

# THE FINE STRUCTURE OF VON EBNER'S GLAND OF THE RAT

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## ABSTRACT

The fine structure of von Ebner's gland was studied in untreated rats and rats stimulated to secrete by fasting-refeeding or injection of pilocarpine. Cytological features were similar to those reported for pancreas and parotid gland. Abundant granular endoplasmic reticulum filled the basal portion of the cell, a well-developed Golgi complex was located in the vicinity of the nucleus, and the apical portion of the cell was filled with dense secretory granules. Dense heterogeneous bodies resembling lysosomes were closely associated with the Golgi complex. Coated vesicles were seen in the Golgi region and also in continuity with the cell membrane. Granule discharge occurred by fusion of the granule membrane with the cell membrane at the secretory surface. Successive fusion of adjacent granules to the previously fused granule formed a connected string of granules in the apical cytoplasm. Myoepithelial cells were present within the basement membrane, and nerve processes were seen adjacent to acinar and myoepithelial cells. Duct cells resembled the intercalated duct cells of the major salivary glands.

## INTRODUCTION

The surface of the mammalian tongue is well supplied with glandular elements, predominately of a mucous nature. Located beneath the large circumvallate papillae on the dorsum, and the foliate papillae on the sides of the tongue, are a small group of branching tubuloalveolar glands (3, 10, 43) known as von Ebner's gland. They are classically described as serous in nature (3, 11, 29) and their ducts open into the trough at the base of the papillae.

The function of von Ebner's gland has often been described as one of washing out taste substances from the trough and readying the taste receptors in the walls of the papilla for a new stimulus (5, 11, 36). Their secretions have been implied to be of importance in the sense of taste because of their enzyme content (2). The present study was undertaken to determine the ultrastructural char-

acteristics of these glands and their mode of secretion.

## MATERIALS AND METHODS

Von Ebner's glands were obtained from the tongues of 5- to 8-week-old male Sprague-Dawley rats, weighing 170-270 g, maintained on laboratory chow and water ad libitum. Specimens from animals in which secretion was stimulated were also included. Food was withheld from some animals for 24-48 hr, then they were refed and sacrificed at times from 5 min to 15 hr after feeding. Other animals received intraperitoneal injections of pilocarpine (160 mg/kg) and were sacrificed at times from 5 min to 4 hr after injection.

A variety of fixation procedures was used. The most reliable fixation was obtained by vascular perfusion through the heart of an anesthetized animal (50 mg/kg sodium pentobarbital, intraperitoneally) which was artificially respired with a mixture of 95% oxygen

and 5% carbon dioxide. Other animals were killed by a blow on the head. In either instance, the tissue was rapidly excised, placed in cold fixative, and cut into 1 mm cubes. Fixatives employed were phosphate-buffered 2.5% glutaraldehyde (28, 50), phosphate-buffered 1% osmium tetroxide (28), or cacodylate-buffered 4% formaldehyde (made from paraformaldehyde powder) or formaldehyde-glutaraldehyde mixtures (4), all at pH 7.3. Aldehyde fixation was carried out for 2-6 hr, followed by postfixation in 1% osmium tetroxide in phosphate or cacodylate buffer for 2-3 hr. Some tissues were stained in block for 2 hr with 0.5 or 2.0% uranyl acetate (24) prior to dehydration. They were dehydrated in ethanol and embedded in Araldite (27).

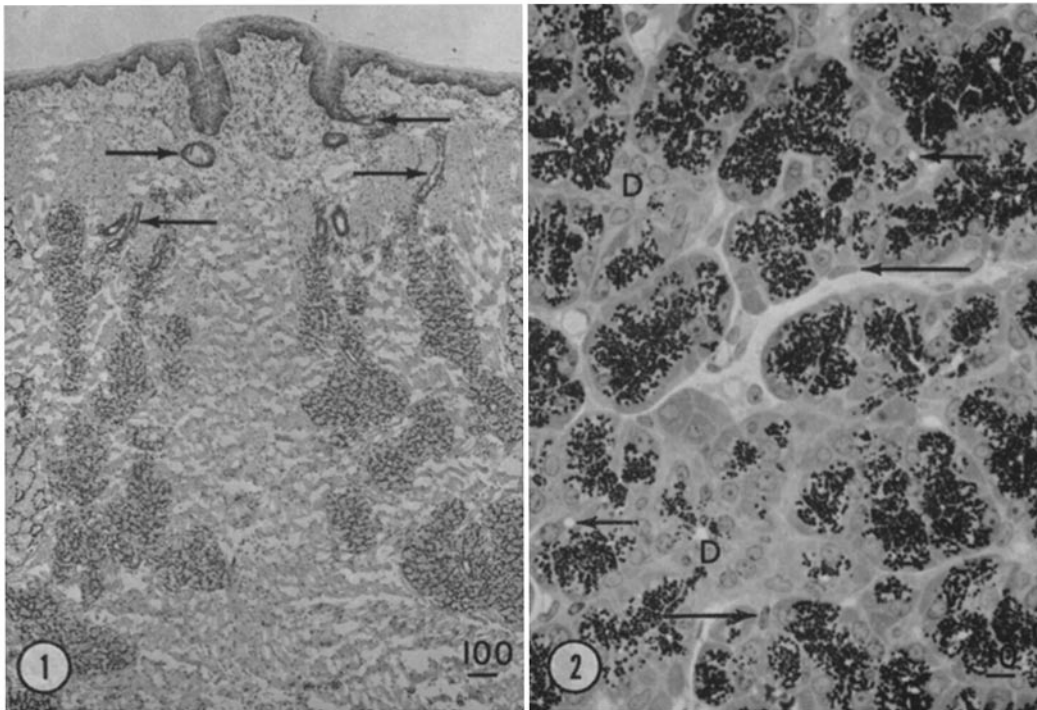
For light microscopy, 6  $\mu$  cryostat sections were fixed in calcium acetate-formalin (26) and stained

with hematoxylin and eosin, and 0.5  $\mu$  sections of Araldite-embedded tissues were stained with toluidine blue. For electron microscopy, thin sections were cut on a Porter-Blum microtome (Ivan Sorvall Inc., Norwalk, Conn.), stained with uranyl acetate and/or lead citrate (49), and examined in a Siemens Elmiskop I at 80 kv. Micrographs were obtained with initial magnifications of 1800 to 33,000.

## RESULTS

### *Light Microscopy*

Typically serous-appearing glands were located beneath the circumvallate papilla (Fig. 1). They were oriented more or less vertically in the tongue between bundles of striated muscle. Ducts ex-



Scale marks on figures are in microns.

