Dept. of Anatomy and Histology, Fac. Vet. Med., Assiut University.

FINE STRUCTURE OF THE SECRETORY END-PIECES OF THE PAROTID SALIVARY GLAND OF THE ONE-HUMPED CAMEL (CAMELUS DROMEDARIUS)

(With 25 Figures)

By
A.O. SALEM and YOUSRIA A. ABDEL-RAHMAN
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التركيب الدقيق للنهايات الإفرازية للغدة اللعابية النكفية في الجمل وحيد السنام

أحمد عمر سالم ، يسريه عبد الغني عبد الرحمن

في هذا البحث تمت دراسة التركيب المجهري الدقيق للنهايات الإفرازية للغدة اللعابية النكفية في ثمانية جمال بالغة من كلا الجنسين. أظهرت الدراسة أن النهايات الإفرازية عبارة عن عني بات مصلية بحقه مبطنة بخلايا هرمية الشكل تفاعلت سلبيا مع كل من معامل شيف والألسين الأزرق. كما أظهرت الدراسة أن هذه العنبيات لها تجاويف متسعة وأن خلاياها المبطنة تميزت بالأنثناءات القاعدية الكثيفة والقنيات البين خلوية المحتوية على مواد إفرازية وكذلك العديد من المتقدرات . و قد احتوت هذه الخلايا أيضا على جهاز جولجي متطور وشبكة أندوبلازمية خشنة والريبوزومات والأجسام الجالة والخيوط السيتوبلازمية وكذلك قليل من الحبيبات الإفرازية المكونة من مادة ليفية دقيقة متجانسة فاتحة الكترونيا. هذا وأوضحت الدراسة أن هذه الحبيبات تفرز بالتسرب أو بالطريقة الشبه مفترزة. الخلايا العضلية الطلائية المغدة السلعابية السنكفية فيالحيوان موضع الدراسة تشبه مثيلاتها في الغدد اللعابية الأخرى ولكنها تُظهر علاقات غير معتادة النهابات الإفرازية ، حيث وجد أن خلية وأحدة تحيط بعنيب تنين أو أربّعة عنيبات متجّاورة في أن واحد. وأظهرت الدراسة كذلك وجود نوع خاص مــن الخَلايا العنيبية بجوار الخلايا العنيبية المألوفة في النهايات الإفرازية ، ويشبه هذا النوع إلى حد ما الخلايا العنبيية المألوفة في تركيبها الدقيق فيما عدا وجود بعض المتقدرات كبيرة الحجم ولها أعراف انبيبية أو انبيبية حويصلية بالإضافة إلى وجود بعض حويصلات الشبكة الاندوبلازميــة الملســـاء. هذا وأظهرت هذه الفلايا علاقة بالخلايا العضلية الطلائية والمدد العصبي. وقد نوقشت وقورنت نتائج هذا البحث مع مثيلاتها في الحيوانات والإنسان.

SUMMARY

The secretory end-pieces of the parotid salivary gland of the camel were examined by light and transmission electron microscope. They consisted of pure serous acini lined with pyramidal-shaped cells with large rounded basally located nucleus. These cells reacted negatively with alcian blue and PAS. The parotid acinar cells were surrounded wide lumen and their lining cells were characterized by extensive basal infoldings, well-developed intercellular canaliculi containing secretory substance and numerous mitochondria. In addition, a well-developed Golgi-apparatus, RER, ribosomes, cytoplasmic filaments, lysosomes, centrioles and secretory granules were observed. The latter were few and characterized by homogenous electron-lucent fine fibrillar matrix. Beside exocytosis, these secretory materials showed evidence of an apocrine-like mode of secretion. The myoepithelial cells displayed the common features of the analogous cells of the other salivary glands. Unusual relations to the secretory acini, that were enclosed, were observed, where one cell surrounded either two or four closely relating acini within a continuous basal lamina. Special kind of acinar secretory cells were recognized among the ordinary acinar cells. They were few and somewhat ultrastructurally resembled the ordinary ones except of the presence of some large tubular or tubulovesicular mitochondria and vesicles of smooth endoplasmic reticulum. They were often observed in relation to myoepithelial cells and nerve terminals. The latter were also seen in close relation to the secretory acini but they did not penetrate the basal larnina, while lymphocytes were intercellularly demonstrated. The obtained results were compared with those of other animal species as well as man.

