

Histochemical characterization and distribution of mucosubstances and enzyme activity in the lingual salivary glands of the one-humped camel (*Camelus dromedarius*)

B. M. Jarrar¹N. T. Taib¹

JARRAR (B. M.), TAIB (N. T.). Caractérisation histochimique et localisation des mucosubstances et leur activité enzymatique dans les glandes salivaires du dromadaire (*Camelus dromedarius*). *Revue Elev. Méd. vét. Pays trop.*, 1989, 42 (1) : 63-71.

Des recherches histologiques et histochimiques ont été effectuées sur les glandes salivaires du dromadaire (*Camelus dromedarius*) afin d'en déterminer les structures et la répartition, la composition chimique et les activités enzymatiques. Des glandes de Weber et des glandes de von Ebner ont été trouvées dans la langue du dromadaire, mais les glandes apicales de Nünh étaient absentes. Les glandes de von Ebner se sont révélées séromuqueuses et d'architecture tubulo-acineuse ; elles sécrètent des mucosubstances neutres, des sialomucines et un peu de sulfomucines. Les glandes de Weber sont mucoséreuses et de type tubulaire ; elles sécrètent des mucosubstances neutres, des sialomucines et des sulfomucines résistantes à la hyaluronidase. Ces deux types de glandes ont montré une activité enzymatique variable pour les phosphatases acides et alcalines, l'adénosine triphosphatase, les estérases non spécifiques, l'anhydrase carbonique et la déhydrogénase succinique. Aucune activité pour la β -glucuronidase, la lipase, les cholinestérases et l'aminopeptidase n'a été détectée et seule une légère activité amylolytique pour l' α -amylase a été révélée dans les glandes de von Ebner. *Mots clés* : Dromadaire - *Camelus dromedarius* - Glande salivaire - Histologie - Enzyme.

INTRODUCTION

Although the one-humped camel (*Camelus dromedarius*) is still one of the least studied mammals, yet considerable histochemical investigations were carried out on its major salivary glands, the parotid and the mandibular glands (1, 6, 20, 23, 24, 25, 38). On the other hand, the histochemistry of its minor salivary glands has been recently investigated in the labial (36) and in the palatine salivary glands (37).

In the present study, another group of minor salivary glands of the one-humped camel, the lingual salivary glands are being histologically characterized and their histochemical composition and histoenzymatic activities investigated.

1. Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.

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MATERIAL AND METHODS

The heads of sixteen adult camels, 9 males and 7 females, each immediately were removed following slaughter at the Riyadh abattoir. The whole tongue of each animal was divided into small pieces and were quickly immersed into containers each with one of the following fixatives : cold (4 °C) 10 p. 100 buffered formalin (pH 7.8) with 2 p. 100 calcium acetate, alcoholic Bouin's fluid, Rossman's fluid and Zenker's fluid. They were thoroughly washed in running water and processed for sectioning at 5-6 μ m thickness. Paraffin sections were stained with haematoxylin-eosin and with Mallory trichrome for histological examination. The secretory cells of the glands were characterized by the method of GABE and ST-GIRONS (8).

Paraffin, as well as fresh unfixed frozen sections were used for the following histochemical reactions :

α -glycol rich mucosubstances : Periodic acid Schiff (PAS) technique (10), Best's carmine for glycogen (2), PAS after acetylation blockade (18), PAS after acetylation-deacetylation (27), PAS after phenylhydrazine treatment (32) and PAS after treatment with chloroform and methanol.

Acid mucosubstances : Alcian blue 1 p. 100 (AB) at pH 2.5, AB at pH 1.0 and AB at pH 0.4 (16), for the selective characterization of sulfomucins.

Distinction between acidic and neutral mucosubstances : AB (pH 2.5)-PAS (21) and AB (pH 1.0)-PAS (32).