**FIGURE 1** Section through the circumvallate papilla of the rat tongue. Von Ebner's gland is located beneath the papilla, dispersed among the bundles of muscle. Ducts can be seen extending toward the trough around the papilla (arrows) and, on the right, one duct is seen opening into the trough. Portions of the mucous glands of the tongue are seen lateral to von Ebner's gland. Hematoxylin and eosin.  $\times 40$ .

**FIGURE 2** 0.5  $\mu$  section of Araldite-embedded tissue. The acinar cells are packed with densely-staining granules. Their nuclei are located basally, and nucleoli are prominent. Apparent vacuoles in the basal portions of some cells (small arrows) are probably lipid droplets. Myoepithelial cell nuclei (large arrows) are elongated and closely applied to the base of the acinus. Two small ducts can be seen (D), and capillaries and nuclei of connective tissue cells are located between the acini. Toluidine blue.  $\times 400$ .

tended toward the circumvallate papilla, opening into the trough around it.

At higher magnification (Fig. 2), large numbers of deeply stained secretory granules were seen in the apical two-thirds of the acinar cells. The nuclei of the acinar cells were round with a prominent nucleolus and were situated in the basal half of the cell. Occasionally an unstained vacuole was seen in a cell. The droplet could be stained by Oil Red O and presumably contained neutral lipid. The acinar lumen and intercellular canals could be identified in some cases. Closely applied to the base of the acinus were myoepithelial cells, identified by their elongated nuclei.

Between the acini were vascular and connective tissue elements, and ducts of varying size. The duct cells were cuboidal, smaller than the acinar cells, and sometimes contained granules. Mucous cells were occasionally seen within the walls of the larger ducts (3). The ducts varied in thickness from one to four or five cells as they approached the trough of the circumvallate papilla.

### *Electron Microscopy*

**ACINAR CELLS:** The acinar cells were packed with numerous electron-opaque secretory granules, located mainly in the apical two-thirds of the cells (Fig. 3). They were homogeneous, finely granular, membrane-bounded, and ranged in size from 0.5 to 1.7  $\mu$ .

Granular endoplasmic reticulum (ER) was present throughout the cell, randomly arranged in the apical region, but in highly ordered parallel arrays in the basal portion (Fig. 3). The cisternae contained a finely granular or flocculent material. Occasionally dilated cisternae were observed, and often certain elements of ER had a vesicular appearance. Transitional elements of the ER were found near the Golgi complex (Figs. 4, 5). Small vesicles 400–700 A in diameter appeared to bud off of the ribosome-free portions of the transitional ER.

Mitochondria were numerous and were scattered throughout the cell or were aligned along the cell membranes (39) (Fig. 3).

In many cells one or more electron-lucent lipid inclusions were present (Fig. 3), found most often in the basal portion of the cell. Some inclusions were membrane bounded; others were not. Their usual size ranged from 1 to 3  $\mu$ , although some as large as 5–6  $\mu$  were observed. One or more mitochondria were usually found adjacent to an inclu-

sion. The lipid appeared homogeneous in osmium tetroxide-fixed tissues; however, in aldehyde-fixed tissues, it was not always well preserved and the inclusions appeared as empty or partially empty vacuoles.

A well-developed Golgi complex was found lateral or apical to the nucleus (Figs. 3–6). In some cells two or three Golgi regions were recognized. They usually consisted of four to five lamellae, some of which were dilated and appeared empty or contained a light flocculent material. The dilated lamellae were in close relation to the transitional elements of the ER (Figs. 4, 5). One or more condensing vacuoles (7, 22, 23) were present in the Golgi region (Figs. 4–6). They were 0.8–2.0  $\mu$  in diameter, their content varied in density from a light flocculent material to a density approaching that of the mature secretory granules, and they were bounded by a membrane which was irregular in outline and often showed images suggestive of fusion with or fission of small vesicles. Occasionally what appeared to be a connection between a Golgi lamella and the condensing vacuole was observed (Fig. 6).

Two other types of organelles were regularly seen in the Golgi region. One type was a granule 0.2–0.4  $\mu$  in diameter, a number of which were present (Fig. 4). The other type was a coated vesicle, 500–1800 A in diameter, a population of which occurred, (Figs. 4, 7), the vesicles often being continuous with the Golgi lamellae. Coated vesicles were also found throughout the cell, occasionally showing continuity with the cell membrane (Fig. 11).

A heterogeneous group of lysosome-like bodies were regularly found in the acinar cells (Figs. 3, 7–10). Structures which resembled autophagic vacuoles contained recognizable organelles. The most common type of dense body contained numerous vesicles, whorls or scrolls of membranes with a 120–160 A periodicity, or a hexagonally-packed tubular-crystalline structure with a center-to-center spacing of about 200 A. Often these structures contained electron-lucent vacuoles of various sizes, suggestive of lipid content. The dense bodies were found throughout the cell, but were most numerous in the Golgi region.

Each acinus contained a lumen and numerous intercellular canals. The secretory surfaces of the cell contained a few small microvilli (Fig. 11). The cytoplasm beneath the surface was fibrillar in nature, and occasionally microtubular profiles