Key words: Fine structure, secretory end-pieces, parotid gland, myoepithelial cells, camel.

INTRODUCTION

The parotid salivary gland has been heavily investigated at the ultrastructural level in many mammalian species including man. The parotid salivary gland of herbivores is large and produce large amount of watery secretion which moistens the food (Mandel, 1987). Saliva has an important role in controlling the bacterial flora in the oral cavity (Stolte

and Ito, 1996). Furthermore, the lysozymal enzyme secreted by the serous cells hydrolyses bacterial cell walls (Fawcett, 1994). In herbivores, the parotid gland was ultrastructurally examined in bovine (Shackleford and Wilborn, 1969; Takano, Suzuki and Kanazawa, 1978; Suzuki, Nishinakagawa, Otsuka and Mochizuki, 1981a), sheep (Patterson, Lloyd, Peterson and Titchen, 1973; Patterson, Peterson and Titchen, 1976; Van Lennep, Kennerson and Compton, 1977), goat (Suzuki, Kamei and Otsuka, 1975; Takano, Kamiakito, Arai, Akita, Kutsuzawa, Ebashi and Ueki, 1977; Suzuki, Nishinakagawa and Otsuka, 1981b), horse (Suzuki and Otsuka, 1977) and donkey (Salem, Yousria Abdel-Rahman and Abou-Elmagd, 1995). On the other hand, Stolte and Ito (1996) compared the parotid gland acinar cells ultrastructurally in nine wild ruminant species in order to determine whether the morphological features were correlated with the feeding type.

In the available literature no previous publication was found concerning the fine structure of the parotid gland of the camel. In this study the secretory end-pieces of the parotid gland of the one-humped camel (Camelus dromedarius) was investigated with the aid of transmission electron microscope and compared with those of other species.

MATERIAL and METHODS

Samples of the parotid gland of eight apparently healthy adult camels of both sexes and of different ages were obtained from Assiut-Slaughter House. These samples were employed for both the light and electron microscopical studies.

For light microscopy, the pieces of the gland were fixed in Bouin's fixative and processed for paraffin embedding. 5μ thick paraffin sections were stained with haematoxylin and eosin, periodic acid-Schiff and alcian blue (Mowry, 1956).

For electron microscopy, small pieces were fixed by immersion in a mixture of 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) for 4 hours at 4°C (Karnovsky, 1965). They were rinsed in 0.1 M cacodylate buffer, pH 7.2 and post-fixed with cacodylate buffered 1% osmium tetroxide for further 2 hours. After dehydration in ethanol, the pieces were embedded in Epon-Araldite. Semithin sections were cut on LKB ultramicrotome and stained with toluidine blue as well as alcian blue & PAS (Boeck, 1984). The ultrathin

sections were double stained with uranyl acetate and lead nitrate and observed with JEOL 100 XCII electron microscope.

RESULTS

Light microscopy:

The secretory end-pieces of the parotid salivary gland of the camel were formed of numerous rounded or ovoid acini. They were lined with about 5-7 pyramidal- or truncated pyramidal-shaped secretory cells possessing large vesicular rounded basally located nucleus (Fig. 1). These cells did not reacted with either periodic acid Schiff or alcian blue staining methods (Fig. 2).

Electron microscopy:

The pyramidal-or truncated pyramidal-shaped acinar cells surrounded a wide central lumen and were separated inconstantly from the basal lamina with the interposing myoepithelial cells and their processes (Figs. 3 & 4). The acinar lumen was provided with numerous microvilli of varying length (Figs. 4, 5, 8 & 14). Apically, the adjacent cells were attached together with zonula occludens, zonula adherens and desmosomes (Fig. 5). The lateral plasma membranes of the neighbouring cells were joined together with interdigitations through cytoplasmic processes, which extended into very narrow intercellular spaces (Figs. 5, 6 & 11) as well as desmosomes. The acinar cells were characterized by the presence of numerous intercellular canaliculi along the entire lateral plasma membranes (Figs. 3, 4, 6 - 9, 17 & 18). These canaliculi appeared round or oval, lined with numerous microvilli and bounded at both ends with zonula occludens and zonula adherens (Fig. 6). The intercellular canaliculi were observed communicating with the acinar lumen (Fig. 7) where the junctional complex was somewhat basally displaced. Bridge-like cytoplasmic process was observed crossing the intercellular canaliculi, which extended from one cell joining the opposite one by means of desmosomes (Fig. 8). Secretory granules were seen mostly in close contact with the intercellular canaliculi (Figs. 7-9) as well as within their lumina (Fig. 9). The basal plasma membrane of the secretory cells was extensively infolded. These folds were long slender-like parallel to each other and attached to the smooth basal lamina with hemidesmosomes (Fig. 10). At the places where the

