THE FINE STRUCTURE OF BRUNNER'S GLANDS IN THE MOUSE

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ABSTRACT

Examined with the electron microscope, the secretory cells of the submucosal glands of Brunner in the mouse present a curious combination of the fine-structural features of both serous and mucus-secreting cells. The cells have numerous mitochondria, abundant basal ergastoplasm, dense secretory granules that bear a superficial resemblance to pancreatic zymogen granules, and an unusually extensive Golgi apparatus. The prominence of the lamellar, vesicular, and vacuolar elements of the Golgi complex facilitates detailed observation of these components. More evident than in other glandular cells, aggregates of small vesicles appear to represent the transitional elements and are vehicles for transport of the product between the ergastoplasm and the Golgi complex. The numerous vesicular evaginations of smooth-surfaced regions on cisternae of the rough-surfaced endoplasmic reticulum and accumulations of innumerable vesicles of similar size in the area between the nearest profiles of the ergastoplasm and the Golgi complex support this contention. The cytological characteristics and physiologic properties of Brunner's glands in various species are discussed briefly. It is concluded that the submucosal glands of the mouse are excellent material for exploration of the ultrastructural correlates of both protein and carbohydrate secretion, and it is suggested that their secretion may have functions other than those generally attributed to them, namely, chemical and mechanical protection of the duodenal surface epithelium.

INTRODUCTION

The fine structure of the submucosal duodenal glands of Brunner has thus far been described only for the guinea pig (4) and the cat (25). The principal cell of these glands in the guinea pig is reported to be a typical mucous cell while in the cat it has many of the characteristics of serous cells. In the mouse, described here, Brunner's glands are coiled, tubular merocrine glands that extend 1 to 3 mm beyond the pyloric sphincter (11). They are usually classified as mucous glands because they are analogous to the duodenal submucosal glands of other species, and because of their viscous, carbohydrate-rich secretion which, as far as is known, lacks digestive enzymes. However, the cells of Brunner's glands in the mouse strongly resemble serous cells in their fine structure. As in the cat, they are rich in organelles, have an abundant basal ergastoplasm, small apical secretory granules, and a system of intercellular canaliculi—features which set them apart from cells generally acknowledged to be mucus secreting (21, 27). Electron microscopic studies make it apparent that the traditional categorization of exocrine secretory cells as either mucous or serous is inadequate. Glands with intermediate cytological characteristics are being recognized with increasing frequency and should be separately designated. The murine Brunner's glands appear to belong in this intermediate category. It is the purpose of the present electron...
A microscopic study to establish the normal fine structure of this cell type in the mouse as a basis for further exploration of the specific role of the unusually prominent Golgi apparatus in the elaboration of its complex secretory product.

MATERIAL AND METHODS

Fourteen male mice of the AKD-2 strain were used (Jackson Memorial Laboratory, Bar Harbor, Maine). The animals ranged from 8 to 26 weeks in age and 30 to 44 gm in weight. They were either continually maintained on a diet of Purina chow or fasted for 24 hours prior to sacrifice. Two animals received 100 microcuries of tritiated l-leucine intraperitoneally in a diluent of 12.5 per cent ethyl alcohol and 0.9 per cent saline and were sacrificed 15 and 45 minutes after injection. The results of the autoradiographs made from these animals are to be reported elsewhere. Inasmuch as the injections of labeled amino acids had no effect upon the appearance of Brunner's glands, tissues from these animals are included with those of untreated mice in the present description of the normal ultrastructure of the gland.

The animals were killed by cervical dislocation after light ether anesthesia. The abdomen was rapidly incised, the first portion of the duodenum excised and minced into 1- to 2-mm blocks in a few drops of cold 1.33 per cent osmium tetroxide buffered to pH 7.25 to 7.45 with s-collidine (1). The buffered fixa-

Figure 1 A diagram of a Brunner's gland cell from the mouse duodenum.
tive contained 0.025 to 0.045 gm of sucrose (2) and approximately 0.03 mg of calcium chloride per cubic milliliter. The tissue was then transferred to vials containing fresh fixative at 4°C in which it remained for 2 hours with occasional agitation. After decanting the fixative, the tissues were rapidly dehydrated in a series of increasing concentrations of cold ethanol beginning with 70 per cent and brought slowly to room temperature in absolute ethanol. Dehydration was completed with two additional 15-minute changes of absolute ethanol and two 15-minute changes of propylene oxide. Embedding was in Epon containing fresh fixative at 4°C in which it remained approximately 0.03 mg of calcium chloride per cubic milliliter. The tissue was then transferred to vials of Epon 812, dodecenyl succinic anhydride, and nadic methyl anhydrides, respectively, containing 1.5 per cent 6,4,6-tri(dimethylaminoethyl) phenol. Sections displaying silver-to-gold interference colors were cut with glass knives on a Porter-Blum microtome and picked up on uncoated 150-200 mesh copper grids. The sections were stained with lead acetate for 1 minute followed by lead citrate (33) for 3 to 5 minutes. Electron micrographs were made with an RCA EMU 3E or 3F microscope. Preparations for light microscopy were cut at 1 micron and stained with 5 per cent toluidine blue in borax or by the periodic acid–Schiff (PAS) reaction.

