Fine Structure of the Bovine Parotid Gland

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Abstract. The bovine parotid glands were examined by transmission electron, scanning electron and light microscopies. The acinar lumina of the gland were notably spacious. Since the acinar cells were acidophil and reacted with periodic acid Schiff (PAS) but not stained with alcian blue (AB), they were thought to be serous in nature. The acinar epithelium was composed of light cells, dark cells and specific light cells. The light cells and dark cells contained various granules, many mitochondria and sparsely scattered RER short segments. The specific light cells had no granules along with few cytoplasmic organelles. The intercellular tissue spaces and canaliculi were well developed. The acinar cells extended many long microvilli into the lumina and into the intercellular spaces. The intercalated duct epithelium was composed of light cells alone, with no secretory granules. The striated duct epithelium also consisted of light cells, dark cells and specific light cells. These cells had no secretory granules. Their apical surfaces were characterized by the two different features, in that some were flat or folded and others had ovoidal or spheroidal protrusions of various sizes. In addition, between the epithelial cells of the striated duct and their basal lamina, there were special basal cells which had acidophil, PAS-positive and AB-negative granules with various electron densities.

A few reports have been published on histologic and histochemical structures of the bovine parotid gland which secretes a large amount of saliva and which presumably has some relations with the digestive process of the rumen [7, 9, 10].

Although the "continuous flow" of secretion of the ruminant parotid saliva has been shown [3-5], descriptions of ultrastructure of the gland itself have so far been based chiefly on the acini, with little emphasis on the intercalated and striated ducts [12, 25, 26]. Moreover, no attempt has been made to observe the gland by scanning electron microscopy.

In the present study, fine structures of

both the acini and the intercalated and striated ducts of the bovine parotid gland were examined by transmission and scanning electron microscopes and by a light microscope, with additional discussion on the relationships between the structure and the function of the gland.

Materials and Methods

Seven adult cows (4 Japanese black and 3 dairy cows) were killed by cutting the common carotid artery under local procaine anesthesia.

For transmission electron microscopy (TEM), tissue blocks of the central part of the parotid gland were removed quickly and fixed in a mixture of 1.25% glutaraldehyde and 1% osmium tetroxide adjusted to pH 7.4 with phosphate buffer. Then the blocks were dehydrated in a graded series of

ethanol and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEM-100 B and a JEM-100 C transmission electron microscope.

For scanning electron microscopy (SEM), the tissue blocks were cut into smaller blocks of about $5 \times 5 \times 20$ mm. These smaller blocks were fixed in a mixture of 2% glutaraldehyde and 5% paraformaldehyde buffered with phosphate. Then they were immersed in 40% dimethylsulfoxide, frozen in Freon 22, fractured in liquid nitrogen, dehydrated by ethanol, and transferred into isoamyl acetate. After the dehydration was complete, the blocks were transferred into liquid CO_2 and dried by the critical point drying method. Some dried samples were coated either with gold or with carbon and gold palladium, and were examined with a HSM-2A and a JSM-25 scanning electron microscope.

For light microscopy, the tissue blocks were fixed in Zenker-formol or Bouin's fluid, embedded in paraffin and sectioned at $6 \mu\text{m}$. The sections were stained with hematoxylin-eosin, periodic acid Schiff reagent (PAS) or pH 2.5 alcian blue (AB).

Results

Acinus: Acinar cells were reacted with PAS but not stained with AB. By TEM, they consisted of 3 principal cell types; light, dark, and specific light. By SEM, on the fracture surface of the gland, the acini and the striated ducts were clearly distinguished (Fig. 1).

In each acinus, the light cells were predominant in number. They had many variable secretory granules of various densities in the supranuclear and apical regions in the cytoplasm (Fig. 2). The granules of moderate and high densities were relatively few and most of them were enveloped with thin limiting membranes. The granules of low density appeared to be fused with one another in most cases. The granules having their matrix of low density appeared similar to mucous granules. By SEM, some acinar cells had a few smooth spherical granules (Fig. 3). Again by TEM, Golgi apparatuses were relatively well developed in the supranuclear region. The granules of low density

were occasionally located closely to the Golgi membranes (Fig. 4). Short segments of rough endoplasmic reticula (RER) were dispersed throughout the cytoplasm.

