958, **102,** 1.
35. PALAY, S. L., and KARLIN, L. H., An electron microscopic study of the intestinal villus. I. The fasting animal, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 363.
36. REVEL, J. P., NAPOLITANO, L., and FAWCETT, D. W., Identification of glycogen in electron

micrographs of thin tissue sections, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 575.

37. PATZELT, V., Die feinere Ausbildung des Menschlichen Darmes von der Fünften Woche bis zur Geburt, *Z. Mikr. Anat. Forsch.*, 1931, **27,** 269.
38. JENNINGS, M. A., and FLOREY, H. W., Autoradiographic observations on the mucous cells of the stomach and intestine, *Quart. J. Exp. Physiol.*, 1956, **41,** 131.
39. CLARA, M., Beiträge zur Kenntnis des Vogeldarmes. V. Die Schleimbildung im Darmepithel mit besonderer Berücksichtigung der Becherzellenfrage, *Z. Mikr. Anat. Forsch.*, 1926, **6,** 256.
40. FLOREY, H. W., Electron microscopic observations on goblet cells of the rat's colon, *Quart. J. Exp. Physiol.*, 1960, **45,** 329.
41. SEDAR, A. W., Electron microscopy of the oxyntic cell in the gastric glands of the bullfrog, *Rana catesbana*. II. The acid-secreting gastric mucosa, *J. Biophysic. and Biochem. Cytol.*, 1961, **10,** 47.
42. NOVIKOFF, A. B., and ESSNER, E., The liver cell, *Am. J. Med.*, 1960, **29,** 102.
43. SCOTT, B. L., and PEASE, D. C., Electron microscopy of the salivary and lacrimal glands of the rat, *Am. J. Anat.*, 1959, **104,** 115.
44. PARKS, H. F., On the fine structure of the parotid gland of mouse and rat, *Am. J. Anat.*, 1961, **108,** 303.
45. ZIPKIN, R., Beiträge zur Kenntnis der Gröberen und feineren Strukturverhältnisse des Dundarms von Inmus Rhesus, *Anat. Hefte*, 1903, **23,** 115.
46. CORDIER, R., Contribution à l'étude de la cellule de Ciacco-Masson et de la cellule de Paneth, *Compt. rend. Soc. biol.*, 1923, **88,** 1227.
47. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. I. Isolation and enzymatic activities of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 203.
48. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. II. Functional variation in enzymatic activity of microsomes, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 309.
49. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. III. *In vivo* incorporation of leucine-1-C^{14} into the proteins of cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 557.
50. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. IV. Chemical and metabolic investigation of the ribonucleoprotein particles, *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 1.
51. SIEKEVITZ, P., and PALADE, G. E., A cytochemical study on the pancreas of the guinea pig. V. *In vivo* incorporation of leucine-1-C^{14} into the chymotrypsinogen of various cell fractions, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 619.
52. CARO, L. G., Electron microscopic radioautography of thin sections: The Golgi zone as a site of protein concentration in pancreatic acinar cells, *J. Biophysic. and Biochem. Cytol.*, 1961, **10,** 37.
53. CARO, L G , Electron microscopic radioautography of thin sections: The Golgi complex as a site of concentration of newly synthesized proteins in pancreatic exocrine cells, Program of First Annual Meeting of the American Society for Cell Biology, Chicago, 1961, 29.