myoepithelial cells were interposed, these infoldings became small and attached to these cells with desmosomes.

Intracellularly, the most prominent feature of the acinar secretory cells of the parotid gland of the camel was the vast number of mitochondria that each contained (Figs. 3 & 4). They represented a large percentage of the cell cytoplasm scattering throughout it and especially concentrated along the lateral plasma membranes as well as the intercellular canaliculi (Figs. 8 & 11). Numerous scattered long cisternae of rough endoplasmic reticulum and well-developed supranuclear Golgiapparatus consisting of 3-4 complexes were seen. Each complex formed of 4-5 semicircular cisternae and associated vesicles (Fig. 12). The secretory cells also contained few membrane-bounded secretory granules, which characterized by their homogenous electron-lucent fine fibrillar matrix (Fig. 13). They discharged their content by simple exocytosis into the acinar lumen (Fig. 14) as well as into the intercellular canaliculi (Fig. 9). Pleomorphic apocrine-like protrusions were observed extending into the acinar lumen. These protrusions were covered with smooth plasma membrane lacking nearly microvilli. Their content was homogenous electron-lucent resembling nearly those of the secretory granules. On the other hand, a large electron-lucent secretory mass was recognized filling the acinar lumen (Fig. 16).

The secretory cells contained also a pair of centeriole (Fig. 15), lipid droplets, lysosomes, cytoplasmic filaments and numerous ribosomes.

The nucleus of the acinar secretory cells was large and somewhat basally located. The nucleoplasm contained few marginal heterochromatin and chromatin islands. The nuclear membrane and its pores as well as the nucleoli were distinct (Figs. 3, 4 & 17-19).

The myoepithelial cells were demonstrated surrounding the secretory acini (Figs. 3, 4 & 17-19). They consisted of cell body containing the nucleus and many cytoplasmic processes extending between the secretory cells. They occupied mostly by parallel streams of myofilaments giving them more electron-dense appearance. Mitochondria, short RER-cisternae, ribosomes, lipid droplets and attachment sites within the myofilaments were observed (Figs. 18 & 19). The myoepithelial cells were attached to the secretory cells with cytoplasmic interdigitations and desmosomes and to the basal lamina with hemidesmosomes.

The myoepithelial cells presented unusual relations to the secretory portions, where one myoepithelial cell was observed enclosing with their processes either two (Fig. 17) or four neighboring acini (Fig. 18). In this condition, the acini as well as the myoepithelial cell were enclosed by a continuous basal lamina (Figs. 17-19).

Special kind of acinar cell was recognized often within the secretory end-pieces (Figs. 20-24). They were few, where one cell was incorporated within an acinus among the ordinary acinar cells. They were also pyramidal- or truncated pyramidal-shaped, rested on the basal lamina and reached the acinar lumen. They resembled somewhat the ordinary acinar secretory cells, but they were characterized by the presence of some large mitochondria of tubular or tubulovesicular cristae. The latter were longitudinally (Fig. 23) and circumferentially arranged (Fig. 24). It also contained small vesicles of smooth endoplasmic reticulum, short RER-cisternae and secretory granules of homogenous electron-lucent fine granular matrix. They were often seen in close relation to the myoepithelial cells and the non-myelinated nerve terminals (Figs. 20 & 22).

Migratory lymphocytes were commonly observed within the intercellular spaces of the parotid acinar cells (Fig. 11). Bundles of non-myelinated nerve terminals were frequently demonstrated between the adjacent acini near the basal lamina. They contained mitochondria as well as clear and dense vesicles (Fig. 25). These nerve terminals could not seen penetrating the basal lamina or in direct contact with the acinar cells.