The lumen of each acinus was wide and the intercellular canaliculi were well developed (Fig. 2). Numerous long microvilli extended into the lumen (Figs. 2, 3, 5), closely to which many granules were accumulated. In the lumen, there were a few dense granules enveloped with a limiting membrane (Fig. 5), apocrine processes (Fig. 6) and numerous secretory materials (Fig. 7). The lateral and basal borders of the acinar cells had a large number of microvilli-like cytoplasmic processes, as a prominent feature of the intercellular tissue spaces (Fig. 8).

The dark cells were very rare, having the electron dense cytoplasm and granules of high and moderate densities (Fig. 9). The features of cytoplasmic organelles were almost similar to those of the light cells.

The specific light cells were less in number than the light and dark cells, characterized by their electron lucent cytoplasm (Fig. 10). Short segments of RER were dispersed as in the foregoing light and dark cells, but mitochondria with flattened cristae were very few and scattered sparsely in the cellular margin. The electron density of the nuclei was less than that of other cell types. Free ribosomes were observed sparsely. The specific light cells did not contain any granules.

Intercalated duct: The epithelium of the duct was PAS-positive and AB-negative, consisting of cuboidal light cells alone (Fig. 11).

The light cells had a few vacuoles and vesicles of low and moderate densities in the apical portion, but did not contain secretory granules. Golgi apparatuses were not well developed, but short segments of

RER, free ribosomes and mitochondria were dispersed. Many microfilaments existed around the nucleus. The lateral and basal borders of the cells were relatively flat. By SEM, short microvilli were studded sparsely on the luminal surfaces.

Striated duct: The epithelium of the duct consisted of light cells, dark cells and specific light cells (Fig. 12), and was reacted with PAS but not stained with AB.

The light cells were predominant in number and had fine vacuoles and moderately dense vesicles in the supranuclear and apical regions, though without any secretory granules. Golgi apparatuses were not well developed. RER and free ribosomes were distributed sparsely, but mitochondria were relatively numerous. A large number of microfilaments existed in the supranuclear region. The typical basal striations (so-called basal infoldings) were not well developed. The lateral borders of the duct cells were relatively flat.

The dark cells were few, containing many vacuoles but no secretory granules. Golgi apparatuses were poorly developed. Free ribosomes and RER were very few. Mitochondria were more numerous than those of the light cells, but most of them were swollen (Fig. 12).

The specific light cells were fewer than the dark cells, containing fine vacuoles and vesicles in the apical portions. Short segments of RER, a few mitochondria, Golgi apparatuses and free ribosomes were scattered sparsely. On the apical surfaces of these cells, leaf-like and apocrine processes were observed (Fig. 12).

By SEM, the apical surfaces of the striated duct cells appeared pentagonal or hexagonal, characterized by the two features, in that some were flat or folded (Fig. 13) and others had variously sized spheroidal protrusions (Fig. 14).

The special basal cells were located between the epithelial cell of the striated duct and the basal lamina (Figs. 14, 15). These cells contained acidophil, PAS-positive and AB-negative granules. By TEM, they had many granules of two kinds; round or amorphous granules containing vesicular materials of various densities and round homogenous granules of high density (Figs. 16, 17). Most cells had one kind of these granules, and very few cells contained both kinds of granules. Any evidence as for release of these granules was not detected. Golgi apparatuses consisted of flattened lamellae and vacuoles, closely accompanied by variously sized vacuoles. Free ribosomes and slightly dilated RER were scattered. The special basal cell-surfaces adjacent to the striated duct cells were relatively flat, but occasional interdigitations of neighboring cells were observed.

Discussion

The acinar cells of the bovine parotid gland are said to be extraordinarily serous in type with no resemblance to serous cells of other salivary glands [9, 10], just in harmony with the present finding of AB-negativeness. Hence, the parotid acinar cells of cattle and sheep have come to be classified into a special type of serous cells [11]. Although the bovine parotid acinar cells were recently reported to have an extremely small quantity of neutral or weak acid mucopolysaccharides and to be categorized in seromucous type [7], our observations still support the view that the bovine parotid acini are serous in type, not seromucous.