Observations

General Histological Organization

Brunner’s glands in the mouse consist of coiled tubules arranged in aggregations 1/2 to 1 millimeter in diameter and randomly distributed in the submucosa around the circumference of the duodenum. The cross-section of each tubule is comprised of six to ten cells around a narrow lumen. The cells have a truncated pyramidal form, 12 to 16 μ across the base and 13 to 17 μ in height. They are, in general, of a single type except for rare argentaffin, Paneth, or goblet cells which may be found near the openings of the glands into the crypts of Lieberkühn. The cells vary somewhat in their appearance in different functional conditions. After fasting, they are low columnar in form and contain abundant secretory granules. After feeding, they tend to be cuboidal and contain relatively few secretory granules.

The Surface and Internal Fine Structure of the Cells

The luminal surface of the gland cells is not highly specialized, but does have a few short microvilli (Fig. 3). These are irregular in their orientation and distribution except that a single prominent microvillus is invariably found adjacent to the junctions of neighboring cells. The plasmalemma of the free surface is a typical unit membrane about 100 A thick. Its innermost dense layer and intermediate light zone are more clearly resolved than in the membranes on the lateral and basal surfaces of the cell. The outer surface is characterized by the presence of a superficial layer of radially oriented fine filaments. This layer is most obvious on the microvilli and its components appear to correspond to the filamentous exocertificates first described on gall bladder epithelium by Yamada (35) under the term “antennulae microvillares.” A similar surface coating was subsequently observed by others on the epithelia of the urinary bladder (3, 30), stomach (19), acini and ducts of the pancreas (9), and elsewhere. The interior of the microvilli occasionally shows faint longitudinal striations suggesting the presence of a fibrillar internal component.

For the most part, the cells are in close lateral contact over the entire height of the epithelium but, at the angles where three cells meet, the free surfaces not infrequently extend some distance down the sides of the cells to form short edge-canals or secretory canaliculi. Microvilli are longer and more numerous on this part of the surface and their extensive interdigitation all but occludes the lumen of the narrow intercellular canals. Elsewhere along the free surface, adjacent cells are in close apposition and united by typical junctional complexes consisting of a tight junction (zonula occludens) near the lumen, an intermediate junction (zonula adherens), and desmosomes (macula adherens) arranged in that sequence (7, 9). With the fixation employed here, the intercellular cleft is narrowed to 100 A or so, but does not appear to be entirely obliterated at the site of the zonula occludens. The membranes diverge to the usual 200-A distance in the region of the zonula adherens and at the maculae adherentes and elsewhere on the opposing lateral surfaces. The cytoplasm immediately subjacent to these specialized regions of the junctional complex is condensed as usual, and reinforced by a feltwork of fine tonofilaments, but these are quite localized and are not continuous with a recognizable terminal web.

The lateral cell surfaces below the junctional complex are relatively straight but become extensively interdigitated near the base. In se-
tions parallel to the cell base, it can be seen that there is considerable intercrescence of slender flutings at the corners in which three or more cells meet (Figs. 2, 5). Except for occasional shallow infoldings, the plasmalemma at the cell base is uncomplicated and rests upon a moderately dense basement lamina ("basement membrane") 500 to 800 A thick.

The nucleus is spherical or ovoid and situated in the basal half of the cell. Sections often include one or two nucleoli that are organized with the denser amorphous regions of the nucleolonema in the center and the less dense, granular regions around the periphery. The chromatin is usually dispersed but in some cells, especially from fasted animals, small irregular masses of heterochromatin are distributed around the periphery of the nucleus. The nuclear envelope is interrupted by a variable number of nuclear pores (Fig. 6) of which there may be from two to half a dozen, unevenly spaced around the circumference. In some sections of nuclei none is encountered. It is the author's impression that there is a tendency for the pores to occur with greater frequency along the upper pole of the nucleus near the Golgi complex. An interruption in the continuity of the peripheral chromatin is always found opposite the fenestrations in the nuclear envelope, and in favorable sections the "pores" are seen to be closed by a very thin diaphragm that is generally thinner than the unit membranes that make up the rest of the nuclear envelope. In some areas, the outer membrane of the nuclear envelope bears ribosomes on its cytoplasmic surface and is occasionally seen to be in continuity with cisternae of the endoplasmic reticulum.