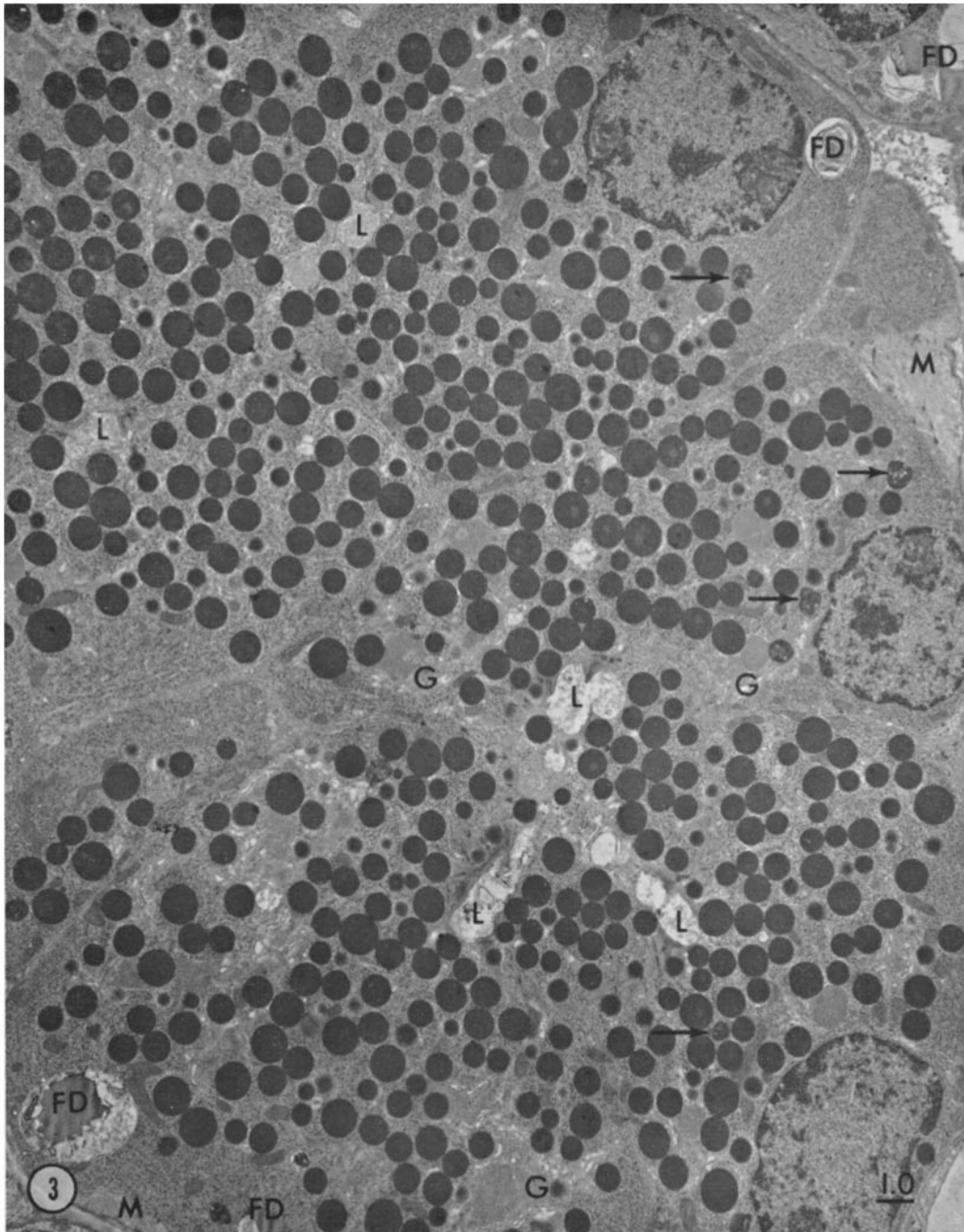


FIGURE 3 Major portion of an acinus from a rat fasted for 24 hr; parts of at least nine acinar cells are seen. Secretory granules fill the apical two-thirds of the cells, and the nuclei are located basally. The endoplasmic reticulum is arranged in parallel arrays in the basal portions of the cells. Intercellular canals and part of the acinar lumen are evident (L). Condensing vacuoles are located in the Golgi regions (G). Numerous heterogeneous bodies and autophagic vacuoles are present (arrows). Myoepithelial cell process (M) are located peripherally but within the basement membrane. Lipid droplets (FD). Formaldehyde-glutaraldehyde-osmium tetroxide.  $\times 5500$ .

were observed. Adjacent cells were joined by well-defined attachment complexes at their secretory surfaces, and the lateral surfaces were joined by occasional desmosomes and interlocking processes of cytoplasm.

Preliminary experiments to stimulate secretion showed results similar to those obtained by Ichikawa in the perfused canine pancreas (20). Fusion of the granule membrane with the cell membrane at the secretory surface occurred. Fusion of the membranes of adjacent granules produced a string of connected granules in the cell (Figs. 13, 14). As many as six to seven granules were seen fused together. The fused granules appeared less dense than the nonfused granules, suggesting dilution of their contents. The cytoplasm around fused granules was finely fibrillar in nature, as was the cytoplasm beneath the secretory surface in nonstimulated cells. Fusion of granules to each other was not seen in nonstimulated cells.

**MYOEPIITHELIUM AND NERVES:** Myoepithelial cell processes and nuclei were seen within the basement membrane surrounding the acinar and ductal cells (Figs. 3, 12), and desmosomes were occasionally seen between the myoepithelial processes and the epithelial cells. The structure of the myoepithelial cells was similar to that of myoepithelial cells of the salivary and lacrimal glands (25, 39, 44, 47, 48).

Unmyelinated axons invested by Schwann cells were abundant in the connective tissue spaces. Within the parenchyma of the gland, nerve processes were found adjacent to acinar cells and/or myoepithelial cells (Fig. 12). They usually fitted into depressed areas of the acinar and myoepithelial cell surfaces. Occasionally a nerve process

within the parenchyma was seen surrounded by a rim of Schwann cell cytoplasm.

**DUCTS:** The ducts were similar in structure to the intercalated ducts of the major salivary glands (39, 44) (Fig. 15). No portions of the ducts corresponded to the striated ducts of the major salivary glands. The ducts were surrounded by one or two layers of myoepithelial cells. Nerve endings were rarely seen in contact with duct cells.

The duct cells were smaller than the acinar cells, and their nucleus was usually round, but often had an indentation of cytoplasm. An abundant supply of ER, numerous mitochondria, free ribosomes, a well-developed Golgi complex, a few lysosome-like bodies, and bundles of fine filaments characterized their cytoplasm. Along the lateral cell membrane desmosomes were numerous, the apical surface was studded with short microvilli, and the basal surface was often thrown into irregular, interdigitating folds of cytoplasm. A few dense granules ranging from 0.2 to 0.9  $\mu$  in size were usually found near the apical surface. One type was round and finely granular, and the other type was oval or irregular in shape, denser, and also finely granular.

