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HISTOMORPHOLOGICAL AND HISTOCHEMICAL STUDIES OF THE HARDERIAN GLANDS OF THE ONE-HUMPED CAMEL

(*Camelus dromedarius*)

(With 3 Tables, 2 Plates and 15 Figs.)

By

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دراسات هستولوجية وهستوكيميائية على غدد الهارديريان في الجمال وحيد السنام

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أجريت هذه الدراسة على غدد الهارديريان في الجمال وحيد السنام ووجد أن هذه الغدة تنتمي إلى النوع المركب الأنبوبي الحويصلي . تتكون النهايات المفردة للغدة من ثلاث أنواع حسب نوع الخلايا المكونة له . كما لوحظ وجود خلايا طبيعية ، خلايا دهنية ، صفائح غضروفية ونسيج ليمفاوي في غدة الهارديريان .

SUMMARY

The Harderian glands of the one humped camel are compound tubuloalveolar glands. The glandular end-pieces were of three types. The first type (78%) was exclusively lined by light cells. The second type (15%) was lined by both light and dark cells. However the third type 7% was lined exclusively by dark cells. Lymphoid tissue, plasma cells, pigment cells, adipocytes and cartilagenous platelets were demonstrated within the harderian gland.

PAS positive materials, acid-mucopolysaccharides and sudanophilic materials were also demonstrated within the cells of the glandular end pieces and the ducts of these glands.

INTRODUCTION

The role of the Harderian gland in protecting the eye ball and its involvement in reproduction in different animal species and birds have been described by several authors (WETTEBERG, GELLER and YAWILER, 1970; REITER and KLEIN, 1971; THIESSEN, GLANCY and GOODWIN, 1976 and BURNS, 1979).

WOODHOUSE and RHODIN (1963); HOFFMAN (1971); KUHNEL (1974) and FAHMY, SHAHIEN and KANDIL (1979) mentioned that the harderian gland of mice, golden hamsters, swine and one-humped camels respectively were compound tubuloalveolar gland.

This work is carried out to study the histomorphological and the histochemical features of the Harderian gland in one-humped camels not only to discern the designation of this gland in these terrestrial vertebrate but also to throw light on the reliable function of this gland in such animal which live an adverse climatic condition.

MATERIAL and METHODS

The Harderian glands of 40 normal healthy camels ranging 6 months-10 years old were freshly collected from Cairo and Bani Adi slaughter houses. The age of these animals were judged by the appearance of the teeth according to the description given by WAHBY (1938). During the removal of the Harderian gland, the general location and shape were noted. The average length, width, thickness and weight of the gland were recorded. Materials were fixed in calcium formol, Carnoy's fluid and Bouin's fluid. After proper fixation, the materials were dehydrated, cleared and embedded in paraffin wax. Serial sagittal, frontal and transverse sections of about 5-7 μ m were cut and stained with haematoxylin and Eosin, Crossman's trichrome, Weigert elastica, PAS technique and Sudan black (DRURY and WALLINGTON, 1980).

Paraffin sections 5-7 μ m thickness stained with haematoxylin and eosin of 14 glands of camels were analysed (Volume Percentage of the parenchyma, connective tissue, cartilage, adipose tissue and lymphatic tissue) by differential point counting as described by WEIBEL (1979).

The micromorphological examination includes measuring the diameter of the secretory end-pieces, number of the cells/end-piece, height of the glandular epithelium and nuclear volume of the glandular epithelium in 17 glands of camels.

Measurements were carried out with an eye piece micrometer disc calibrated on a stage micrometer to the nearest micron.

The results were statistically analysed according to SNEDECOR and COCHRAN (1967).

RESULTS

a- Histomorphological features :

The Harderian gland of the one humped camel was represented by a large and well developed elongated pear shaped structure situated, ventrally within the orbit, postero-medial to the eye ball (Plate 1). It beared two surfaces, an outer convex surface and an inner concave surface. Each gland recorded mean length, width and thickness of 26, 15 and 11 mm; respectively. The Harderian gland weighed 4.39 ± 0.05 and 4.5 ± 0.02 in both male and female camel; respectively.