The mitochondria appear as ovoid or elongate structures lodged between the profiles of the endoplasmic reticulum. Unusually long or branched forms are not uncommon. There are usually ten to fifteen mitochondria in any given section of a cell. Their cristae are moderately numerous and frequently extend the full width of the organelle (Fig. 8). Mitochondrial granules about 500 A in diameter are present in small numbers in the intercristal matrix. Two kinds are distinguishable. One is irregular in shape and appears to be made up of a number of minute compartments bounded by thin denser borders (Fig. 7). The other type is regular in outline, solid and extremely dense (Fig. 5).

The Golgi apparatus consists of three major elements — smooth-membraned cisternae or lamellae, vacuoles, and vesicles (5, 6, 8, 27) (Fig. 4). The distribution and appearance of the Golgi components vary with cellular activity. In general, they are compact in their organization and characterized by prominent parallel arrays of lamellae when the gland is less active (Fig. 3), while in stimulated glands (Figs. 2, 5) they are more dispersed with vesicles constituting the predominant component. In glands at both extremes of physiological activity, however, many of the cells have a Golgi pattern intermediate between the typical compact and the typical

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**Figure 2** This low power electron micrograph which includes portions of four Brunner's gland cells reveals the fluted, interdigitating pattern of adjacent cell membranes (CM). The distribution of numerous aggregates of Golgi lamellae, vesicles, and vacuoles (GO) is typical for gland cells from animals which were stimulated by feeding. The uniformly dispersed nuclear chromatin, the appearance of the nucleolus, and the stippling of the dense secretory granules are characteristic for gland cells from both fed and fasted animals. \( \times \) 15,000.

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FIGURE 3 The short microvilli are sparse and irregular in their orientation and distribution on the luminal surface formed by the three gland cells in this electron micrograph. The narrow lumen contains material similar to that in the membrane-bound secretory granules in the apices of the cells. The Golgi apparatus in this section lateral to the nucleus is composed of parallel arrays of lamellae enclosing a space occupied by vesicles, vacuoles, and a few mature secretory granules; the configuration is characteristic of gland cells from fasted animals. × 8,000.

dispersed forms. In sections passing through the supranuclear region, transverse to the cell axis, the Golgi region is recognized as a large circular area outlined by curving parallel aggregations of Golgi cisternae. In sections through the cell axis, a flattened elliptical or crescentic area is similarly outlined by packets of cisternae. Visualized in three dimensions, the lamellar elements of the Golgi complex thus appear to form the discontinuous wall of an indented and partially collapsed, hollow sphere with the curvature of the juxtanuclear cisternae conforming to the convex contour of the nucleus.

The parallel lamellar arrays of Golgi elements consist of seven to ten membrane-limited cisternae. The membranes are thinner than the plasma-

FIGURE 4 The three major elements of the Golgi apparatus—lamellar arrays of smooth-surfaced cisternae, vacuoles (VAC), and vesicles—are illustrated in the paranuclear region. The cisternal profiles have bulbous terminal expansions. Vacuoles partially filled with dense material and the mature secretory granules (G) are found between the nucleus (NUC) and the lamellae, while clear vacuoles are present localized on the side nearest the lateral cell membrane. Vesicles cluster around the margins of the cisternae. × 43,000.
lemma, and the flat cavities that they enclose vary in width from 150 to 300 Å and are 150 to 250 Å apart (Fig. 4). The packets of cisternae vary greatly in their extent. Some are only about a micron long in section, but others are much longer and may pursue an undulant course across the entire width of the cell. Accumulations of vesicles cluster around the margins of the assemblages of cisternae, and there is no indication of continuity of membranes from one group of lamellae to another. Most of the cisternae have a lumen of low density, but some have a content of appreciable electron opacity and of a texture resembling that of the secretion granules located in the concavity of the Golgi complex. There is an indication of functional polarity within the lamellar packets, in that it is invariably the innermost one or two cisternae that contain this dense material. The outermost cisterna is often so extensively fenestrated that it resembles a row of vesicles (Fig. 5). The cisternal profiles often have bulbous terminal expansions and fusiform enlargements along their length (Fig. 4). These have a content that varies from barely detectable to a concentration and density comparable to that of mature secretory granules. It is clear that by further expansion and repletion these expanded segments of cisternae give rise to secretion vacuoles and, in turn, to granules, presumably by further addition and concentration of product. It is not uncommon to find vacuoles of secretory material with tail-like outpocketings or “buds” (Figs. 7 and 8). A profusion of vesicles of similar size is often found between these terminal elements of the ergastoplasm and the nearest packet of Golgi cisternae. Similar observations in pancreas and other protein-secreting gland cells have invited the speculation that the vesicles are budded off from the agranular areas of the ergastoplasm and that they transport quanta of the cell product to the Golgi complex (25, 36). The present study supports this view. There are many transition zones present.