#### DISCUSSION

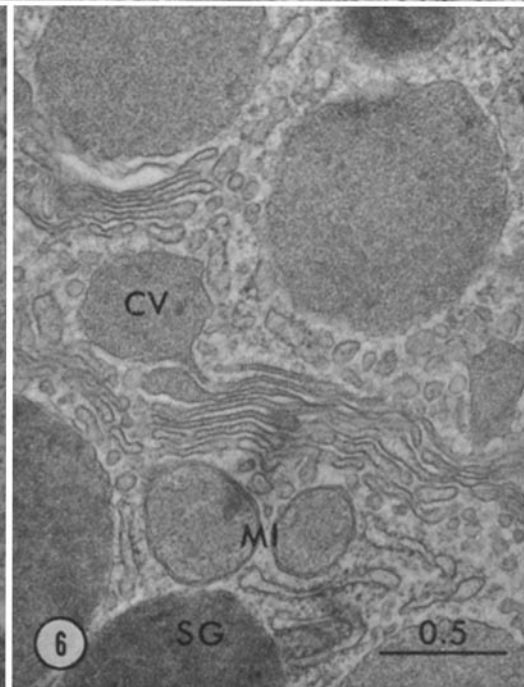
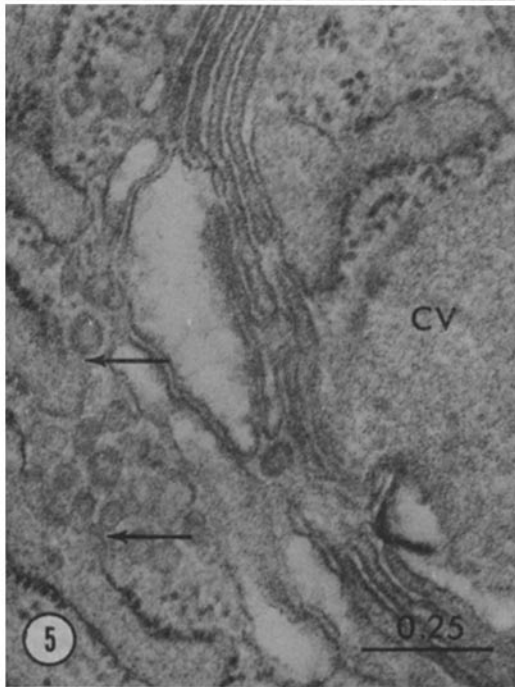
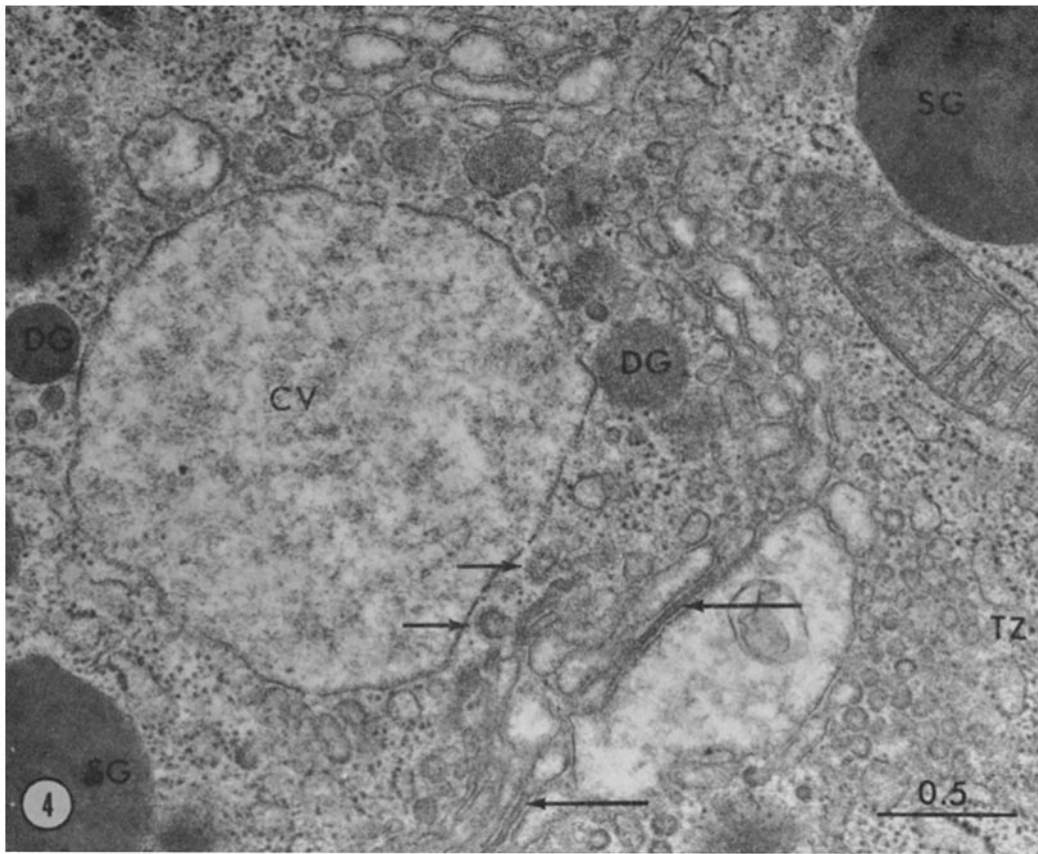
Von Ebner's gland was found to possess an abundant granular endoplasmic reticulum, transitional elements of the ER in close association with numerous small vesicles of the Golgi region, a condensing vacuole with an irregular bounding membrane and content of variable density, and numerous dense, membrane-bounded secretory granules located in the apical two-thirds of the acinar cells. These cytological features, which are