The Harderian glands of the one-humped camel were of the compound tubulo-alveolar variety. Each gland was surrounded by two capsules; an outer capsule which was formed mainly of adipocytes, and an inner capsule which consisted of fibroelastic connective tissue containing several blood vessels and myelinated nerve fibers. The inner capsule gave rise to several septa, dividing the gland into various lobules.

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The diameter of the end-pieces was neither sex- nor age-linked (Table 1). The glandular cells were pyramidal or columnar in shape and contained large, rounded basally-situated nuclei. The cells exhibited distinct boundaries. Regarding the staining affinity of their cytoplasm, two types of glandular cells could be recognized; light and dark cells (Fig. 1). The light cells were relatively abundant and possessed poorly stained cytoplasm. The amount of light cells was more abundant in she-camels.

According to the arrangement of the light and dark cells within the glandular end-pieces, three types of end-pieces could be distinguished. The first type constituted about 78% of the glandular end-pieces where they were exclusively lined by light cells. The second type constituted about 15% of the glandular end-pieces and they were lined by both light and dark cells. The least numerous third type, consisting about 7% of their total number, was small in size and exclusively lined by dark cells.

The least, numerous dark cells presented coarse eosinophilic granules within their cytoplasm. Myoepithelial cells with oval nuclei and cytoplasmic process were demonstrated between the basement membrane (Fig. 2). The intralobular ducts were lined by a single layer of tall columnar cells. The cytoplasmic luminal portion demonstrated coarse acidophilic granules (Fig. 3). Some oval or goblet-like mucus-secreting cells as well as few pigment cells were demonstrated at the intralobular ducts before they joined the interlobular ducts. The interlobular ducts were lined by 1-2 layers of epithelial cells. The outer layer was formed of cuboidal cells with small rounded vesicular centrally located nuclei. The luminal layer was of the columnar cells with large rounded or oval centrally located nuclei. The lining epithelium of the interlobular ducts contained cylindrical or dome shaped mucus secreting cells which possessed vacuolated cytoplasm and small rounded, deeply stained basal nuclei.

The wall of the latter ducts showed various pigment cells and was surrounded by a relatively thick layer of connective tissue infiltrated by numerous lymphocytes (Fig. 4). The main ducts were represented by four large ducts which opened on the bulbar conjunctival surface of the gland. They were lined by 3-4 cells layers. The basal layer was formed of cuboidal cells with rounded centrally-located nuclei. The intermediate layers were formed of polygonal cells and contained small deeply stained nuclei. The superficial layer was formed of columnar cells with oval, small deeply stained basally situated nuclei. The wall of these ducts presented several mucus-secreting cells and pigment cells which both increased in amount gradually towards the outlet of these ducts on the bulbar conjunctival surface (Fig. 5).

The interstitial tissue presented fibroblasts, numerous plasma cells (Fig. 6), lymphocytic infiltration, macrophages, eosinophilic granulocytes and monocytes. The number of plasma cells was obviously higher in old specimens. In addition the plasma cells in lymphatic nodules were relatively more numerous in female than in male camels. The lymphocytic infiltration were demonstrated in the vicinity of the bulbar conjunctival surface, between the end pieces and also around the intra and interlobular ducts (Fig. 7 a,b). Sometimes numerous lymphocytes might arrange themselves around various arterioles forming structures closely resembling the splenic corpuscles

(Fig. 8 a,b). Some plasma cells and macrophages might demonstrate themselves within these lymphocytic structures as well as the connective tissue around them. The volume percentage of the lymphoid tissue decreased gradually with the advancement of age (Table 2 and Plate 2). The Harderian glands of the camel were traversed by several platelets of Hyaline cartilage. The volume percentage of these cartilagenous platelets decreased by the advancement of age (Table 2 and Plate 2). Several pigment cells demonstrated themselves within the interlobular connective tissue, interlobular ducts, main ducts and the bulbar conjunctival surface epithelium (Fig. 9 a,b,c).

