

LIGHT AND ELECTRON MICROSCOPY OF BUCCAL SALIVARY GLANDS OF THE DROMEDARY CAMEL

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ABSTRACT

The study was aimed to investigate the light and electron microscopic structure of buccal salivary glands in the dromedary (*Camelus dromedarius*). Anatomically, the dromedary camel buccal glands were well-developed and were located on the lateral side of the cheek. Three parts (groups) of the buccal glands could be observed: dorsal, middle and ventral parts. Histologically, the secretory units were tubule-acinar, surrounded by myo-epithelial cells and supported by connective tissue. Whereas, the dorsal glands were purely mucous and the ventral glands were purely serous in secretion, the middle ones were muco-serous with predominant mucous cells. Ultra-structurally, the glandular cells of the serous secretory units (type I cells) contained numerous microvilli, many electron-dense secretory granules, abundant rough endoplasmic reticulum and mitochondria. On the other hand, the mucous-secreting cells (type II cells) appeared with numerous electron-translucent supra-nuclear granules and flat basal nuclei; dilated tubules of rough endoplasmic reticulum; fewer mitochondria and Golgi bodies were also observed. The obtained results have been compared with the previous reports on salivary glands of camel and other mammalian species.

Key words: Buccal glands, dromedary camel, histology, microscopy, ultra-structure

The mammalian species have two types of salivary glands, i.e. major and minor salivary glands (Grossman and Yousem, 2010; Som and Brandwein Gensler, 2011; Kessler and Bhatt, 2018; Maher *et al*, 2020). Several morphological studies have been carried out on major salivary glands of the dromedary camel (Nawar and El-Khaligi, 1975; Khalil, 1989; Al-Asgah *et al*, 1990; Mansouri and Atri, 1994; Nabipur *et al*, 2003; Mursal *et al*, 2016; Rezk and Shaker, 2017). However, minor salivary glands of the camel have received little attention (Taib and Jarrar, 1989; Nabipour, 2011). Therefore, the current study is an attempt to reveal morphological structure of the buccal salivary glands in the adult dromedary camel, using light and transmission electron microscopy.

Materials and Methods

The general histology and ultrastructure of buccal glands was done in 9 healthy camels obtained from Alsallam slaughterhouse, Omdurman, Sudan. For histological study, samples were quickly taken

and washed in normal saline and were fixed in 10% buffered formalin. Samples were later dehydrated in a series of graded, alcohol, cleared in xylene and embedded in paraffin wax. Sections (5µm thick) were stained by haematoxylin and eosin (H&E), Toluidine blue and Masson's Trichrome as described by Culling (2013) to study the histological features.

Transmission electron microscopic study was performed on small tissue pieces (0.3 cm X 0.3 cm) taken from buccal salivary glands; these were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer (pH 7.4). Later, these were post-fixed in 1% osmium tetroxide for 1 hour, washed in Millonig's buffer, dehydrated in graded ethanol and propylene oxide series and embedded in Epon. Ultrathin sections of 50- 90 nm were cut using a diamond knife, mounted on uncoated grids and stained first with uranyl acetate and then with lead citrate (Robinson and Gray, 1990). The image examination was carried out in Philips XL transmission electron microscope.

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Results and Discussion

Anatomical findings

The buccal salivary glands in dromedary camels were found well-developed and composed of 3 parts or groups, i.e. dorsal, middle and ventral parts and were located on the lateral side of the cheek (Fig 1). These were bordered by the dorsal buccal branch of the facial nerve dorsally, the ventral buccal branch of the facial nerve ventrally, labial salivary gland rostrally and the maxillary vein and mandibular salivary gland caudally and caudo-ventrally, respectively. The middle buccal part is the largest of the 3 parts of buccal glands and is located between the dorsal buccal part, ventral buccal part, maxillary vein and mandibular salivary gland dorsally, ventrally, caudally, and caudo-ventrally, respectively.

Histological findings

Each part of the buccal glands was surrounded by a dense connective tissue that sent connective tissue septa dividing it into lobes and lobules (Fig 2A). Each lobule contained tubulo-acinar secretory units with simple cuboidal epithelial lining was surrounded by myoepithelial cells (Fig 2B). The duct system started with the intercalated ducts which drained the secretory units and were lined by simple squamous epithelium; the excretory ducts were connected to the striated ducts that were lined by simple cuboidal or columnar epithelium; striated ducts lead to larger interlobular ducts in the connective tissue septa that had stratified cuboidal or columnar epithelium and wide irregular lumen (Figs 2A).