Distinction between sulfomucins and sialomucins : Aldehyde fuchsin (AF) and AF-AB (pH 2.5) (34) ; weak (25 °C, 4 hr), mild (37 °C, 4 hr) and strong (60 °C, 4 hr) methylation-saponification-AB (pH 2.5) (33) ; acid hydrolysis (0.1N HCl 60 °C, 4 hr)-AB (pH 2.5) (29) ; Azure A (0.2 p. 100) toluidine blue (TB) 0.003 p. 100 buffered at pH 1.7 and 3.4 (14) ; critical electrolyte concentration (CEC) technique for extinction of alcianophilia at pH 5.6 in the presence of gradual concentration of MgCl₂ (31).

Enzymes digestion tests : they were performed by using PAS followed by α -amylase to exclude any possible glycogen (16, 19), neuraminidase type V from *Vitrio cholerae* followed by AB pH 2.5 (35) or TB pH 3.7 ; testicular hyaluronidase followed by AB

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pH 1.0 (32) or TB pH 2.0; ribonuclease followed by AB pH 2.5 (15). In each case control sections were incubated for the same length of time at the same temperature in buffer solutions without the enzyme.

Enzyme activities: The calcium cobalt method for alkaline phosphatase (9); the lead nitrate method for acid phosphatase (28); the Ogawa and Mayahara method for mitochondrial adenosine triphosphatase (28); the α -naphthyl acetate method for non-specific esterases (28); the modified tetrazolium method for succinic dehydrogenase (13); the McCABE and CHAYEN method for aminopeptidase (17); the naphthol-AS method for β -glucuronidase (11); the tween method for lipase (28); the Häusler method for carbonic anhydrase (28); the acetylthiocholine method for cholinesterases (28) and the modified starch film for amylase (28). In the control sections the substrates were omitted.

RESULTS

The tongue of the one-humped camel is covered with a cornified stratified epithelium of variable thickness. A dense lamina propria is continuous with the connective tissue partitions along the lingual muscles. The mucosa of the dorsal epithelium is differentiated into filiform, fungiform and circumvallate papillae. A lyssa-like structure which is formed of thick connective tissue bundles is passing along the ventral surface of the tongue. The lingual glands of this animal are composed of both von Ebner's glands and Weber's glands.

Von Ebner's glands

These glands occur beneath the circumvallate papillae and they open into a trench surrounding each of them. These tubulo-acinar glands are seromucous in nature and of holocrine type. Superficially situated glands occur amongst the connective tissue and are sometimes surrounded with stromal striated muscle bundles. Deep ones occur mainly amongst adipose tissue. The endpieces are surrounded by a thin basement membrane and are drained by a system of branching ducts, the intercalated ducts, lined by flattened cells. The epithelium of the striated ducts is simple cuboidal, while interlobular excretory ducts were not discernible (Fig. 1a). The secretory epithelium of the glands consists of a series of cells with relatively large nuclei. The apical portions of the cells are filled with almost eosinophilic granules.

Mucosubstances histochemistry

As shown in table I, von Ebner's glands of the camel show moderate PAS reaction only in the apical portion of their cells (Fig. 1b), which is not susceptible to α -amylase digestion but is completely abolished by phenylhydrazine and by acetylation treatment. It is weakly restored by acetylation-deacetylation-PAS sequential techniques. Moreover, the apical portions of these cells have exhibited a weak alcianophilia at pH 2.5 but have neither stained with Best's carmine nor with alcian blue at either pH 1.0 or 0.4. In the sequential PAS-AB (pH 2.5) and PAS-AB (pH 1.0) staining procedure, bluish purple and red staining were observed at pH 2.5 and pH 1.0, respectively. In both of the AF and AF-AB (pH 2.5 and pH 1.0) sequential techniques, the glands were weakly stained with alcian blue at pH 2.5. This alcianophilia was partially abolished by acid hydrolysis, neuraminidase, weak methylation and ribonuclease digestion. Whereas a moderate alcianophilia was restored by weak methylation-saponification sequential techniques. The apical portions of the glandular cells exhibited an alcianophilia at only 0.1M MgCl₂ concentration (CEC technique) and were orthochromatic with Azure A and toluidine blue at pH 1.7 and 3.4.