Several unilocular adipocytes were found either singly distributed or in various aggregations inbetween the secretory end-pieces and or within the interlobular connective tissue (Fig. 8 a).

b- Histochemical characteristics :

The secretory end-pieces of the Harderian gland, showed PAS positive materials. The cytoplasmic apical portion of the glandular cells presented several PAS positive granules. The intensity of the reaction differed not only within the individual cells of the end-pieces but also between the different end-pieces within the various lobules (Fig. 10). Some cells lining the intralobular ducts demonstrated PAS positive granules within the apical portion of their cytoplasm (Fig. 11). These reaction increased obviously within the cells of both the interlobular and the main ducts (Figs. 10, 12, a,b and 13 a,b). Most of these were observed delivering their contents within the lumen of the ducts.

The bulbar conjunctival epithelium presented PAS - positive reaction at the upper half/or quarter of its cells (Fig. 14). Some glandular end-pieces showed strong reaction to AB specially at the apical portion of their cells. In addition the AB reaction was strongly observed within some cells lining the intralobular, interlobular and main ducts (Table 3).

About 40% of the end-pieces showed weak to moderate reaction to AB/PAS technique. These reaction was demonstrated at the apices of the glandular cells as well as within the lumena of different end-pieces. Also PAS/AB positive materials were demonstrated within the cells lining the intralobular, interlobular and the main ducts.

Various sudanophilic materials were demonstrated within the cells of both the glandular end-pieces and intra and interlobular ducts. The sudanophilia was represented either in the form of scattered droplets of various sizes and or dispersed within the cytoplasm of the aforementioned cells (Fig. 15 a,b).

The intra and interlobular ducts demonstrated intense sudanophilic materials more than that observed within the glandular end pieces.

Table (1): Quantitative analysis of the parenchyma of the Harderian gland of the one humped camel.

Animal No.	Age (year)	Sex	Diameter of the end-pieces (um)	Number of the glandular culis/ end-piece	Hight of the glandular epithelium (um)	Volume of the nuclei of the glandular epithelium (um) ³
1	1	M	34.25	10	15.37	65.47
2	3	M	35.12	10	15.25	84.32
3	3	F	33.12	8	15.62	72.43
4	3	F	33.5	9	15.52	65.47
5	4	F	40.12	11	15.87	93.21
6	6	F	36.5	12	15.5	67.4
7	4	F	36.5	11	15.25	96.42
8	4	M	34.12	9	15.37	84.23
9	6	F	33.37	10	15.62	65.47
10	7	M	30.87	9	14.62	69.47
11	7	M	33.62	9	14.55	68.47
12	8	F	38.5	10	17.37	69.47
13	9	F	34.87	10	15.62	72.63
14	10	F	39.62	11	17.12	90.21
15	10	F	29.87	11	12.87	65.47
16	10	F	35.85	12	16.5	94.20
17	10	M	36.21	12	14.62	65.47

Plate (1):

M= Male

F= Female

Posterior view of the left eye ball of the one humped camel.

- 1- Lacrimal gland. 2- Harderian gland. 3- Nictitating membrane.
 4- Optic nerve. 5- Dorsal rectus muscle. 6- Dorsal oblique muscle.
 7- Ventral rectus muscle. 8- Ventral oblique muscle. 9- Lateral rectus muscle.
 10- Median rectus muscle. 11- Retractor oculi muscle.

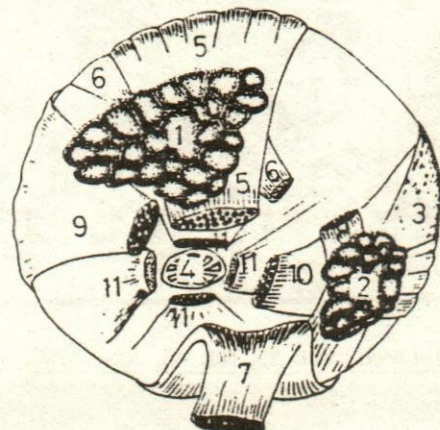


Plate [1]

LEGEND OF FIGURES

Fig. (1): A glandular end-piece of the Harderian gland in the one-humped camel, showing light and dark cells. (Haematoxylin and eosin stain, oc 10X, ob. 100X).

