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Structure and histochemistry of the sublingual salivary glands of the one-humped camel (*Camelus dromedarius*)

AL-ASGAH (N.A.), JARRAR (B.M.), TAIB (N.T.). Structure et histochimie des glandes salivaires sublinguales du dromadaire (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (4) : 519-527

Des études morphométriques, histologiques et histochimiques ont été conduites sur les glandes salivaires du dromadaire d'Arabie (*Camelus dromedarius*). Les glandes sont du type acino-tubulaire, formées de nombreux lobules comprenant deux types de cellules : mucoséreuses et séromuqueuses. Les cellules mucoséreuses constituent l'élément sécréteur essentiel de la glande ; les cellules séromuqueuses sont beaucoup plus rares et forment des associations d'acini. Les premières sécrètent et élaborent de grandes quantités de mucosubstances neutres, des mucomucines et un peu de sulphomucines. A l'inverse, seule la portion apicale des secondes révèle une activité faible ou modérée dans la sécrétion de mucosubstances neutres ou acides. Les tests histoenzymatiques utilisés ont révélé une activité très importante de la phosphatase alcaline, de la déhydrogénase succinique, de l'aminopeptidase et d'estérases non spécifiques. On a décelé une faible activité de l'oxydase cytochrome et de la péroxydase, et aucune activité de la lipase triacylglycérase, de la ß-glucironidase et de l'amylase. La signification fonctionnelle de ces résultats fait l'objet d'une discussion. *Mots clés* : Glande salivaire-Glycoprotéine-Histoenzymologie-Histochimie-*Camelus dromedarius*-Arabie Saoudite.

INTRODUCTION

The one-humped camel (*Camelus dromedarius*) is one of the least studied mammals, yet, several histochemical investigations have been carried out on its parotid and mandibular salivary glands (1, 8, 9, 16, 35, 36, 37, 54). On the other hand, the histology and histochemistry of its minor salivary glands have only been recently investigated (19, 50, 51, 52). The sublingual glands have received little attention (6, 8, 9, 15, 17, 54) and many data are insufficient and highly contradictory. The present study gives a detailed histochemical characterization of these glands.

MATERIALS AND METHODS

Histology

The sublingual salivary glands of 15 adult camels (9 males and 6 females) were removed immediately after slaughter and put into containers with different fixatives : cold (4 °C) 10 % buffered formalin (pH 7.8) with 2 % calcium acetate, Bouin's fluid, Gendre's fluid and Zenker's fluid. The tissues were throughly washed in running water and processed for sectioning at 5-6 µm thickness. Paraffin sections were stained for histological examination with haematoxylin-eosin and with Mallory's trichome stains for histological examinations, whereas the secretory cells of the gland were characterized by the method of GABE and St-GIRONS (11).

Morphometric measurements

Three items were calculated :

– the acinar area, $A = \pi (D_1 . D_2)/4$, where D_1 is the largest diameter of the acinous, D_2, the smallest diameter of the acinous ;

– the cellular height, $h = (D_1 . D_2 - d_1 . d_2)/4$, where D_1 is the largest perpendicular tubule axis ; D_2, the largest perpendicular duct axis ; d_1, the axis of the tubule ; d_2, the axis of the duct lumen ;

– the nuclear ellipsoid volume was calculated from the formula of VALERI *et al.* (53), $V = 0.35341326\ D_1 . D_2 . \sqrt{D_1 . D_2}$, where D_1 is the largest axis of the nucleus, D_2, the shortest perpendicular axis of the nucleus.

The diameter of 20 acini of the glands from each animal was measured with the aid of an eye-piece micrometer.

Histochemistry

Parrafin-embedded and fresh frozen but unfixed samples were used for the following histochemical reactions.

Neutral mucosubstances

Periodic acid Schiff (PAS) technique (13), PAS after salivary digestion (30), PAS after amylase digestion (26), Best's carmine (2), PAS after acetylation-deacetylation (39) and PAS after treatment with chloroform and methanol.

Acid mucosubstances

Alcian blue (AB) at pH 2.5, AB at pH 1.0 and AB at pH 0.4 (26, 32).