Ductal cells were negative to all tests used to detect mucosubstances.

Enzymes histochemistry

Alkaline phosphatase: The activity of this enzyme appeared to be stronger in the ductal cells rather than in the acinar cells (Fig. 1c).

Acid phosphatase: The reactivity of this enzyme was generally moderate in the cytoplasmic granules of the acinar cells and no reactivity was detectable in the tubular cells (Fig. 1d).

Adenosine triphosphatase: This enzymatic activity showed prominent in both acinar and ductal cells (Fig. 1e).

Non-specific esterases: A moderate reaction of this group of enzymes was seen in acinar cells but intense activity was detectable in ductal cells.

Carbonic anhydrase: Weak to moderate reaction appeared evenly distributed throughout the cells of the secretory endpieces as well as throughout the tubular epithelium.

Succinic dehydrogenase: This enzyme showed a moderate reaction in acinar cells as well as in ductal epithelium (Fig. 1f).

α -amylase: A weak amylolytic activity was seen in acinar cells.



Fig. 1a : Von Ebner's glands of *C. dromedarius* stained with haematoxylin eosin. x 240.*

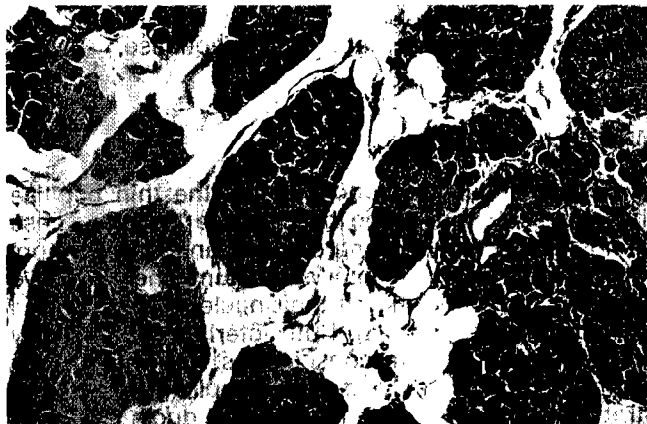


Fig. 1b : Von Ebner's glands of *C. dromedarius* stained with PAS. x 350.*

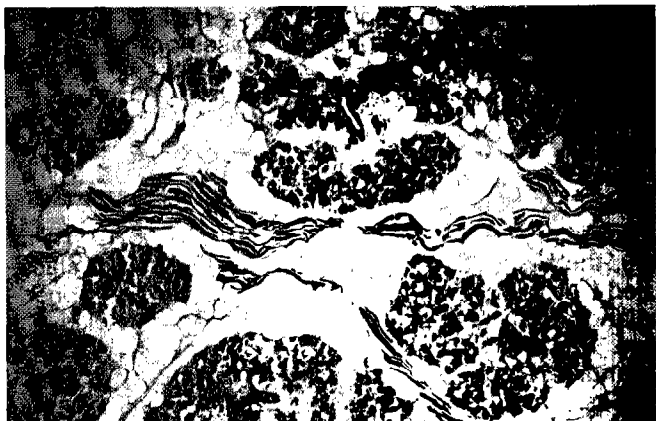


Fig. 1c : Von Ebner's glands of *C. dromedarius* stained with cobalt method for alkaline phosphatase. x 350.*

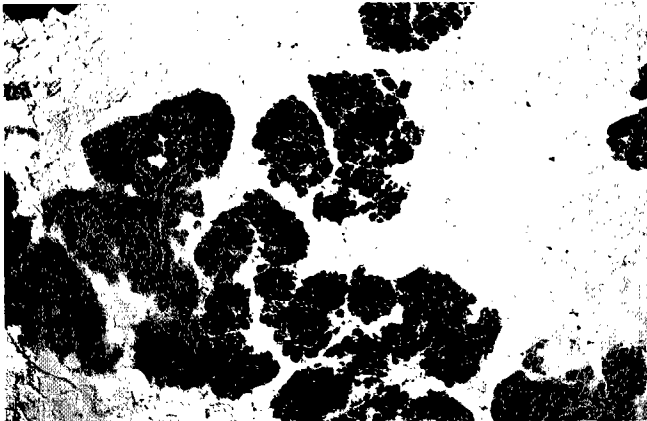


Fig. 1d : Von Ebner's glands of *C. dromedarius* stained with lead nitrate method for acid phosphatase. x 240.*