There were 2 main types of glandular secretory units detected in the camel buccal glands: the mucous secretory units and serous secretory units. The mucous secretory units had relatively short cells with light-stained basophilic cytoplasm, flat basal nuclei and wide lumen. The serous secretory units consisted of tall (pyramidal) cells with dark acidophilic cytoplasm, spherical central nuclei and a narrow lumen. Generally, the dorsal part of buccal glands had mucous secretory cells (Fig 3A); a few secretory units, however, showed serous cells arranged as serous demilunes (Fig 3A). The secretory units of the middle part were muco-serous with predominant mucous cells (Fig 3B). The secretory units of the ventral part were serous in nature (Fig 3 C).

Ultrastructural findings

Two main types of cells were identified in the camel buccal glands secretory units depending on their electron-density of cytoplasmic granules:

Type I:

This type contained small and rounded electron-dense granules that were concentrated in the apical cytoplasm and lumen; numerous microvilli projecting from the apical cell membrane into the lumen were also observed in this type. Diffuse rough endoplasmic reticulum and mitochondria with different shapes and sizes were present around the nuclei were also observed. The nucleus was eccentric and round with diffuse chromatin scattered among heterochromatin. This cell type was present in the serous secretory units of the ventral part and in the serous demilune and secretory units of the middle part of the buccal salivary glands (Fig 4).

Type II:

It contained numerous mucous electron-translucent supra-nuclear granules of different sizes and round shapes. The nucleus was pushed by the mucous granules towards the basal part of the cells. Dilated tubules of rough endoplasmic reticulum were located laterally, basally or between the mucous granules and the nucleus. A few mitochondria, supra-nuclear rough endoplasmic reticulum and sparse Golgi bodies were also observed. This cell type was found in the secretory units of the dorsal parts and the mucous secretory units of the middle parts of the buccal salivary glands (Fig 5).

In the middle part of the buccal salivary glands, many cells showed many electron-translucent granules with different sizes and shapes filling the upper part of the cells; several rounded electron-dense granules were also observed between the light granules and the basal nucleus (Fig 6).

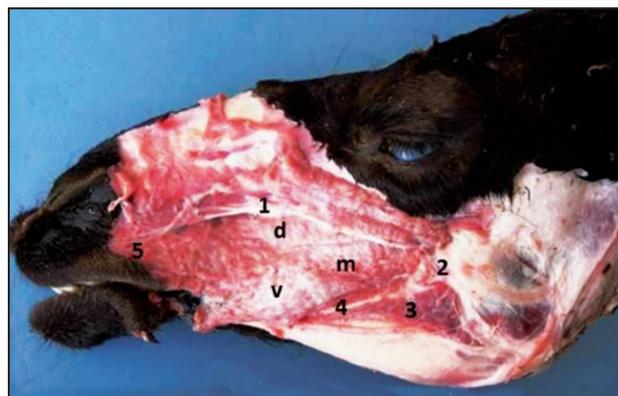


Fig 1. Left view of unfixed dromedary camel head showing the three parts of the buccal salivary glands: dorsal part (d), middle part (m), ventral part (v). Note the dorsal buccal branch of the facial nerve dorsally (1), the maxillary vein (2) and mandibular salivary gland (3), the ventral buccal branch of the facial nerve ventrally (4) and labial salivary gland (5).