Distinction between acidic and neutral mucosubstances

AB (pH 2.5)-PAS (33) and AB (pH 1.0)-PAS (46).

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Reçu le 24.10.1989, accepté le 29.7.1990.

Distinction between sulphomicins and sialomucins

Aldehyde fuchsin (AF) and AF-AB, pH 2.5 (48) ; weak (25 °C, 4 h), mild (37 °C, 4 h) and strong (60 °C, 4 h) methylation-saponification-AB, pH 2.5 (47) ; acid hydrolysis (0.1N HCl 60 °C, 4 h) -AB (pH 2.5) (41) ; Azur A (0.2 %, toluidine blue (TB) 0.003 % buffered at pH 1.7 and 3.4 (22) ; critical electrolyte concentration (CEC) technique for extinction of alcianophilia at pH 5.6 in the presence of gradual concentration of Mg^{++} (44).

Enzymes digestion tests

– amylase-PAS (30) ; neuraminidase (sialidase, *Vibrio cholerae* type V)-AB (pH 2.5) (49) ; hyaluronidase (testicular)-AB (pH 2.5) (46) ; ribonuclease digestion (25) ; neuraminidase-TB (pH 3.7) and hyaluronidase-TB (pH 2.0) were employed. In each case control sections were incubated for the same duration and at the same temperature in buffer solutions without the enzyme.

Proteins

Mercuric bromophenol blue method (27), ninhydrin Schiff (55), chloramine-T Schiff and PAS after trypsin digestion (40).

Methods used for testing enzyme activities

Calcium cobalt for alkaline phosphatase (12) ; lead nitrate for acid phosphatase (40) ; OGAWA and MAYAHARA's method for mitochondrial adenosine triphosphatase (40) ; WACHSTEIN and MEISEL's method for membrane-bound adenosine triphosphatase (24) ; naphthyl acetate for non-specific esterases (40) ; modified tetrazolium method for succinic dehydrogenase (21) ; McCABE and CHAYEN's method (28) for aminopeptidase, naphtol-AS method (14) for ß-glucuronidase ; the tween method for triacyglycerol lipase ; HAÜSLER's method for carbonic anhydrase ; DAB method for peroxydase and modified starch film for amylase (40). Control sections were incubated in the buffer solutions alone without the substrates.

RESULTS

The sublingual salivary glands of *C. dromedarius* are of a polystomatic type composed of a series of small yellow lobules loosely held together in two separate flattened structures along the root of the tongue and covered by the myohyoid muscle. They extend from the level of the third to the fifth lower jaw tooth and have 17-19 excretory ducts that open independently on either side of the floor of the *cavum oris proprium*. The glands are tubulo-acinar with two types of acini, a dominant mucoserous type and an occasional seromucous one (photo 1). The former are large (3086 μm^2 in area) having wide lumina and lined with cuboital to columnar cells (average nuclear volume 54.3 μm^3, average cell height 20.9 µm) with occasional myoepithelial cells surrounding the acini. The latter acini are small (acinar area 477 μm^2)

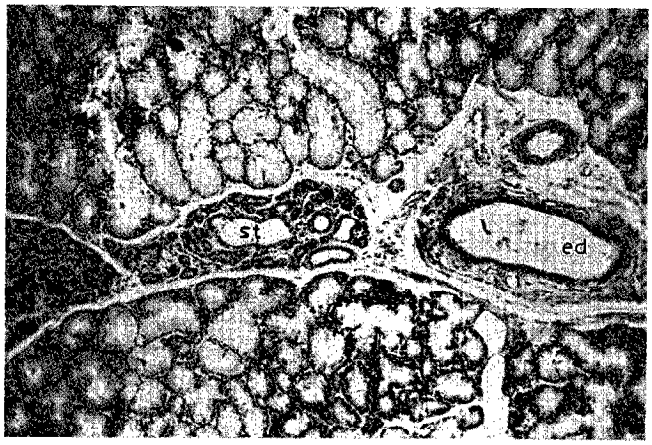

Photo 1 : Sublingual salivary glands of C. dromedarius *stained with haematoxylin-eosin (x 850).*