Fig. (2): End-pieces of the Harderian gland of the one-humped camel, demonstrating myoepithelial cell (arrow) between the glandular cells and the basement membrane. (Haematoxylin and eosin stain, oc. 10X, ob. 100X).

Fig. (3): A part of the wall of the intralobular duct of the Harderian gland of the one-humped camel. (Haematoxylin and eosin stain. oc. 10X, ob. 100X).

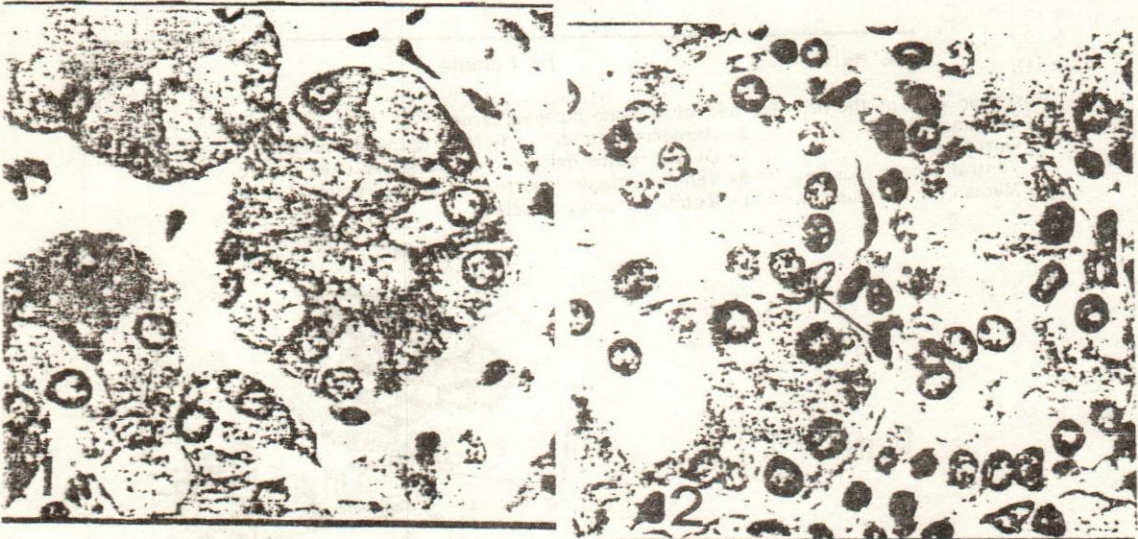
Fig. (4): An interlobular duct of the Harderian gland of the one-humped camel. Notice the heavy lymphocytic infiltration around the wall of the duct. (Haematoxylin and eosin stain, oc. 10X, ob. 16X).