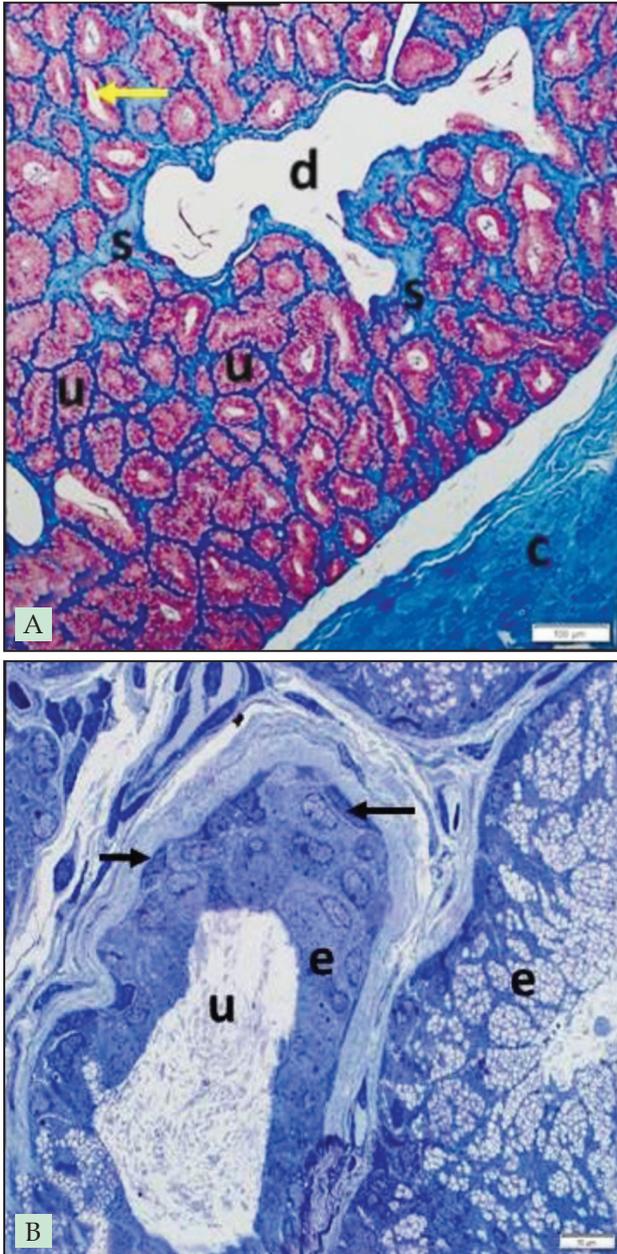


Fig 2. A. Photomicrograph of the ventral buccal glands surrounded by connective tissue (t) with septa (s), tubule-acinar secretory units (u), intercalated duct (black arrow), striated duct (yellow arrows) and interlobular duct (d). Masson trichrome stain. Image bar= 100µm.

B. Photomicrograph of the middle buccal glands showing secretory unit (u) lined by simple cuboidal epithelium (e). Note the myoepithelial cells (arrows) between the basement membrane and basal cell membrane (arrows). Toluidine blue satin. Image bar= 20µm. bar= 10µm.

Several morphological studies have been performed on the minor salivary glands in the different mammalian species (Parida and Das, 1991; Frith and Townsend, 1997; Stinson and Calhoun, 1993; Hand *et al*, 1999; Mohammadpour, 2010; Sadi, 2013).