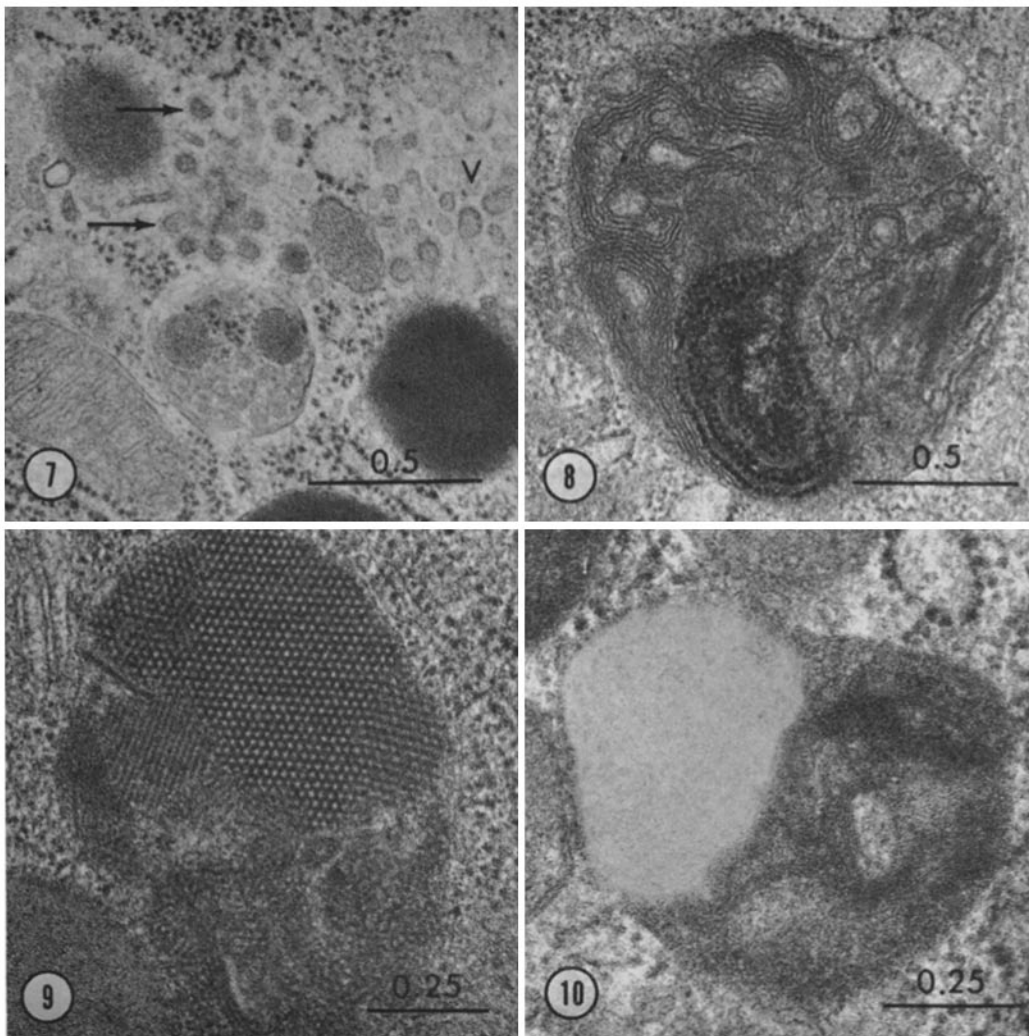
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**FIGURE 4** Golgi region of an untreated rat. The condensing vacuole (*CV*) is larger than the adjacent dense secretory granules (*SG*) and is filled with a flocculent material. Small granules of various density are present (*DG*). Transitional elements of the ER (*TZ*) are seen, with portions partially devoid of ribosomes. Numerous small vesicles are present at both faces of the Golgi complex, and a few coated vesicles can be distinguished (small arrows). Narrow Golgi lamellae with a dense content (arrows) can be seen between more dilated portions. Glutaraldehyde-osmium tetroxide.  $\times 36,500$ .

**FIGURE 5** Golgi region of an untreated rat. Transitional elements of the ER closely approach the Golgi lamellae. Small vesicles appear to bud off of the ribosome-free portions of the transitional elements (arrows). Condensing vacuole (*CV*). Glutaraldehyde-osmium tetroxide.  $\times 70,500$ .

**FIGURE 6** Golgi region of an untreated rat. Continuity between a Golgi lamella and a condensing vacuole (*CV*) is suggested. Mitochondria (*MI*) are located near the Golgi lamellae. Mature secretory granules (*SG*). Osmium tetroxide.  $\times 33,700$ .





FIGURES 7-10 Examples of autophagic vacuoles and heterogeneous dense bodies and their usually close association with the Golgi membranes of the acinar cells.

FIGURE 7 Untreated rat. An autophagic vacuole contains two small dense granules, ribosomes, and numerous small vesicles. Vesicles (V) of the Golgi complex are seen at the right, and numerous coated vesicles (arrows) are located near the autophagic vacuole. Formaldehyde-osmium tetroxide.  $\times 46,200$ .

FIGURE 8 2 hr after pilocarpine injection. The dense body contains scroll-like patterns of lamellae and ribosome-studded ER membranes. Glutaraldehyde-osmium tetroxide.  $\times 43,800$ .

FIGURE 9 Untreated rat. A dense body with a tubular-crystalline inclusion. The clearly crystalline portion has approximately a 200 A center-to-center spacing. Glutaraldehyde-osmium tetroxide.  $\times 64,800$ .

FIGURE 10 Untreated rat. A dense body with a few faint lamellar profiles in a finely granular matrix appears to be forming a lipid droplet. Glutaraldehyde-osmium tetroxide.  $\times 71,800$ .