DISCUSSION

The present investigation revealed that, the secretory portions of the camel parotid gland reacted negatively with alcian blue and PAS. Similar, results were also recorded in the parotid gland of sheep (Van Lennep et al., 1977) and donkey (Salem et al., 1995) and consequently the parotid gland were considered as pure serous gland. In the same animal, Dellmann and Fahmy (1968) confirmed the present results, while El-Khaligi (1974) stated that, they were seromucoid in nature, showing different phases of secretory cycle and reactivity. On the other hand, the bovine parotid gland showed contradictive classification. It was classified as extraordinary serous (Shackleford, 1963), special serous (Shackleford and Wilborn, 1968), seromucous gland (Mori, 1978)

or pure serous gland (Suzuki et al., 1981a) depending on its carbohydrate histochemistry. Based on the ultrastructural criteria, the parotid glands of wild ruminants were classified as serous gland (Stolte and Ito, 1996). However, that of the morten Carnivora was seromucous, since their granules have a gray stain (Junior and Masuko, 1998).

In the contrary, to the non-ruminant animals as horse (Suzuki and Otsuka, 1977) and donkey (Salem et al., 1995), the acini of the camel parotid gland were characterized by wide central lumina, well-developed basal infoldings, numerous intercellular canaliculi and mitochondria. Similar results were observed in the parotid gland of sheep (Van Lennep et al., 1977), bovine (Suzuki et al., 1981a), goat (Suzuki et al., 1981b) as well as in grass and roughage eaters wild ruminant species (Stolte and Ito, 1996). Suzuki et al. (1981a & b) stated that, such characteristics are due to continuous flow of the parotid saliva in ruminants. Van Lennep et al. (1977) mentioned that, the parotid gland of the sheep and presumably all ruminants, including the camel secrete large amounts of fluid and very little protein. In agree with Van Lennep et al. (1977) as well as Stolte and Ito (1996), the parotid gland of the camel could be considered as an electrolyte-secreting gland based on its ultrastructural characteristics.

The present study revealed numerous intercellular canaliculi containing secretory granules. Similar results were recorded in the parotid gland acinar cells of the sheep (Van Lennep et al., 1977), bovine (Suzuki et al., 1981a), goat (Suzuki et al., 1981b), donkey (Salem et al., 1995) and wild ruminants (Stotte and Ito, 1996). The canaliculi were observed also in the Von Ebner's glands of rat (Hand, 1970), man (Riva-Testa, Cossu, Lantini and Riva, 1985) and camel (Salem, 1996). The opinion of the latter author was acceptable, as these canaliculi play a role in the process of secretion. This is confirmed by the presence of secretory material within the canaliculi and their communication with the acinar lumen.

The secretory granules of the parotid acinar cells of the camel were few, consisting of fine homogenous electron-lucent fibrillar matrix, simulating that observed in some wild ruminant parotid glands as African buffalo, Pere David's and nyala (Stolete and Ito, 1996). While in the rest of the wild ruminant studied, the granules were electron-dense or contained electron-dense core. In human (Riva and Riva-Testa, 1973), sheep (Van Lennep et al., 1977), bovine and goat (Suzuki et al., 1981a &

b, respectively) two to three types of secretory granules were observed in their acini according to their electron density. However, those of the donkey (Salem et al., 1995) contained electron-dense granules surrounded by fine granular margin. It suggested that the granule morphology of the parotid gland is species-dependent.

In addition, to the simple exocytosis, the secretory materials of the parotid gland of the camel were also released into the acinar lumen by apocrine-like process. Similar results were observed in the parotid gland of bovine and goat (Suzuki et al., 1981a &b) as well as in those of wild ruminants (Stolte and Ito, 1996). The latter authors suggested that this mode of secretion might be typical for ruminants, what for camel is also accepted.