The present study shows that the acinar epithelium is composed of light cells, dark cells, and specific light cells. Among these, most of the light and dark cells contain 2:

or 3 kinds of granules. The observed morphologic differences in these granules may reflect their chemical differences. Pale granules located closely to the Golgi apparatus are presumably immature. In contrast, dense granules located closely to the acinar lumen as well as to the intercellular canaliculi are thought to be mature.

Both TEM and SEM reveal that the acini have wide lumina, intercellular canaliculi and well-developed long microvilli extending into the lumina, in which many spherical materials and apocrine processes are included. The lumina of the bovine parotid acini are notably spacious in particular, as compared with those of non-ruminant animals [6, 17, 20, 21]. Such characteristics of the bovine parotid acini are considered to be closely related to the continuous salivation, as was previously pointed out by studies of the goat parotid gland [16, 18].

The intercalated duct of the bovine parotid gland has its epithelial cells reacted weakly with PAS but not stained with AB, as was already reported in cattle and sheep [11]. By TEM, our observations confirm that there are no electron dense granules in the intercalated duct cells of the bovine parotid gland, in line with observations for cattle, sheep [11] and goat [16, 24].

The striated duct of the bovine parotid gland has its peculiar component cells which contain relatively numerous mitochondria and a few fine vacuoles and vesicles in the supranuclear and apical regions of the cytoplasm. In relation to this, in the monkey parotid gland, similar fine vacuoles and vesicles are found and assumed to be secretory materials [6]. The apical surfaces of epithelial cells of the bovine striated ducts are very variable in appearance. Such a wide apical surface variation may reflect changes in secretory function of respective cells, closely associated with a large amount

of salivation in ruminants as reported for the goat [18].

The striated duct of the bovine parotid gland has its special basal cells which often contain acidophil granules. These cells were formerly described as "mast cells" [28] and also as "intrastriated duct cells" resembling mast cells [9]. Incidentally, seemingly very similar cells, globule leucocytes, are situated among the intestinal epithelia [27]. So-called brilliant cells [1, 15] found in the liver are also very similar, but are said to be originated from smooth muscle cells. Thus, the relationships between the special basal cells described here and the mast cells, globule leucocytes and brilliant cells should be investigated in more detail.

Concerning the dark cells noted in the acini and striated ducts, many descriptions have been accumulated for various animals [12, 16, 20, 21]. Similar dark cells are also described in the mandibular glands [13, 14, 19, 30]. In sympathectomized rats, the dark cells are reported to be an exclusively major component of the acini of the parotid gland [29]. There is another report that in the pancreatic exocrine part, the dark cells are artificial products in making the specimen preparations [2]. However, it is reasonable to consider that the dark cells found in the present study are healthy constituents together with other component cells of the acini and the striated ducts of the bovine parotid gland, because the number of the dark cells is quite small and because they have morphologically clear-cut characteristics which can be clarified only by TEM level.

As regards the specific light cells, similar cells are present in the parotid glands of goat [16], dog [16] and rabbit [21] and in the mandibular glands of horse [22] and rabbit [23]. These cells have electron lucent cytoplasm containing few cytoplasmic or-

ganelles. Their nuclei are lower in electron density than those of the light cells. These findings of the specific light cells are similar to those of autolytic hepatocytes [20], and hence, lead us to assume that they are in the course of degenerative changes.