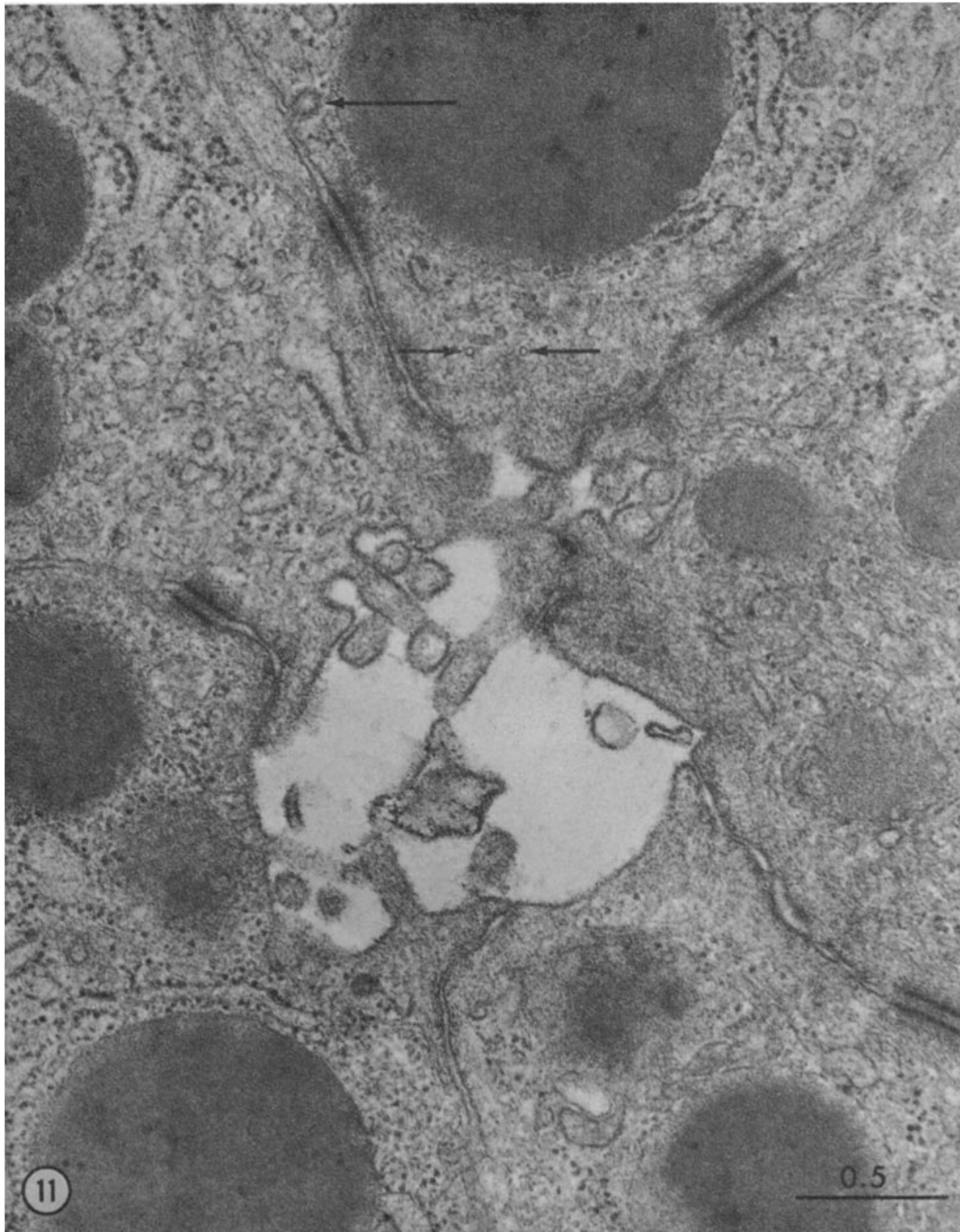


FIGURE 11 Apical region of five acinar cells of an untreated rat. The cells are joined by well defined attachment complexes consisting of a *zonula occludens* and one or more *maculae adherentes* (desmosomes). The intermediate junction (*zonula adherens*) is poorly developed. A few short microvilli project into the lumen. The cytoplasm beneath the apical plasma membrane is fibrillar in nature and reasonably devoid of cellular organelles. Note the coated vesicle in continuity with the lateral cell surface (arrow), and microtubular profiles (small arrows). Glutaraldehyde-osmium tetroxide.  $\times 43,700$ .



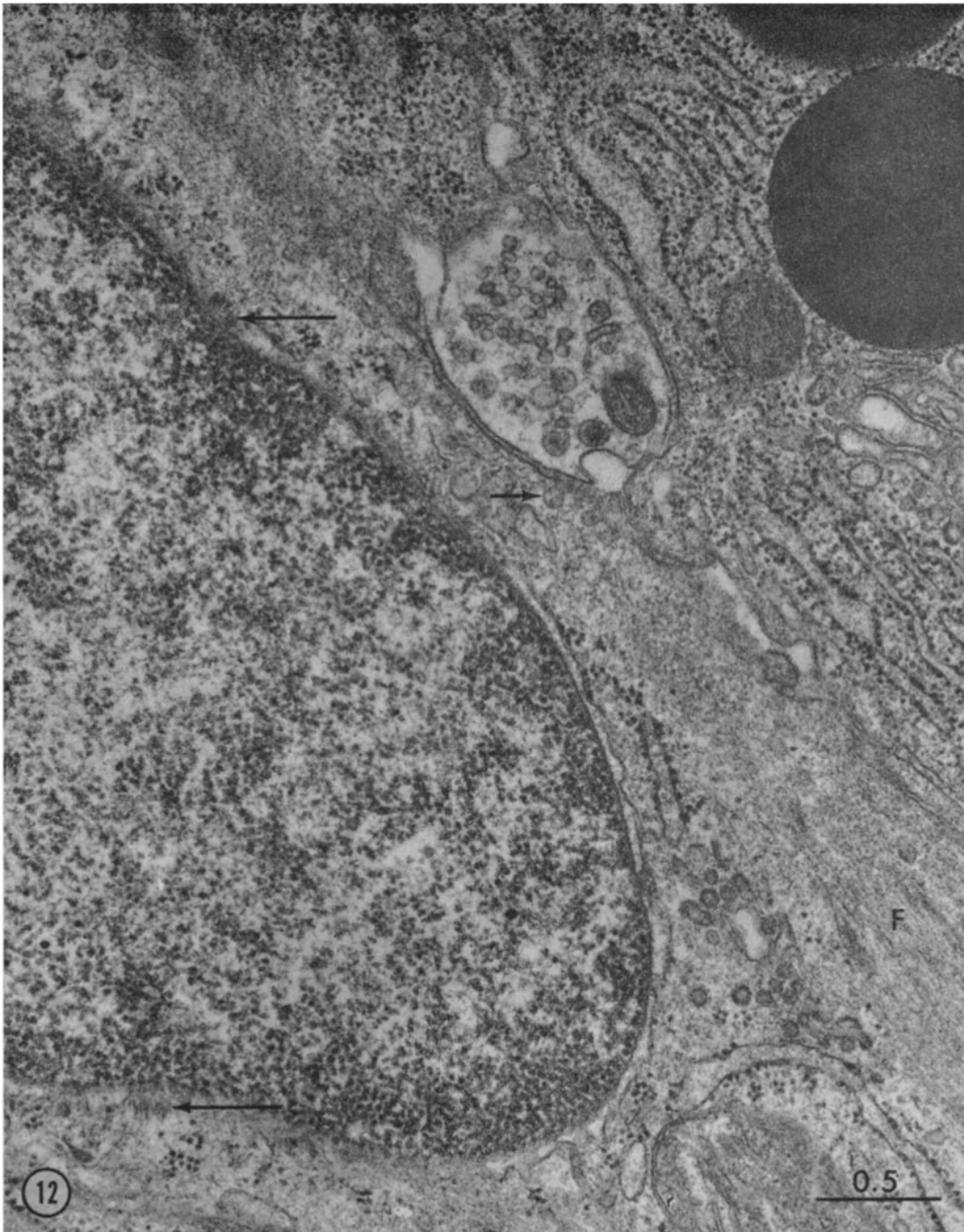


FIGURE 12 A nerve process containing a mitochondrion, small dense granules, and small vesicles lies adjacent to the basal surface of two acinar cells and the parenchymal surface of a myoepithelial cell. The perinuclear cytoplasm of the myoepithelial cell has a few profiles of ER and Golgi membranes, a mitochondrion, and free ribosomes. Cytoplasmic filaments (*F*) fill the myoepithelial cell processes, and occasionally seem to converge upon the nuclear membrane (arrows). Vesicular invaginations of the plasma membrane of the myoepithelial cell are common (small arrow), but few are seen here. Glutaraldehyde-osmium tetroxide.  $\times 36,000$ .