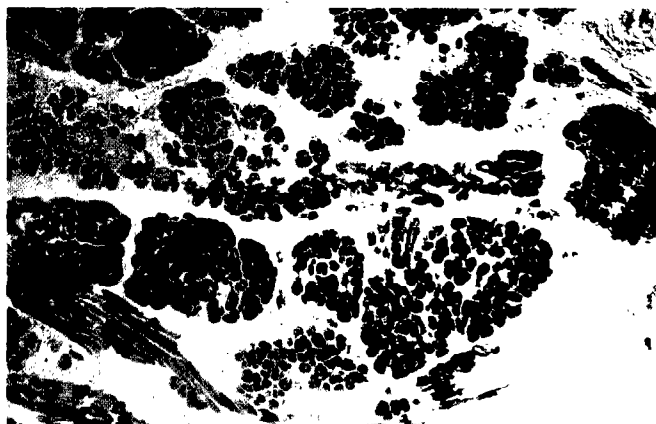


Fig. 1e : Von Ebner's glands of *C. dromedarius* stained with Ogawa and Mayahara method for mitochondrial adenosine triphosphatase. x 300.*

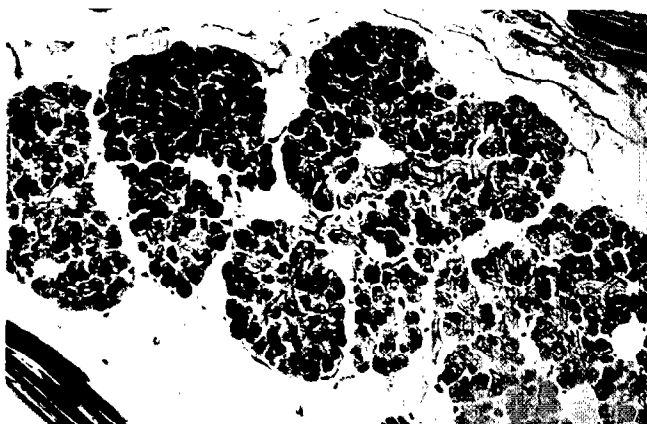


Fig. 1f : Von Ebner's glands of *C. dromedarius* stained with the modified tetrazolium method for succinic dehydrogenase. x 350.*

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These glands showed no reaction for β -glucuronidase, lipase, cholinesterases and aminopeptidase.

Weber's glands

Weber's glands are found beneath the thin papillae that are scattered at the base of the tongue. These glands are composed entirely of branched secretory tubules lined by a columnar epithelium. The cells have an alveolar cytoplasm and their nuclei are situated in their basal pole (Fig. 2a). Both interlobular excretory and striated ducts are conspicuous. The interlobular excretory ducts are lined by a simple or a pseudo-stratified epithelium. The intercalated ducts are indiscernible. These glands are surrounded by connective tissue and muscle bundles that are highly vascularized especially with arteries and capillaries.