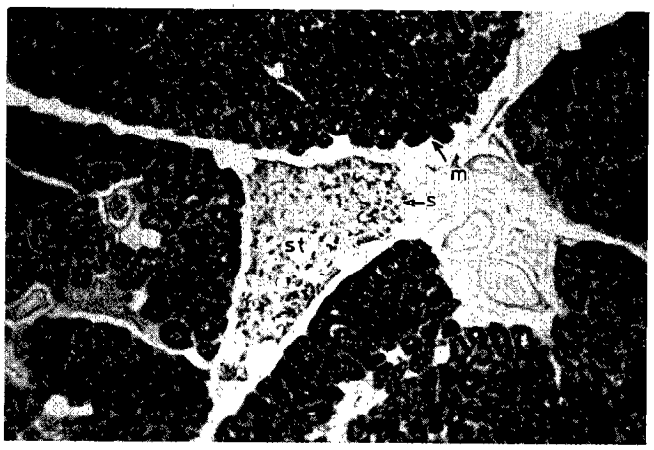

Photo 2 : Sublingual salivary glands of C. dromedarius *stained with PAS (x 475).*

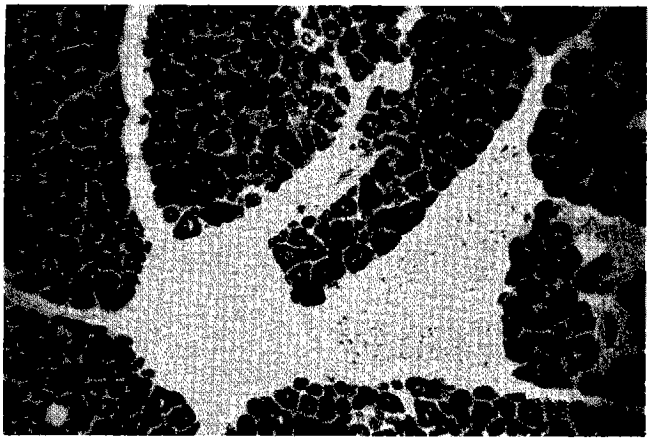

Photo 3 : Sublingual salivary glands of C. dromedarius *stained with AB (pH 2.5) (x 475).*

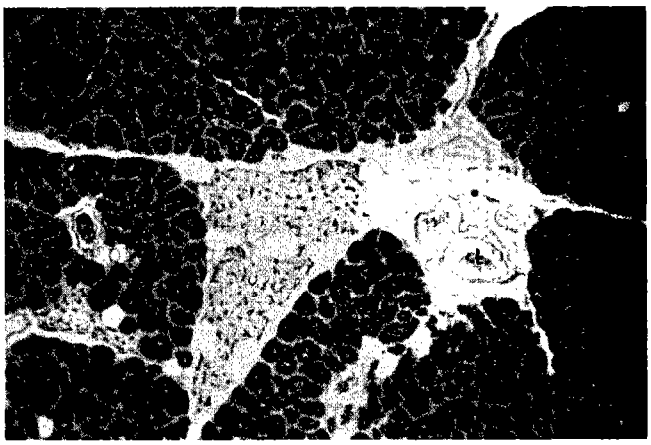

Photo 4 : Sublingual salivary glands of C. dromedarius *stained with AB (pH 2.5)-PAS (x 475).*

Photo 7 : Sublingual salivary glands of C. dromedarius *stained with Ogawa and Mayahara for adenosine triphosphatase (x 570).*

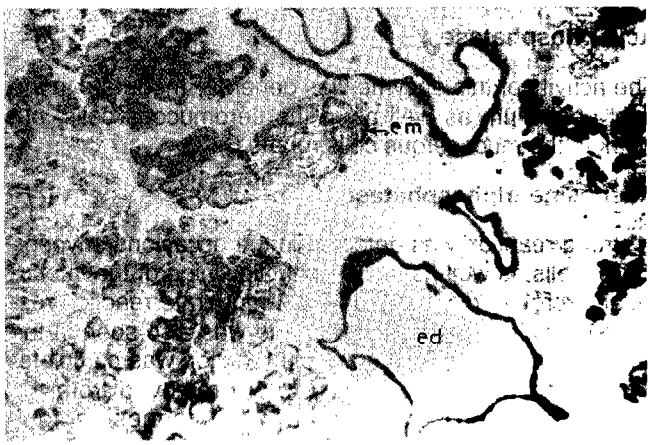

Photo 5 : Sublingual salivary glands of C. dromedarius *stained with cobalt method for alkaline phosphatase (x 475).*