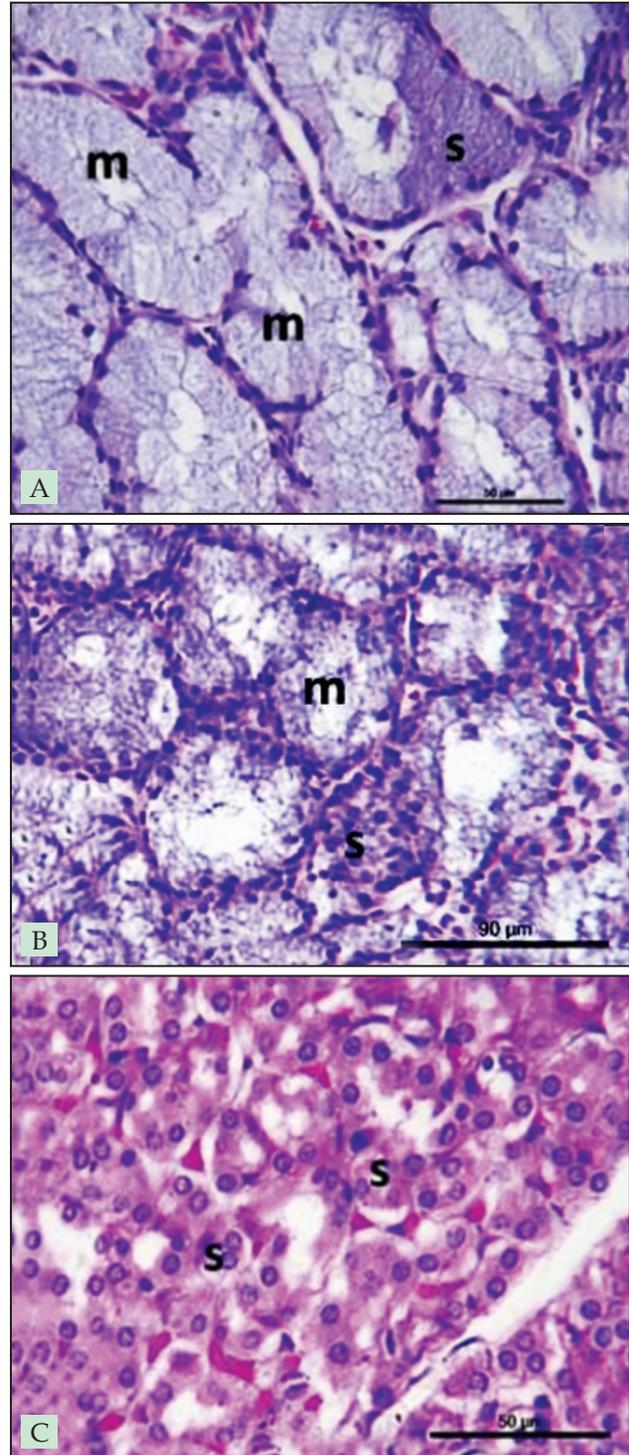


Fig 3. A. Photomicrograph of the dorsal buccal glands showing mucous secretory units (m). Note the serous demilune (s). H&E stain. bar= 50µm.

B. Photomicrograph of the middle buccal glands showing mucous secretory units (m) with light cells and flat basal nuclei narrow lumina, and serous secretory units with dark cells and round nuclei H&E stain. bar= 90µm.

C. Photomicrograph of the ventral buccal glands showing serous secretory units (s) of serous units lined by dark cells with round nuclei H&E stain. bar= 50µm.

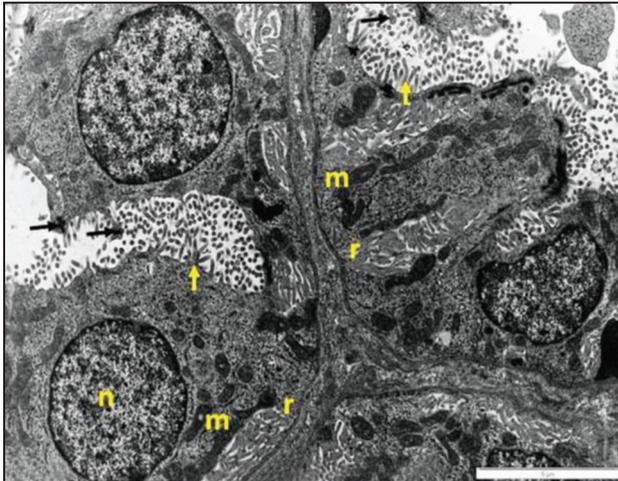


Fig 4. Transmission electron micrograph of the ventral buccal glands showing numerous microvilli (yellow arrows) projecting in the lumen and dark and small secretory granules (black arrows) in the apical cytoplasm and lumen. Note the numerous mitochondria (m) and diffused rough endoplasmic reticulum (r) around the round and central nuclei (n). Image bar= 5 μ m.

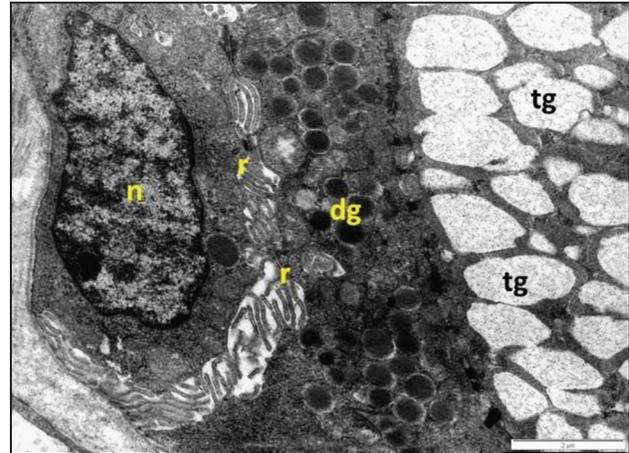


Fig 6. Transmission electron micrograph of the middle buccal gland showing electron-translucent granules (tg) occupying the upper part of the cells. Small and rounded electron-dense granules (dg) are concentrated between the electron-translucent granules and the basal nucleus (n). Note the oval nucleus (n), well-developed supranuclear rough endoplasmic reticulum (r) and Golgi body (g). Image bar= 2 μ m.