Mucosubstances histochemistry

As shown in table I, the secretory granules in all cells of the Weber's glands stained strongly with PAS (Fig. 2b). The PAS reactivity was slightly sensitive to α -amylase digestion, but was partly abolished by phenylhydrazine and completely abolished by acetylation. PAS reactivity of these glands was restored by acetylation-deacetylation-PAS sequential techniques. They also stained weakly with Best's carmine. Moreover, these glands stained strongly with Alcian blue at pH 2.5 and 1.0, but a faint alcianophilia occurred at pH 0.4. A bluish purple colour appeared with the PAS-AB (pH 2.5) sequence and PAS-AB (pH 1.0) staining procedure (Fig. 2c). In the AF-AB (pH 2.5 and pH 1.0), the glands reacted strongly at both dyes and at both pH levels. The alcianophilia observed at pH 2.5 was partially abolished by acid hydrolysis, neuraminidase digestion and weak methylation. It is completely abolished by both moderate and strong methylation and was resistant to hyaluronidase and to ribonuclease digestion. However, alcianophilia was completely restored by weak methylation followed by saponification and to a lesser extent by moderate and strong methylation-saponification. The cells exhibited a moderate alcianophilia with 0.1 M and 0.2 M $MgCl_2$ while a weak alcianophilia was observed with 0.3 M $MgCl_2$. They did not react with 0.4 M $MgCl_2$. They showed a moderate metachromatic reaction with Azure A and toluidine blue at pH 3.4 and pH 1.7.

Ductal cells did not react negatively to all previously mentioned tests.

Enzyme histochemistry

Alkaline phosphatase : A moderate reaction was seen in the secretory tubules of these glands while the tubular cells responded almost negatively.

Acid phosphatase : This enzyme was found diffused at a low level throughout the cells of secretory tubules but a significant level of the enzyme was seen in the interlobular excretory and striated ducts (Fig. 2d).

Adenosine triphosphatase : This enzyme showed prominent activities in the ductal cells as well as in the cells of the secretory tubules.

Non-specific esterases : A faint reaction was seen in the secretory cells of the glands, but a strong reaction was observed in the epithelium of the striated ducts and to lesser extent in the epithelium of the interlobular excretory ducts (Fig. 2e).

Succinic dehydrogenase : Weak to moderate reaction was seen in the cytoplasm of the secretory tubules but a strong reaction was observed in ductal cells.

Carbonic anhydrase : A diffused weak reaction was only seen in the secretory tubules.

No amyolytic activity was detected in these glands and similar to von Ebner's glands no activity for β -glucuronidase, lipase, cholinesterases or aminopeptidase was seen.

DISCUSSION

According to ZIMMERMANN (40) and FAHRENHOLZ (7), the mammalian lingual salivary glands are classified into von Ebner's and Weber's glands. Von Ebner's glands form lobules among the lingual muscles and are related to gustatory papillae whereas Weber's glands are either scattered in the tongue or grouped at its base. In addition, some mammals possess Nühn's glands (Blandin's glands) which are located under the surface of the tip of the tongue on each side of the frenulum (3).

In the present study, both von Ebner's glands and Weber's glands were detected in the tongue of the one-humped camel but no Nühn's glands. The latter, have been reported in man (3) and in insectivorous bats but not in frugivorous bats (22). They seem to be related to the feeding habits of the animal. The location and structure of the lingual glands of the one-humped camel are similar to those described in the common marmoset *Callithrix jacchus*, but Weber's glands of the latter are surrounded by serous demilunes (30).

Using the criterion of GABE and ST-GIRONS (8), the present histochemical investigations prove that von Ebner's glands of the one-humped camel contain seromucous secretory cells whilst Weber's glands contain mucoserous ones. Hence, these glands of the one-humped camel are different from those of other mammals studied. Weber's glands of the rat, mouse, hamster, guinea-pig, rabbit, armadillo, sloth, cat, dog,



Fig. 2a : Weber's glands of *C. dromedarius* stained with haematoxylin & eosin. x 240.*