Photo 8 : Sublingual salivary glands of C. dromedarius *stained with the modified tetrazolium method for succinic dehydrogenase (x 750).*

Photo 6 : Sublingual salivary glands of C. dromedarius *stained with lead nitrate method for acid phosphatase (x 475).*

Photo 9 : Sublingual salivary glands of C. dromedarius *stained with naphthyl acetate method for non-specific esterases (x 1050).*

with narrow lumina and lined with cuboidal to pyramidal cells (average nuclear volume 75.9 µm³, average cell height 13.3 µm).

The interlobular excretory ducts are large (average diameter 0.8-1.1 mm), and lined with simple cuboidal epithelium cells (average nuclear volume 71.8 µm³, average cell height 18.1 µm) with acidophilic cytoplasm and spherical nuclei (table I). Occasional interlobular striated ducts lined with simple cuboidal cells are seen in the mucoserous acini together with delicate intercalated ducts lined with flattened epithelium, but no goblet cells.

TABLE I Morphometric measurements of the acini and interlobular excretory ducts of the sublingual salivary glands of the one-humped camel (**Camelus dromedarius**).

	Area	Average diameter *	Average nuclear volume **	Average cell height **
Mucoserous acini	3 086 µm²	—	54.3 µm³	20.9 µm
Seromucous acini	477 µm²	—	75.9 µm³	13.3 µm
Interlobular excretory ducts	—	0.8-1.1 mm	71.8 µm³	18.1 µm

* Diameter is used in the calculation of the acinar area.
** Axis refens to the calculation of the cellular height and of the nuclear volume.

The secretory granules of the mucoserous cells were strongly stained with PAS (photo 2) which was resistant to saliva or amylase digestion. It was partly abolished by phenylhydrazine and completely by acetylation treatment, to be restored by acetylation-deacetylation-PAS sequential techniques. Moreover, the glands were strongly stained with Alcian blue at pH 2.5 (photo 3), but less and no alcianophilia occurred at pH 0.1 and 0.4, respectively. A bluish purple coloration developed with the sequential AB (2.5)-PAS staining procedure (photo 4), but was almost red with the AB(1.0)-PAS sequential techniques. The alcianophilia formed at pH 2.5 was partially abolished by acid hydrolysis, neuraminidase digestion and weak methylation, completely abolished by both moderate and strong methylation but was resistant to hyaluronidase as well as to ribonuclease digestion. However, alcianophilia was completely restored by weak methylation-saponification techniques and to a lesser extent by moderate and strong methylation-saponification techniques. Moderate alcianophilia developed with 0.1 M and not with 0.2 M $MgCl_2$ or above using the CEC techniques. A metachromatic reaction developed with Azur A and toluidine blue at pH 3.4 while orthochromasia occurred at pH 1.7.

The seromucous cells showed a weak to moderate activity for PAS, AB (2.5) and AB (1.0), but the reactivity was restricted to the apical portions only. A moderate purple color was formed with the AB (2.5)-PAS sequential technique, but almost no metachromasia or azurophilia was observed at either pH 3.4 or 1.7. However, basophilia in the cytoplasm of the glandular cells was not affected by ribonuclease digestion.

Protein histochemistry

The endpieces of the glands were affected by trypsin digestion and gave a moderate response to protein detection tests.

Enzyme histochemistry

Alkaline phosphatase

The enzyme activity was moderate in the ductal system (photo 5) and to a lesser extent in the secretory endpieces, together with some reaction in the scarce myoepithelial cells of the glands.

Acid phosphatase

The activity of this enzyme was generally moderate in the duct epithelium as well as in the seromucous cells, but weak in the mucoserous cells (photo 6).