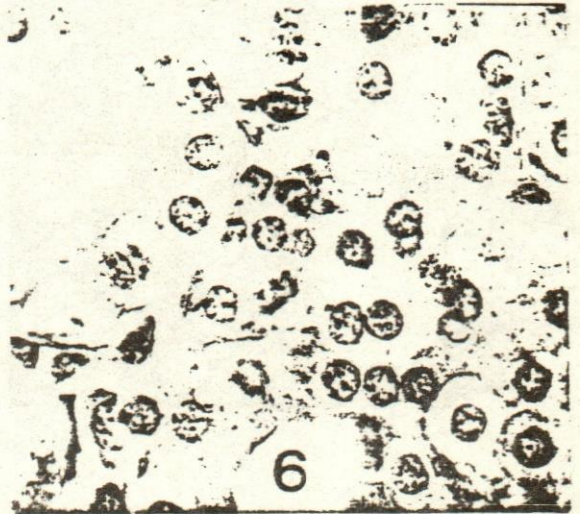
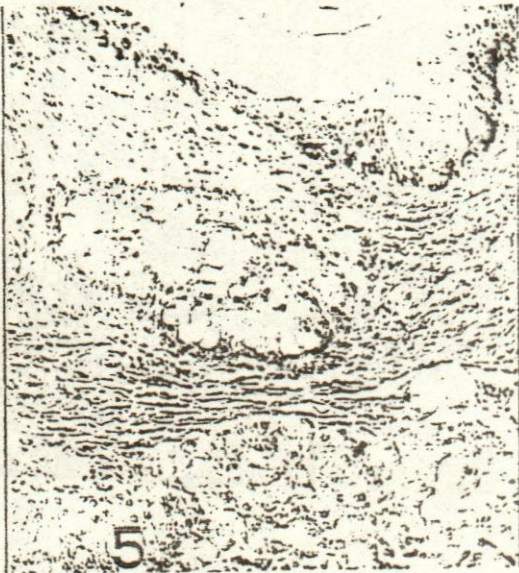
Fig. (5): The main duct of the Harderian gland of the one-humped camel short before its opening on the bulbar conjunctival surface. (Haematoxylin and eosin stain, oc. 10X, ob. 10X).

Fig. (6): Several plasma cells within the interstitial tissue of the Harderian gland of the one-humped she camel. (Haematoxylin and eosin stain, oc. 10X, ob. 100X).

Fig. (7 a,b): Lymphocytic infiltration represented by several lymph nodules in the vicinity of the bulbar conjunctival surface. (Haematoxylin and eosin stain, a- oc. 10X, ob. 16X. b- oc. 10X, ob. 40X).

Fig. (8 a,b): A lymphatic nodule inbetween the glandular end-pieces consisting of numerous lymphocytes aggregating around an arteriole. Notice the aggregation of adipocytes within the interlobular connective tissue. (PAS Haematoxylin technique. a- oc. 10X, ob. 16X. b- oc. 10 X, ob. 40X).





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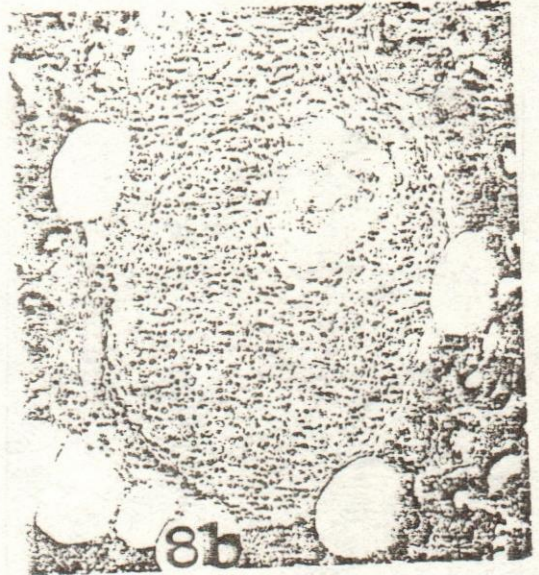
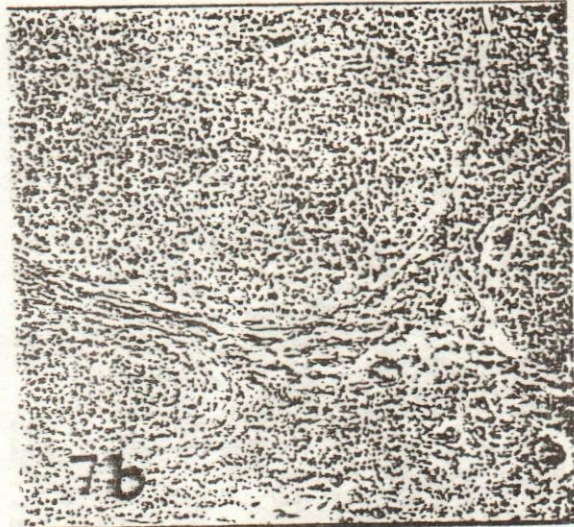


Plate (II): Diagrammatic illustration showing the volume percentage of the different constituents of the postnatal Harderian gland of the one-humped camel.