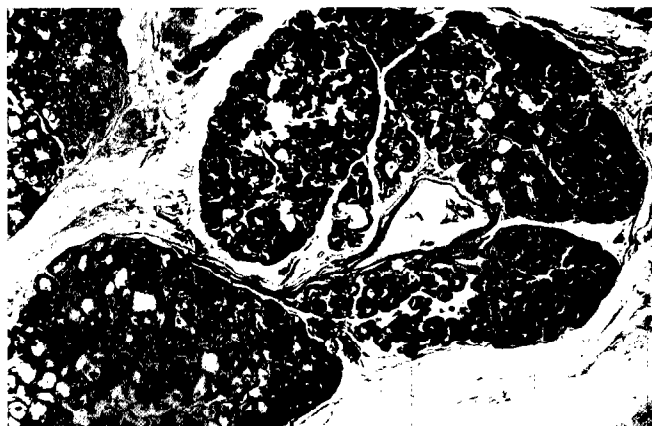


Fig. 2b : Weber's glands of *C. dromedarius* stained with PAS. x 350.*

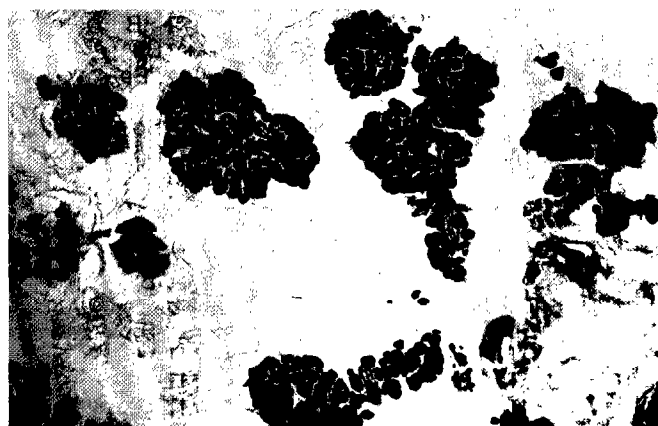


Fig. 2c : Weber's glands of *C. dromedarius* stained with AB (pH 2.5)-PAS. x 240.*

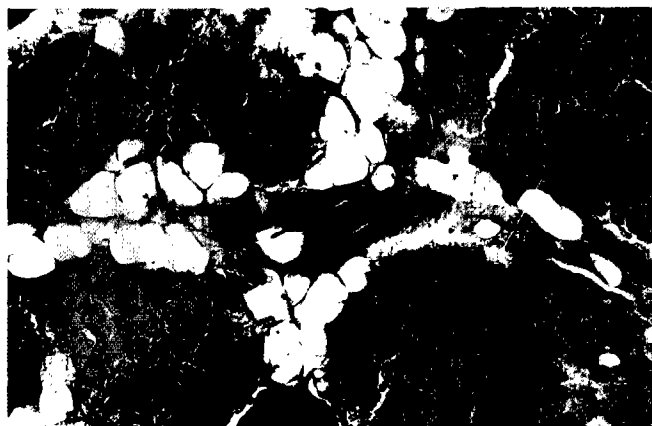


Fig. 2d : Weber's glands of *C. dromedarius* stained with lead nitrate method for acid phosphatase. x 420.*

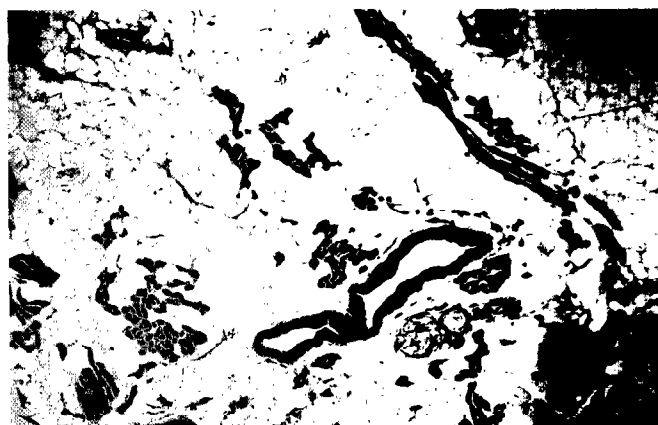


Fig. 2e : Weber's glands of *C. dromedarius* stained with the α -naphthyl acetate method for non-specific esterases. x 500.*

pig and sheep contain only mucous cells, those of the goat, ox and horse mucous cells with serous demilunes ; but the von Ebner's glands of them all consist of only serous cells (26).