Adenosine triphosphatase

A strong reaction was detected in the occasional myoepithelial cells as well as in the epithelial lining of the interlobular excretory ducts (photo 7). A moderate reaction restricted mainly to the cell membrane was also seen in the secretory cells of the glands. The reaction was completely inhibited when calcium ions were removed from the substrate and to some extent when magnesium ions were eliminated. When both ions were omitted, the enzyme activity of the epithelial lining of the ducts was totally inhibited. The reaction was also blocked when the pH of the substrate was lowered to 7.3.

The intralobular striated duct responded negatively to this enzyme.

Succinic dehydrogenase

The activity of this enzyme was very prominant in the ductal system and in the secretory endpieces (photo 8).

Non-specific esterases

A strong reactivity in the ductal system (photo 9) together with a considerable reactivity in the seromucous cells as well as in the occasional myoepithelial cells were seen, but the reaction in the mucoserous cells was weak.

Aminopeptidase

A strong reaction was seen in the epithelium of the excretory ducts with a moderate to weak activity in the secretory endpieces.

Peroxidase

A weak activity was only seen in the seromucous cells.

Cytochrome oxidase

A very weak reaction was observed in the acini and non in the ductal system.

Carbonic anhydrase

A weak activity was only observed in the intralobular striated duct.

The glands responded negatively to amylase, triacylglycerol lipase and ß-glucoronidase.

DISCUSSION

The present results demonstrate that the sublingual glands of the one-humped camel are much smaller than in any other mammal of comparable size. These glands were previously reported to be insignificant and loosely agglomerated (4, 23) as well as to represent the glandula sublingualis minor of other animals, while the sublingualis major is absent (54). Both striated and intercalated ducts were observed in the present study, but EL-KHALIGI (8) did not discern the striated ducts. He observed that the intercalated ducts do extend a long way from the secretory endpieces to join directly the interlobular excretory ducts. However, VAN LENNEP (54) reported that both ducts were absent in the dromedary camel. Intercalated ducts of such glands were, however, scarce in the buffalo (56).

Moreover, the data of the literature on the nature of the sublingual glands in the camel are highly contradictory. They have been reported to be purely mucous (9, 35, 54), or mixed with mucous and serous acini (17) or mainly mucous with a few small lobules forming seromucoid endpieces (8). According to the present detailed histochemical study and to the criterion of GABE and St-GIRONS (11), the secretory endpieces of the sublingual salivary glands of the dromedary are composed of mucoserous and seromucous cells. Hence, these glands are quite different from their counterparts in other mammals such as the mucous glands of the dog and the mouse (3, 5), the mainly serous glands of other canines (42), and the mixed gland of the buffalo (7).

A classification of the types of mucosubstances elaborated by these glands was made by comparing the histochemical data obtained in this study with the classification of mucosubstances proposed by several histochemists (33, 40, 44, 48). According to this, the mucoserous cells secrete and elaborate considerable quantities of neutral mucosubstances, sialomucins and little sulphomucins while the seromucous cells secrete and elaborate little mucosubstances at their apical portions only. The maintenance of basophilia following ribonuclease digestion appears to eliminate polynucleotide phosphate as a source of polyanion which binds the cationic dye in the cytoplasm of the secretory cells. Moreover, the abundance of sialomucins over sulphomucins observed in the secretions of these glands might be of some phylogenetic importance, since sialomucins secretory cells are more primitive than sialidase nonliable or sulphated mucosubstances.

The results also demonstrate considerable enzyme activities in the gland, although the phosphatase activities are less marked than those of other salivary glands of the dromedary (19, 31, 36, 37, 50, 51, 52). It may, however, be related to the buffered composition of the camel's saliva that constitutes an essential medium for food fermentation in the forestomach. On the other hand, the strong activity of alkaline phosphatase, adenosine triphosphatase, succinic dehydrogenase, non-specific esterases and aminopeptidases detected in the epithelial ductal system of the glands, might be related to a high rate of oxidative metabolism or to a role of osmosis needed for the production of saliva or for the resorption of materials through the ductal lumen in order to maintain the necessary pH needed during feeding. The activity of succinic dehydrogenase in the secretory endpieces of the glands might be related with the production of secretions at different tonicities in the formation of mucosubstances.