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要 約

ウシの耳下腺の微細構造について：鈴木秀作・西中川駿・大塚閏一（鹿児島大学農学部家畜解剖学教室）、望月公子（東京大学農学部家畜解剖学教室）——成熟したウシの耳下腺の腺胞・介在部および線条部について、光学顕微鏡ならびに透過・走査電子顕微鏡で観察した。耳下腺の腺腔は広く明瞭で、腺胞細胞は微酸好性で、PAS に弱く反応し、AB に染まらない漿液細胞であった。電顕的に、腺胞細胞は明調・暗調細胞、さらに、細胞小器官の少ない明るい特殊細胞からなり、前二者の細胞は種々の分泌顆粒を有していた。腺腔は広く、細胞間隙ならびに細胞間分泌細管がよく発達し、これらに面する細胞自由面には、よく発達した微絨毛やアポクリン突起がみられた。また、明調細胞の核上部には、多くのミトコンドリアが認められたが、粗面小胞体は短桿状を呈し、その分布は疎であった。介在部は、丈の低いPAS 陽性で、AB に染まらない立方上皮細胞からなり、電顕的には、明調細胞のみからなり、明らかな分泌顆粒は認められなかった。線条部は、PAS 陽性で、AB に染まらない丈の高い円柱上皮細胞からなり、明調・暗調細胞と、さらに明るい特殊細胞がみられた。これらの細胞には、いずれにも明らかな分泌顆粒は認められなかった。管腔面の構造は、さまざまな形状を示し、大きく2つの型がみられた。一つは、短かい微絨毛が比較的少ないものから多数存在するもの、他は、前者と形態的に全く異なり、細胞の頂部が膨隆していた。さらに、線条部の底部には、酸好性で電子密度の異なる種々の顆粒をもつ特殊基底細胞が認められた。

Explanation of Figures

- Fig. 1. Low magnification of scanning electron microscopic image of the fracture surface of the bovine parotid gland. The acini and striated ducts are observed. $\times 280$.
- Fig. 2. TEM. Acini showing the wide lumina and intercellular canaliculi. Secretory granules of high and low density and numerous mitochondria are observed in the cytoplasm. Numerous long microvilli extend into the lumina. $\times 2,800$.
- Fig. 3. SEM. Acinar lumen showing the well-developed microvilli. Acinar cells contain a few smooth spherical granules. $\times 12,000$.
- Fig. 4. TEM. Acinar cell containing well-developed Golgi apparatus. Pale granules are seen closely to the Golgi zone. $\times 14,000$.
- Fig. 5. TEM. Acinar lumen containing a secretory granule. The granule includes a corpuscle of high density and is enveloped with a limiting membrane. $\times 11,200$.
- Fig. 6. TEM. Apical portion of an acinar cell. An apocrine process projects into the lumen. This process contains cored granules enclosed by a single limiting membrane. $\times 11,200$.
- Fig. 7. SEM. Acinus including smooth spherical materials in its wide lumen. $\times 6,260$.
- Fig. 8. TEM. Basal portions of acinar cells. The lateral and basal borders of acinar cells are provided with numerous cytoplasmic processes. $\times 4,780$.
- Fig. 9. TEM. Acinar dark cell showing electron dense cytoplasm. The cell contains granules of high and moderate density. $\times 7,980$.
- Fig. 10. TEM. Acinar specific light cell showing electron lucent cytoplasm. Cytoplasmic organelles are not well developed. $\times 4,800$.
- Fig. 11. TEM. Intercalated duct cells, cuboidal in shape. The epithelia are composed of light cells without secretory granules. $\times 2,600$.
- Fig. 12. TEM. Striated duct cells showing a flat, leaf-like and apocrine process-like expansion in the apical surfaces. Light and dark cells are observed. $\times 3,100$.
- Fig. 13. SEM. Luminal surface of the striated duct. The apical surfaces of the striated duct cells are pentagonal or hexagonal in outline. The surfaces are flat and folded. $\times 1,490$.
- Fig. 14. SEM. Luminal surface of the striated duct. Ovoidal or spheroidal protrusions are observed. Special basal cell containing spherical granules is observed in basal portion. $\times 1,490$.
- Fig. 15. TEM. Special basal cell of the striated duct. The cell is observed between the striated duct cells and basal laminae. $\times 4,500$.
- Fig. 16. TEM. High magnification of granules in the special basal cell shown in Fig. 15. Each granule exhibits a dense granular matrix separated by a large space from the limiting membrane. $\times 18,000$.
- Fig. 17. TEM. High magnification of granules in the special basal cell. Round granules of high density and amorphous materials of moderate density are present. $\times 19,200$.