The apocrine-like process, the presence of secretory materials within the wide acinar lumen and in the intercellular canaliculi as well as the presence of few secretory granules within the secretory cells might indicate that the parotid salivary gland of the camel is highly active gland and characterized by fast as well as massive production of saliva through these different routs. This is very important to accommodate not only the high demand of saliva during mastication, swallowing and rumination but also to adapt the nature of this dessert animal on feeding hard dry and spiny feeding stuffs of low water content. In rat parotid gland, Amsterdam, Ohad and Schramm (1969) confirmed partially this explanation, where injection of isoprenaline as an inducer of secretion causes depletion of secretion granules within the secretory cells and dramatical dilatation of the acinar lumen.

In agree with Cowley and Shackleford (1970), Van Lennep et al. (1977), Suzuki et al. (1981a & b), Chaudhry, Cutler, Yamane, Labay, Sunderraj and Manak (1987) and Salem et al. (1995) the myoepithelial cells of the parotid gland of the camel possessed the ultrastructural features of contractile elements. They revealed unusual relations to the secretory acini where one myoepithelial cell surrounds two secretory acini, simulating that observed by Salem (1996) in the Von Ebner's glands of the same animal. Extraordinary is that, one myoepithelial cell surrounds four secretory acini enclosed in a continuous basal lamina, which was not mentioned till now in the available literature not only in salivary glands of all species but also in the other exocrine glands, which may probably a character of this animal.

It suggested that, this myoepithelial cell performs its contractile action on these acini simultaneously to insure the fast and massive secretion production from this gland as before-mentioned speculated. On the other hand, such phenomenon may also reflect that the contractile activity of the myoepithelial cell of the camel is sufficient enough to effect more than one acinus at the same time.

Special kind of acinar secretory cells were seen among the ordinary ones characterized by the presence of large tubular or tubulovesicular shaped mitochondria and vesicles of smooth endoplasmic reticulum. These cells were not described before either in the secretory acini of the parotid gland or in the other salivary glands.

Mitochondria of tubular or tubulovesicular cristae were observed only in the striated duct cells of the pig parotid gland, indicating that this gland is involved in steroid metabolism (Boshell and Wilborn, 1978). An in vitro biochemical study revealed that pregnenolone and testosterone are modified in the parotid gland of pig (Kadis and Chyn, 1975). On the other hand, Flood (1973) detected hydroxysteroid dehydrogeneases in the maxillary gland of the pig. It can be speculated that these cells probably produce steroidal compounds, sex pheromones that come out with the parotid saliva. Pineda and Faulkner (1980) who isolated steroidal compound from the pig parotid saliva, confirm this speculation.

In conclusion, the parotid salivary gland of the camel was pure serous gland. It simulated that of ruminant animals as an electrolyte-secreting gland characterized by massive salivary secretion to accommodate the feeding nature of this dessert animal. It might also produce pheromones as that of pig.

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LEGENDS

- Fig. 1: Semithin section of the parotid gland of the camel showing serous acini lined with pyramidal-shaped cells with rounded basally located nucleus. Toluidine blue. X 160.
- Fig. 2: Semithin section of the parotid gland of the camel showing negative alcian blue-PAS reaction within the acini. X 160.
- Fig. 3: Electron micrograph of the serous acini and their lining pyramidal-shaped cells with rounded nucleus (N). Lumen (L), intercellular canaliculi (Ic), basal lamina (Bl), basal infoldings (arrow), myoepithelial cell (My). X 4000.
- Fig. 4: Electron micrograph of an acinar cell showing large rounded nucleus (N), numerous mitochondria (M), Golgi complex (G), intercellular canaliculi (Ic), RER-cisternae, and basal infoldings (arrow). Lumen with microvilli (L), basal lamina (Bl), myoepithelial cell (My). X 6758.
- Fig. 5: Higher magnification showing the acinar lumen (L) with numerous microvilli (Mv), zonula occludens (Zo), zonula adherens (Za) and desmosomes (D). Narrow intercellular space (arrow), mitochondria (M), RER-cisternae. X 33333.