According to the classification of mucosubstances proposed by several workers (21, 28, 31, 34), Weber's glands of the one-humped camel elaborate neutral mucosubstances, sialomucins and sulfomucins and von Ebner's glands neutral mucosubstances, sialomucins but little sulfomucins. These glycoproteins especially the neutral ones have been detected in the lingual glands of most mammals studied (4, 5, 26). Sialic acid was detected in the mucous and serous cells of the lingual glands of the armadillo, sloth, pig, sheep, goat, ox and horse whilst sulfomucins were found in the mucous cells of the rat, mouse, hamster,

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guinea-pig, sloth, cat, dog and horse (26). On the other hand, both glands contain sulfomucins and sialomucins in insectivorous microchiropterans, but these substances occur only in Weber's glands of frugivorous bats (39). However, in other mammals

such as the jerboa, *Jaculus jaculus*, von Ebner's glands are devoid of both neutral and acid glycoproteins, but the Weber's glands contain sialomucins and sulfomucins (39).

TABLE I The histochemical reactions in the lingual salivary glands of the one-humped camel *Camelus dromedarius*.

Histochemical reaction	Results	
	von Ebner's glands	Weber's glands
PAS	+, p	+++ , p
α -amylase-PAS	-	-
Best's carmine	-	\pm ?
Acetylation-PAS	Cb	Cb
Acetylation-deacetylation-PAS	+, p	+++ , p
Phenylhydrazine-PAS	Cb	pb
AB(pH 0.4)	-	+, B
AB(pH 1.0)	-	+++ , B
AB(pH 2.5)	+, B	+++ , B
AB(pH 1.0)-PAS	+, p	++ , Bp
AB(pH 2.5)-PAS	+, Bp	+++ , Bp
AF	-	+, p
AF-(AB pH 1.0)	-	++ , Bp
AF-(AB pH 2.5)	+, B	++ , Bp
Acid hydrolysis-AB(pH 2.5)	Cb	pb
W. methylation-AB(2.5)	Cb	pb
W. methylation-saponification-AB(pH 2.5)	\pm , B	+++ , B
M. methylation-AB(pH 2.5)	Cb	Cb
M. methylation-saponification-AB(pH 2.5)	\pm , B	++ , B
S. methylation-AB(pH 2.5)	Cb	Cb
S. methylation-saponification-AB(pH 2.5)	Cb	+ \pm , B
Azure A(pH 1.7)	OT	MT
TB(pH 1.7)	OT	MT
Azure A(pH 3.4)	OT	MT
TB(pH 3.4)	OT	MT
CEC (AB, 0.1 M)	+	+
CEC (AB, 0.2 M)	-	+
CEC (AB, 0.3 M)	-	\pm
CEC (AB, 0.5 M)	-	-
Neuraminidase-AB(pH 2.5)	\pm , Pb	++ , Pb
Neuraminidase-TB(pH 3.4)	OT, Pb	MT, Pb
Hyaluronidase-AB(pH 1.0)	-	+++ , Nb
Hyaluronidase-TB(pH 1.7)	Nb, OT	Nb, MT
Ribonuclease digestion-AB(pH 2.5)	+, B	+++ , B
(Chloroform + methanol)-PAS	+, P	+++ , P
Alkaline phosphatase	++	+ \pm
Acid phosphatase	++	+
Adenosine triphosphatase	++ \pm	++
Non-specific esterases	+ \pm	+
Carbonic anhydrase	+	+
Succinic dehydrogenase	++	+
Amylase	\pm	-
Cholinesterase	-	-
Lipase	-	-
Aminopeptidase	-	-
β -glucuronidase	-	-

Reactions: - negative; \pm faint or negligible; + weak; ++ moderate; +++ intense; Cb complete blockade; M mild; MT metachromasia; Pb partial blockade; Nb no blockade; OT orthochromasia; S strong; TB toluidine blue; W weak; ? doubtful.
Colours: B blue; Bp bluish purple; P pink.