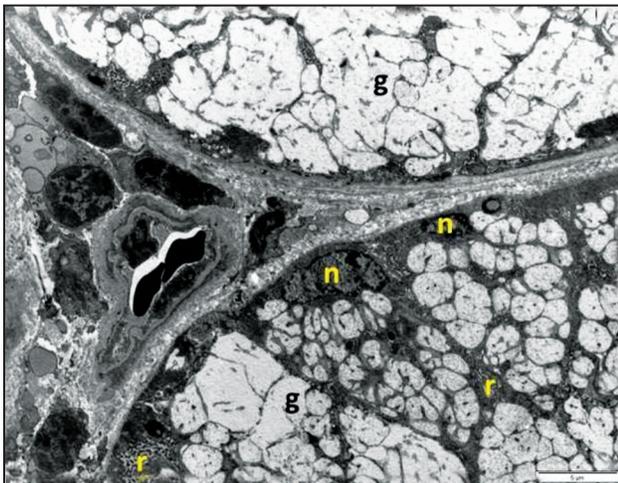


Fig 5. Transmission electron micrograph of the dorsal buccal glands showing light granules (g) filling the upper part of the cells pushing the flat nuclei (n) towards the basal part of the cells. Note the diffused rough endoplasmic reticulum (r) between the granules. Image bar= 5 μ m.

The current anatomical results confirmed the earlier findings in camels (Smuts and Bezuidenhout, 1987), domestic ruminants (Parida and Das, 1991; Singh *et al*, 2011) and horse (Stinson and Calhoun, 1993) and accordingly the buccal salivary glands have three parts, i.e. dorsal, middle and ventral. In contrast, van Lennep (1957) reported that the buccal glands of dromedary camels had two parts, i.e. dorsal and ventral.

Histologically, the present study revealed that each part of the buccal glands of dromedary camel was

covered by a dense connective tissue and supported by connective tissue septa to divide it into lobes and lobules; the lobules were occupied by tubulo-acinar secretory units with myoepithelial cells. These findings are in line with those reported by Taib and Jarrar (1989) and Nabipour (2011) in the buccal glands of camels. The present results showed that the secretory units of camel buccal glands were generally mucous, mixed and serous in the dorsal, middle and ventral parts, respectively. Similar findings have also been stated in the buccal glands of dromedary camels (Nabipour, 2011) and other mammals (Kumar, 2014). However, a few serous demilunes were observed in some secretory units of the dorsal part. Moreover, Taib and Jarrar (1989) claimed that the dorsal part of the glands contains muco-serous acini. In the present study, the dorsal part of the buccal glands in the camels was mucous and the middle part was muco-serous with predominant mucous-secreting cells. Moreover, van Lennep (1957) found that the ventral buccal glands in the same species have a mucous secretion in their upper part. Thus, it could be suggested that the secretion of the buccal glands in dromedary camels is mainly mucous, which might help in lubrication and swallowing of food, protection of oral mucosa and preservation of water. This fact might be of great importance for this animal species which is living in a desert environment that is characterised by scarcity of water and few food.

The two main types of cells (type I and type II) in the secretory units of the camel buccal glands.

Type I, which was found in serous secretory units of the ventral part and middle buccal glands, contained electron-dense granules, numerous microvilli, abundant rough endoplasmic reticulum and mitochondria. These cellular characteristics have also been described in bovine (Shackleford and Wilborn, 1969), rats (Igbokwe, 2018) and camel (Mansouri and Atri, 1994) parotid glands which have purely serous secretory units. Shackleford and Wilborn (1969) have also mentioned that the ultrastructural appearance of camel parotid gland is similar to that of bovine parotid gland. Projection of microvilli into the acinar lumen and presence of intercellular canaliculi could suggest increased secretory surface of the glandular cells; these characteristic features have been considered as an indication of increased production of saliva (Shackleford and Wilborn, 1968, 1969; van Lennep *et al*, 1977).

Type II or mucous cells detected in this study contained numerous electron-translucent supra-nuclear granules and flat basal nuclei; dilated tubules of rough endoplasmic reticulum, a few mitochondria and Golgi bodies. Similar ultrastructural observations have also been reported in the bovine submandibular glands (Shackleford and Wilborn, 1970). In the present study, the serous demilunes showed similar characteristics of the serous secretory units that included many electron-density granules, well-developed rough endoplasmic reticulum and sparse Golgi body. The structure and organelle distribution in the demilunar cells presented in this study has also been reported in other mammalian mandibular salivary glands (Shackleford and Wilborn, 1970; Pinkstaff, 1980).

It could be concluded that most of the histological and ultrastructural features of the buccal salivary glands in dromedary camels resemble those of other mammalian species.

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