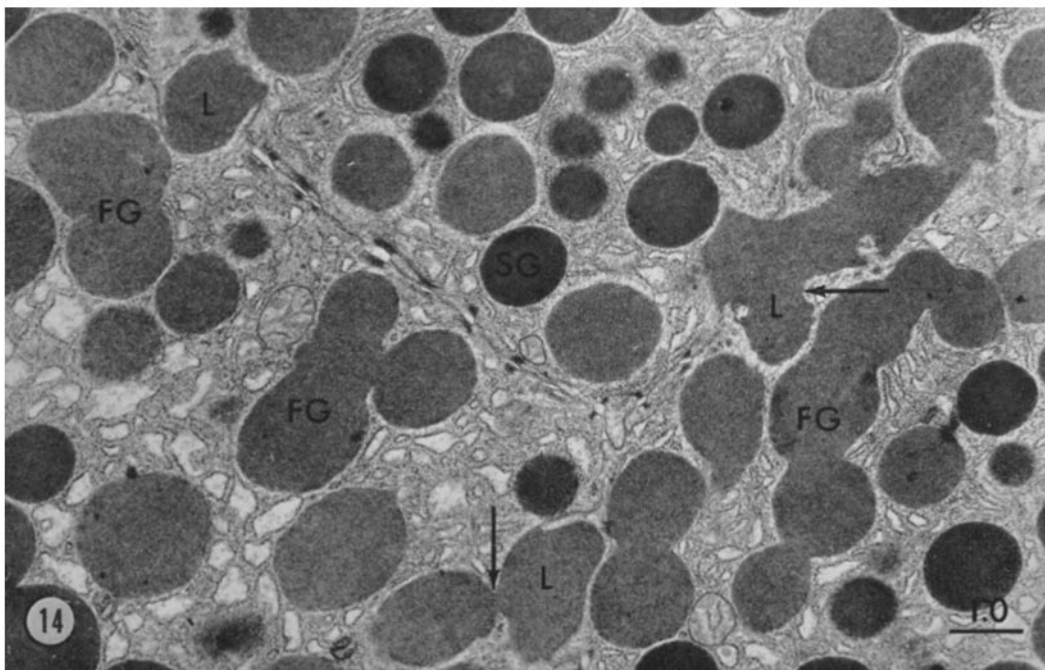
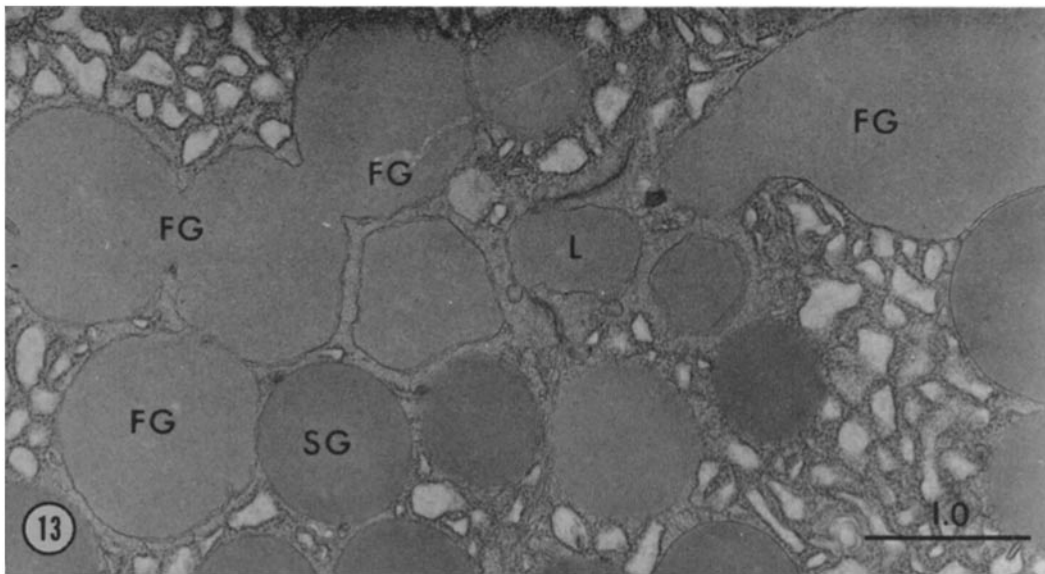


FIGURE 13 5 min after pilocarpine injection. Portions of two cells around an intercellular canal (*L*) are shown. Four fused granules (*FG*) can be seen in the cell on the left, and three to four granules have fused to make the large figure on the right. Connections between the fused granules and the intercellular canal are out of the plane of section. The fused granules appear less dense than the nonfused granules (*SG*). The cytoplasm around the fused granules and the intercellular canal is fibrillar. Glutaraldehyde-osmium tetroxide.  $\times 21,900$ .

FIGURE 14 Rat fasted 48 hr, sacrificed 5 min after refeeding. Portions of three cells and three intercellular canals (*L*) are shown. Fusion between granules and intercellular canals can be seen (arrows). Numerous examples of fused granules can be seen (*FG*), although their connections with the intercellular canals are not visualized. Fused granules are less dense than nonfused granules (*SG*). Osmium tetroxide.  $\times 9000$ .