VOLUME PERCENTAGE OF THE DIFFERENT COSTITUENTS OF THE HARDERIAN GLAND OF THE ONE-HUMPED CAMEL.

Plate [II]

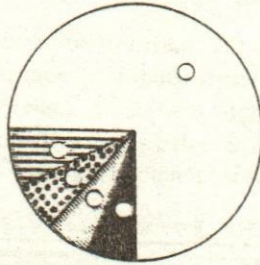


Fig. (9 a,b,c): Several pigment cells within one bulbar conjunctival surface epithelium (a and c), and around the wall of main duct (b).

a- (Haematoxylin and eosin stain). oc. 10X, ob. 40X.

b- (Alcian blue oc. 10X, ob. 100X).

c- (PAS technique, oc. 10X, ob., 40X).

	PARENCHYMA	75.13%
	CONNECTIVE TISSUE	6.17%
	ADIPOSE TISSUE	5.57%
	LYMPHATIC TISSUE	5.61%
	CARTILAGE	7.51%



Fig. (10): The Harderian gland of the one-humped camel demonstrating the PAS reaction within the glandular end-pieces and the interlobular duct. (PAS technique. oc. 10X, ob. 40X).

Fig. (11): The Harderian gland of the one-humped camel demonstrating the PAS reaction within the glandular end-pieces and the intraalobular duct. PAS. technique, oc. 10X, ob. 40X).

Fig. (12 a,b): An interlobular duct within the Harderian gland of the one-humped camel demonstrating the reaction to PAS technique.

Notice the lining cells delivering their contents within the lumen. (b-arrows). The lumen is filled with PAS-positive material.

(PAS technique a- oc. 10X, ob. 6.3X. b- oc. 10X, ob. 40X).

End-pieces Interlobular duct

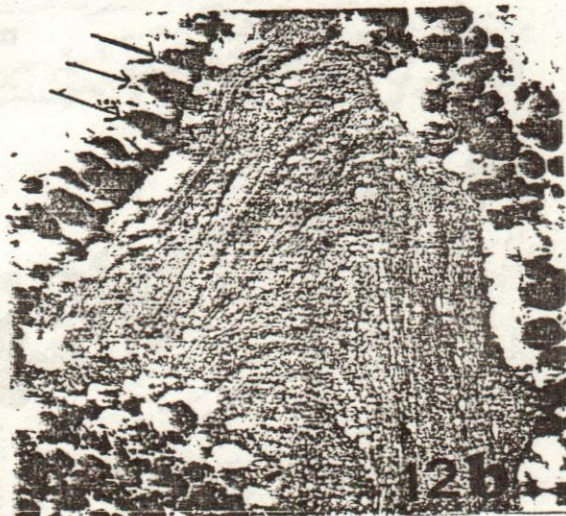
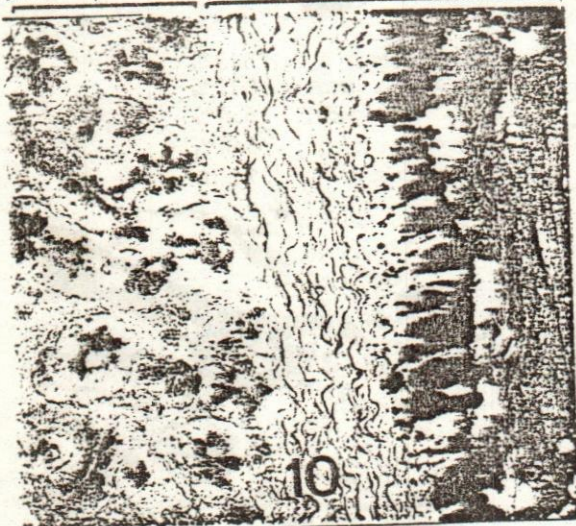


Fig. (13 a,b): The main ducts of the Harderian gland of the one-humped camel, showing the reaction to PAS technique.