The mature secretory granules, PAS-positive as seen in thick sections, are 0.4 to 0.6 μ in diameter and approximately round or oval in section. Their limiting membrane is somewhat irregular in contour, possibly due to some degree of shrinkage during specimen preparation. It is closely applied to the contents which have a uniformly gray, finely stippled appearance in thin sections (Fig. 4). In their size, mode of formation, and the texture of their contents, the secretory granules more closely resemble the prozymogen granules of serous glands than they do the large heterogeneous droplets typical of mucus-secreting goblet cells. The granules are always present in substantial numbers within the limits of the Golgi zone and evidently move out through the spaces between neighboring packets of cisternae to accumulate in the apex of the cell near its luminal surface and are also distributed along their outer and inner surface where they are often aligned in rows. Another site of accumulation of vesicles is in proximity to certain cisternal elements of the ergastoplasm around the periphery of the Golgi region. These cisternae are characteristically in close topographic relation to a mitochondrion and often partially surround it. The surface of the cisterna toward the mitochondrion is usually studded with ribosomes while that facing the Golgi complex is smooth surfaced. At numerous points along the ribosome-free areas of such cisternae there are small 600- to 900-Å outpocketings or “buds” (Figs. 7 and 8). A profusion of vesicles of similar size is often found between these terminal elements of the ergastoplasm and the nearest packet of Golgi cisternae. Similar observations in pancreas and other protein-secreting gland cells have invited the speculation that the vesicles are budded off from the agranular areas of the ergastoplasm and that they transport quanta of the cell product to the Golgi complex (25, 36). The present study supports this view.

Vesicles 400 to 800 Å in diameter are exceedingly numerous throughout the Golgi region. A few are hirsute or alveolate (28), but the great majority are smooth-surfaced vesicles with moderately dense content. They are particularly abundant at the ends of the arrays of cisternae, but

**Figure 5.** Segmented arrays of Golgi lamellae are in linear array in the apical cytoplasm. The inner cisternae of the upper lamellar aggregates are extensively fenestrated so that they resemble rows of vesicles. The vacuoles containing dense material appear to arise from dilatations of the cisternae. A multivesicular body (MVB) is present in the upper left; the lumen (L) of a canalculus is on the right. Notice the single cisternae of ergastoplasm partially surrounding many mitochondria. X 24,000.
Figure 6 This electron micrograph illustrates the frequent distribution of nuclear "pores" (P) along the upper pole of the nucleus near the Golgi complex. Adjacent to the Golgi lamellae a vacuole (DV) is filled with secretory material, its limiting membrane is continuous with a tail-like vestige of a cisterna (arrow). A less dense vacuole (V) and a mature secretory granule (G) surround it. Profiles of the granular endoplasmic reticulum (ER) are in the lateral perinuclear area. × 21,000.

laterally along the intercellular canaliculi. Rarely, granules with a content of less than usual density are encountered in contact with the plasmalemma, in such a way as to suggest a fusion of the limiting membrane of the granule with the cell membrane. In such instances, secretory product can sometimes be seen escaping into the lumen. Empty-appearing vacuoles connected with the lumen by a narrow neck suggest later stages in evacuation of the content of the secretory granules.

Other structures commonly associated with the Golgi apparatus are multivesicular bodies (29) and small pleomorphic osmiophilic bodies presumed to be lysosomes. No less than one out of four sections passing through the Golgi region includes one or more multivesicular bodies which are 300 to 400 mμ in diameter and contain three to twelve small vesicles (Fig. 5). Larger osmiophilic structures occasionally found adjacent to the nucleus correspond to the perinuclear lipid bodies described by Cochrane et al. in Brunner's glands of the guinea pig.