Considerable enzyme activities were detected in the lingual salivary glands of the camel in the present study. These include important phosphatases activities in the secretory cells which might be related to the buffered composition of the camel's saliva that constitute an essential medium for food fermentation in the forestomach. Moreover, strong reactivity of succinic dehydrogenase, acid phosphatase and non-specific esterases, were also detected in the ductal cells of these glands. These might be related to a high rate of oxidative metabolism or to a role in the osmosis needed for the production of saliva or for resorption of materials through duct lumen in order to maintain the necessary pH needed during feeding.

Hence, these lingual salivary glands of the one-humped camel contribute, together with the other salivary glands such as the parotid, the mandibular, the sublingual, the ventral buccal, the labial and the

palatine glands (1, 6, 12, 20, 23, 24, 25, 36, 37, 38) to produce bicarbonate-rich, well-buffered and mucous saliva that furnishes an excellent medium for the lubrication and moistening of lips, buccal cavity and taste buds, as well as for water balance, serum electrolyte control and for the ion exchange needed in synaptic and nerve impulse transmission as well as for regulating food fermentation.

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JARRAR (B. M.), TAIB (N. T.). Histochemical characterization and distribution of mucosubstances and enzyme activity in the lingual salivary glands of the one-humped camel (*Camelus dromedarius*). *Revue Elev. Méd. vét. Pays trop.*, 1989, 42 (1) : 63-71.

The lingual salivary glands of the one-humped camel (*Camelus dromedarius*) have been histologically and histochemically investigated to characterize their structure and distribution, chemical composition and enzyme activities. Both von Ebner's and Weber's glands were detected in the tongue of the camel, but Nüh's apical glands were absent. Von Ebner's glands proved to be seromucous with tubulo-acinar endpieces that secrete neutral mucosubstances, sialomucins but little sulfomucins and Weber's glands are mucoserous and of tubular type. They secrete neutral mucosubstances, sialomucins and hyaluronidase resistant sulfomucins. Both glands showed variable enzyme activity for acid and alkaline phosphatases, adenosine triphosphatase, non-specific esterases, carbonic anhydrase and succinic dehydrogenase. No activities for β -glucuronidase, lipase, cholinesterases and aminopeptidase were detected and only a weak amyolytic activity for α -amylase was seen in von Ebner's glands. *Key words*: Camel - *Camelus dromedarius* - Salivary gland - Histology - Enzyme.

JARRAR (B. M.), TAIB (N. T.). Determinación de las características histoquímicas y localización de las mucosubstancias y su actividad enzimática en las glándulas salivarias del dromedario (*Camelus dromedarius*). *Revue Elev. Méd. vét. Pays trop.*, 1989, 42 (1) : 63-71.

Se efectuaron búsquedas histológicas e histoquímicas sobre las glándulas salivarias del dromedario (*Camelus dromedarius*) para determinar las estructuras y la repartición, la composición química y las actividades enzimáticas. Se observaron glándulas de Weber y glándulas de von Ebner en la lengua del dromedario, pero las glándulas apicales de Nüh eran ausentes. Las glándulas de von Ebner eran seromucosas y de arquitectura tubulo-acinosa; secretan mucosubstancias neutras, sialomucinas y un poco de sulfomucinas. Las glándulas de Weber son mucoserosas y de tipo tubular; secretan mucosubstancias neutras, sialomucinas y sulfomucinas resistentes a la hialuronidasa. Ambos tipos de glándulas mostraron una actividad enzimática variable para las fosfatasa ácidas y alcalinas, la adenosina trifosfatasa, las esterases no específicas, el anidrasa carbónico y la succínica dehidrogenasa. No se encontró ninguna actividad para la α -amilasa en las glándulas de von Ebner. *Palabras claves*: Dromedario - *Camelus dromedarius* - Glándula salivaria - Histología - Enzima.

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