The results also demonstrate the inability of the ductal system of the glands to secrete mucosubstances. This might indicate that the enzymatic reactivity of the ducts is involved in controlling the ionic composition, the tonicity of saliva and the amount of mucosubstances secreted by the endpieces. Moreover, occasional mucoepithelial cells observed in the sublingual glands of rats and buffaloes (7, 43) were also observed in the glands of the camel in the present study. The myoepithelial cells might be involved in membrane transport by speeding up the outflow of saliva by their contraction. They may also have a role in overcoming the peripheral resistance to the secretory cells and the adjacent intercalated ducts.

Hence, the sublingual glands of the dromedary contribute with the other salivary glands (8, 19, 31, 34, 36, 37, 50, 51, 52), to producing bicarbonate-rich, well-buffered and mucous rich saliva that furnishes an excellent medium for the lubricant and moistening of the lips, buccal cavity and taste buds, as well as for the water balance, serum electrolyte control and for the ion exchange needed in synaptic and nerve impulse transmission as well as for regulating food fermentation. Nevertheless, more investigations on histochemistry and ultrastructure of the salivary glands of the camel are needed to understand the remarkable adaptive physiology of this animal to its harsh environment.

ACKNOWLEDGEMENTS

We are grateful to Prof. H.S. HUSSEIN for helpful criticism during the preparation of the manuscript. This research was supported by Research Grant (Zoo/1409/28) from the Research Centre, College of Science, King Saud University, Saudi Arabia.

AL-ASGAH (N.A.), JARRAR (B.M.), TAIB (N.T.). Structure and histochemistry of the sublingual salivary glands of the one-humped camel (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (4) : 519-527

Morphometric, histological and histochemical studies were carried out on the sublingual salivary glands of the Arabian camel (*Camelus dromedarius*). The glands are of the tubulo-acinar type and consist of many lobules that are composed of two types of cells, mucoserous and seromucous. The mucoserous cells form the main secretory units of the gland but seromucous cells are much more seldom and form associated acini. The former cells secrete and elaborate large quantites of neutral mucosubstances, sialomucins and little sulphomucins while only the apical portion of the latter cells shows weak to moderate activity for neutral and acid mucosubstances. The histoenzymological tests employed here detected a considerable activity of alkaline phosphatase, succinic dehydrogenase, aminopeptidase and non-specific esterases, but weak activities of cytochrome oxidase, peroxidase and no activities of triacylglycerol lipase, ß-glucoronidase and amylase. The functional significance of these findings is discussed. *Key words* : Salivary gland D0Histochemistry D0Histoenzymology D0Glycoprotein D0*Camelus dromedarius* D0Saudi Arabia.

AL-ASGAH (N.A.), JARRAR (B.M.), TAIB (N.T.). Estructura e histoquímica de las glándulas salivares sublinguales del dromedario (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1990, **43** (4) : 519-527

Se llevaron a cabo estudios morfológicos, histológicos e histoquímicos sobre las glándulas salivares del dromedario de Arabia (*Camelus dromedarius*). Las glándulas son de tipo acino tubular, formadas por numerosos lobulos, compuestos de dos tipos de células : mucoserosas y seromucosas. Las células mucoserosas constituyen el elemento secretor esencial de la glándula ; las células seromucosas son mucho más raras y forman grupos de acinos. Las primeras secretan y elaboran grandes cantidades de sustancias mucosas neutras, mucocinas y una pequeña cantidad de sulfamucinas. Por otro lado, sólo la porción apical de las seromucosas demuestra un grado de actividad bajo o moderado, para la secreción de sustancias mucosas neutras o acidas. Las pruebas histo-enzimáticas utilizadas demuestran una actividad importante de la fosfatasa alcalina, de la succinil deshidrogenasa, de la aminopeptidasa y de esterasas inespecíficas. Se notó una ligera actividad de la oxidasa citocrómica y de la peroxidasa, pero ninguna actividad de la lipasa triacilglicerasa, de la ß-glucironidasa y de la amilasa. El significado práctico de estos resultados es el objeto de una discusion. *Palabras claves* : glándula salivar D0Glicoproteina D0Histoenzima D0Histoquímica D0*Camelus dromedarius* D0Arabia Saudita.

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