- Fig. 6: Electron micrograph of an intercellular canaliculus showing numerous long microvilli (Mv). Zonula occludens (arrow), zonula adherens (arrowhead), desmosome (D), interdigitations (double arrow), mitochondria (M), secretory granules (S). X 13400.
- Fig. 7: Electron micrograph of a part of the apical portions of three acinar cells showing two intercellular canaliculi (Ic) communicating with the acinar lumen (L) and secretory granules (S). Microvilli (arrowhead), mitochondria (M), lysosome (arrow). X 11725.
- Fig. 8: Electron micrograph of the apexes of two acinar cells showing bridge-like cytoplasmic process (arrow). Intercellular canaliculi (Ic), lumen (L), microvilli (Mv), desmosomes (D), mitochondria (M), secretory granule (S), RER-cisternae, nucleus (N). X 14857.
- Fig. 9: Electron micrograph of a part of an intercellular canaliculus between two acinar cells showing secretory materials within its lumen (arrow). Microvilli (Mv), secretory granules (S) incontact with the canaliculus, desmosomes (D), mitochondria (M), basal lamina (Bl). X 13400.
- Fig. 10: Electron micrograph of a basal portion of an acinar cell showing intensive basal infoldings (arrow), hemidesmosomes (arrowhead), smooth basal lamina (BI) and mitochondria (M). X 15000.
- Fig. 11: Electron micrograph of the basal portion of an acinus showing mitochondria (M) along the lateral plasma membranes. Lymphocyte (Ly) in the intercellular space (Is), cytoplasmic interdigitations (arrow), basal lamina (Bl). X 10666.
- Fig. 12: Electron micrograph of the supranuclear region of an acinar cell showing well-developed Golgi-apparatus (G), vesicles (arrow) and secretory granules (S). Nucleus (N), RER-citernae, mitochondria (M). X 144444.
- Fig. 13: Higher magnification electron micrograph of a membranebounded secretory granule with its homogenous electron-lucent fine fibrillar matrix. X 45692.

- Fig. 14: Electron micrograph of the apex of an acinar cell showing exocytosis of the secretory granule (arrow) into the acinar lumen (L). Microvilli (Mv), secretory granules (S), mitochondria (M), nucleus (N). X 11485.
- Fig. 15: Electron micrograph of the apical portion of an acinar cell showing a pair of centriole (arrow) and lumen (L). X 22166.
- Fig. 16: Electron micrograph of the apexes of acinar cells showing apocrine-like protrusions (asterisk) with smooth plasma membrane (arrowhead) extended into the acinar lumen (L). Intercellular canaliculus (Ic), microvilli (Mv), secretory mass within the lumen (arrow). X 13714.
- Fig. 17: Electron micrograph showing a myoepithelial cell (My) located between two secretory acini (A&B). Lumen (L), continuous basal Iamina (arrow), intercellular canaliculi (Ic). X 4435. Inset: Higher magnification of marked area in Fig. 17: showing continuous basal Iamina (arrow) and hemidesmosomes (arrowhead). X 15000.
- Fig. 18: Electron micrograph showing a myoepithelial cell (My) surrounds four acini (A, B, C & D) within a continuous basal lamina (arrow). Nucleus (N), intercellular canaliculi (Ic). X 3333.
- Fig. 19: Higher magnification of the myoepithelial cell of Fig. 18 showing dense streams of myofilaments as well as the attachment sites (a), RER-cisternae (arrow) and lipid droplet (d). Nucleus (N), hemidesmosomes (double arrow), desmosomes (D), continuous basal lamina (arrowhead). X 6720.
- Figs. 20-22: Electron micrographs showing special kind of acinar cell (Sc) among the ordinary cells (Oc) reaches the lumen (L) and rests on the basal lamina (arrow). It contains characteristic mitochondria (M), vesicles (V), short RER-cisternae (double arrow), secretory granules of fine granular matrix (S). Intercellular canaliculi (Ic), microvilli (arrowhead), myoepithelial cell (My), nerve terminals (Nt), nucleus (N), basal infoldings (double head-arrow). (20): X 10526.

- (21): Higher magnification of marked area A in Fig. 20. X 26600, (22): Higher magnification of marked area B in Fig. 20. X 20000.
- Figs. 23 & 24: Electron micrographs showing the characteristic tubulovesicular mitochondria with longitudinally (23) and circumferentially arranged cristae (24). X 21875.
- Fig. 25: Electron micrograph showing a bundle of non-myelinated nerve (Nt) in close relation to the basal portion of orindary acinar cell containing clear (arrow), dense vesicles (arrowhead) and mitochondria (M). Basal infoldings (double arrow), basal lamina (Bl). X 17173.