Discussion
Brunner's first major paper in 1715, describing the submucosal glands in the duodenum (discovered earlier by Wepfer in 1679), employed the term "pancreas secondarium." About a century later, this term was abandoned as inaccurate and Brunner's name was applied to the glands (17). Efforts to replace the eponym by the official designation glandulae duodenales (PNA) have met with only limited success. These glands, present only in mammals, have been shown to consist of mucous cells in man, ox, sheep, deer, beaver, dog, goat, rat, pig, and guinea pig (12, 13, 21). In the rabbit and horse a serous component has been
reported (23). In the species in which it has been
studied (cat, goat, sheep, rabbit, and pig), the
secretion is clear, viscous, and distinctly alkaline
(pH 8.2 to 9.3). The principal function of the
glands is thought to be to protect the duodenal
mucosa against the erosive effects of the gastric
juice by virtue of the mucoid nature of its secretion,
its alkalinity, and possibly by the buffering capac-
ity of its bicarbonate content (17). No enzymatic
activity involved in the digestive process has yet
been found, although in some species juices
collected from the region of Brunner's glands do
contain a mucolytic enzyme (13, 18). Secretion
of the glands is enhanced by humoral (crude
secretin), nervous (parasympathetic), and me-
chanical stimuli (presence of food or rubbing the
mucosal surface) (13-17, 34).

Although the similarities of the duodenal glands
of various animals have been emphasized in the
past, there has long been physiological evidence of
significant differences among species, and this is
borne out by recent histochemical studies.
Brunner's glands in the cat exhibit marked alkaline
phosphatase activity, while none is demonstrable
in the guinea pig (25, 4). The glands have histo-
chemically demonstrable lipase activity, and this
occurs in species whose glands are histologically of
pure mucous type (34) as well as in those whose
glands have serous cells (17). There also appears
to be species differences in the character of the
mucopolysaccharides and the degree of their
sulphation. In different species, the cells vary in
the intensity of their staining with mucicarmine
(13) and in their metachromasia (20). The
duodenal glands of most species are rich in
sulphated mucopolysaccharides and, therefore,
concentrate radioactive sulphur. Those of the
mouse do not (20).

Electron microscopic studies of Brunner's
glands have revealed fine structural characteristics
that may be correlated with the histochemical
and physiological differences among species. The
glands in the guinea pig consist of typical mucous
cells (4). They contain large globules of secretory
material of low density, the ergastoplasm is
sparse, and mitochondria relatively few. The
cytoplasmic matrix is dense and often contains
dense juxtanuclear lipid bodies that are un-
reactive for acid phosphatase. The cell surface
bears a few short microvilli, but there are no
intercellular canaliculi. In the cat (25) and in the
mouse described here, the cells do not have the
characteristics of typical mucous cells, but in-
stead have cytological features intermediate
between those of mucous and serous cells. There
are small dense secretory granules, abundant
ergastoplasm, and numerous mitochondria. Juxta-
nuclear lipid bodies are rare. The cells are
provided with microvilli of distinctive appearance,
and there are intercellular canaliculi.

Investigators of glandular cells with the electron
microscope have been mainly concerned with
protein secretion. Much has been learned from
these studies and from correlated biochemical
analyses, about the function of ribosomes and the
role of the ergastoplasm in the elaboration of
protein cell products. It is likely that other
organelles are involved in the synthesis and con-
centration of different classes of substances. It is
clear that if we are to understand, in a broader
sense, the respective roles of the various cell
organelles in the complex process of secretion we
must take advantage of opportunities to study the
fine structural basis for synthesis of products of
diverse chemical composition. The mixed nature
of the secretion of Brunner's glands in the mouse
makes this interesting material for studying the
secretion of both carbohydrates and proteins in
the same cell. The morphological observations
reported here are consistent with the thesis that
the protein moiety of the secretion originates in
the ergastoplasm, but the synthesis of the carbo-
hydrate moiety may well reside in the extremely
well developed Golgi complex. Autoradiographic
studies are now in progress to test this hypothesis.
Evidence accumulating from various sources (8,
31, 32) indicates that the widely held view that the
Golgi complex is only concerned with segregation
and concentration of material synthesized else-
where in the cell, is not correct. It seems in-
creasingly likely that the Golgi complex has, in
addition to this function, a significant degree of
synthetic activity of its own, particularly for
complex carbohydrates.

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Figures 7 and 8 Cisternae of the ergastoplasm are frequently in close topographical relation to mitochondria. The surfaces of the cisternae toward the mitochondria are studded with ribosomes, while those facing the Golgi complex are smooth surfaced. "Buds" are numerous along the ribosome-free areas or transition zones of such cisternae and a profusion of vesicles of similar size is often found between these terminal elements of the ergastoplasm and the nearest packets of Golgi cisternae. Fig. 7, × 47,000. Fig. 8, × 24,000.

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