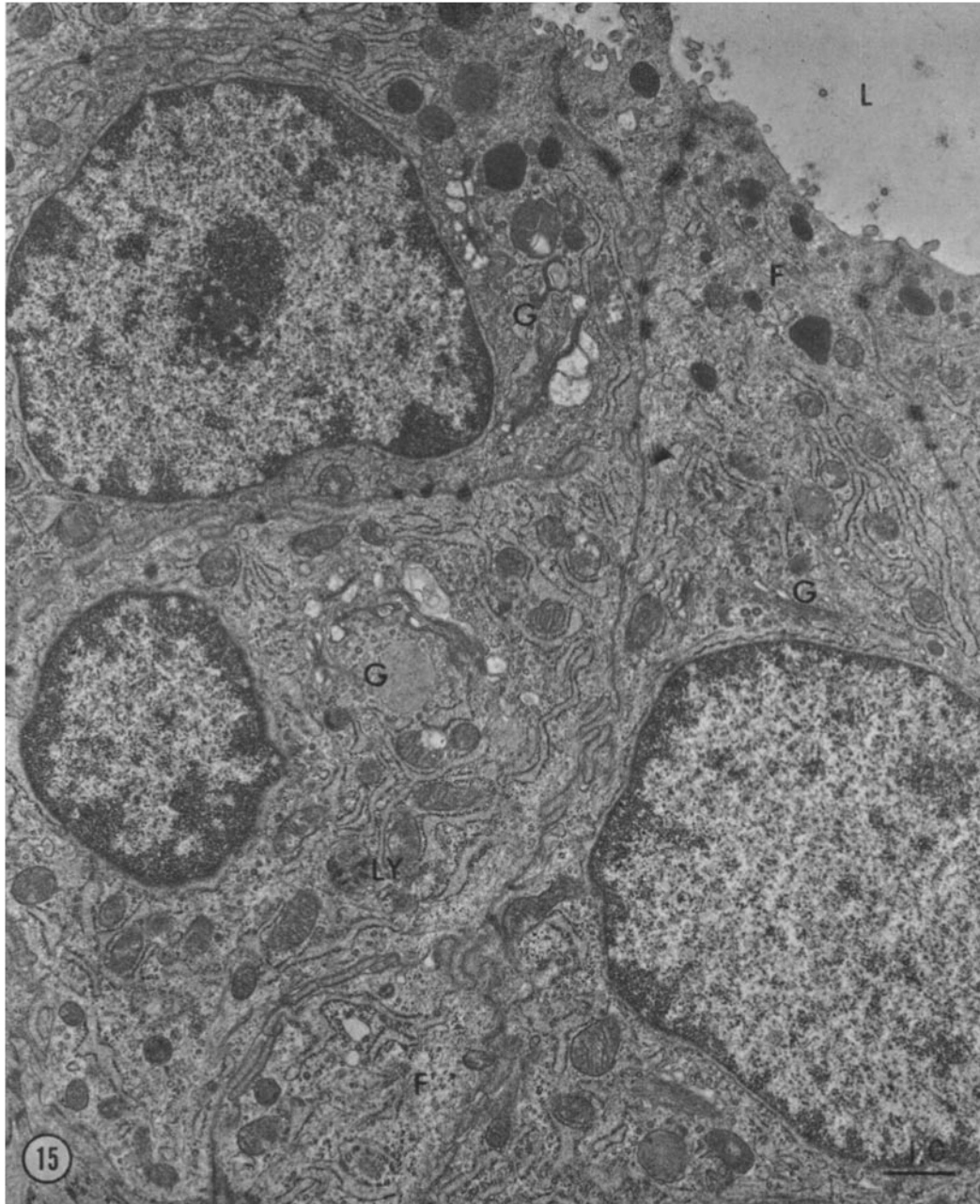


FIGURE 15 A portion of a duct showing the lumen (*L*) and parts of seven cells. Adjacent cell surfaces have interlocking processes and numerous desmosomes. The luminal surface is supplied with a few small microvilli. The Golgi complex (*G*) is well developed, and an occasional coated vesicle is evident. An autophagic vacuole (*LY*) is seen in the cell at the lower left. Numerous cytoplasmic filaments (*F*) are present. Granules of two different types can be seen in the apical regions of the cells. Glutaraldehyde-osmium tetroxide.  $\times 10,200$ .

essentially similar to those of other exocrine secretory glands (7, 23, 38, 39, 44, 45), suggest that the secretory product of von Ebner's gland is proteinaceous, and that its intracellular synthesis and transport is similar to that postulated for the pancreas (7, 22, 23, 38, 40).

In the pancreas, protein synthesis presumably occurs on the ribosomes of the ER; the protein is transferred to the cisternae of the ER (40), and is then transported to the condensing vacuole via the ER cisternae, the transitional elements, and the peripheral vesicles of the Golgi region, which supposedly act as "shuttle carriers" between the ER and the condensing vacuole (22, 23). The Golgi lamellae do not appear to be involved in the transport of protein, but may instead add a second component to the condensing vacuole. In other cells, the Golgi region has been shown to be the site of incorporation of carbohydrate moieties into the secretory product (31, 32). The acid hydrolytic activity associated with newly formed secretory granules in some glands (33, 46) may be contributed by the Golgi apparatus. Another function attributed to the Golgi complex is that of supplying membranes to surround the secretory granules. Occasionally, apparent connections were observed between the Golgi lamellae and the condensing vacuole of von Ebner's gland. The significance of these connections is being explored further.

The Golgi complex has also been implicated in the formation of lysosomes in other cells (9, 12, 15, 34) and may play a similar role in von Ebner's gland. The dense heterogeneous lysosome-like bodies with a content of vesicular and lamellar profiles showed a consistent topographical relationship with the Golgi region. Lysosomes in secretory cells such as von Ebner's gland may participate in removal or degradation of excessive or exhausted membranous material. Fowler and de Duve (14) have shown that extracts of lysosomes isolated from liver are enzymatically capable of degrading the membranes of mitochondria and microsomes. Hicks (17, 18) attributed membrane breakdown to dense heterogeneous bodies in transitional epithelium of the rat ureter and bladder. She was able to demonstrate acid phosphatase activity in some of these bodies, thereby classifying them as part of the lysosomal system. Acid phosphatase and nonspecific esterase have previously been demonstrated in von Ebner's gland by light microscopy (2, 21, 30), and Bogart

(6) has recently shown acid phosphatase activity associated with lipofuscin granules in the rat submandibular gland, some of which show similarities to the dense bodies described in the present study.

Stimulation of secretion by fasting-refeeding or pilocarpine administration showed that granule discharge occurred by fusion of the granule membrane with the cell membrane at the secretory surface. Once fusion had occurred between one granule and the cell membrane, another granule was able to fuse with the first granule. Repetition of this process apparently leads to a string of connected granules in the cell. The density of fused granules was always less than that of granules in the same cell which were not fused. The cytoplasm immediately surrounding the fused granules had the characteristics of the apical cytoplasm in nonstimulated cells, i.e. finely fibrillar and devoid of organelles. These findings indicate, as suggested by other workers (1, 20), that the membrane of the fused granule takes on the properties of the cell membrane, a transformation which enables other granules to fuse with it.

Once the content of the granules has been released, the fused granule membranes appear to be left as an invagination of the now enlarged lumen into the apical cytoplasm of the cell. The fate of this membrane as the cell resumes its former shape is unknown. Fawcett (13) has suggested that an amount of membrane equivalent to that which was added during secretion is withdrawn from the cell membrane at a molecular level and reassembled into visible membrane in the Golgi region. Palade (37) has suggested that in pancreatic acinar cells small "empty" vesicles seen after secretion of zymogen granules might represent return of membrane to a "membrane depot" in the Golgi region. In the parotid gland stimulated to secrete with isoproterenol, Amsterdam et al. (1) have demonstrated numerous vesicles in the apical region of the cell during the reduction of the size of the lumen. Comparable vesicles were not observed in the present study; however, a massive secretion as caused by isoproterenol in the parotid gland was not achieved in von Ebner's gland.

Coated vesicles have been described in many other cells (8, 16, 19, 35, 41, 42) where they are mainly involved in protein uptake from the extracellular space. They have also been observed

to originate from the Golgi complex and to contain hydrolytic enzymes (16). The function of coated vesicles in von Ebner's gland is unknown, but their association with the Golgi complex suggests a possible role in secretory granule or lysosome formation.

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