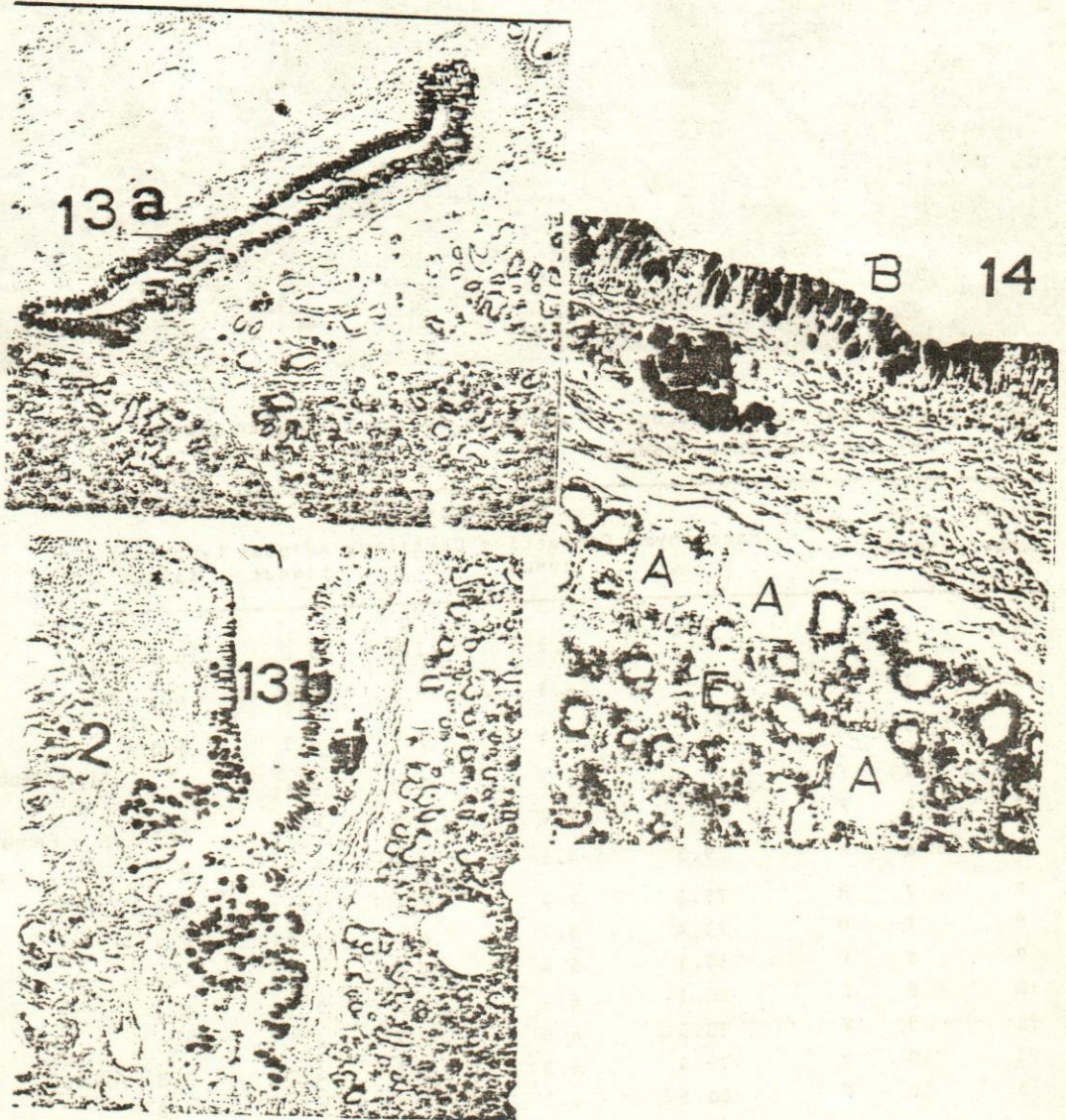
(PAS routine, a- oc. 10X, ob. 6.3X. b- oc. 10X, ob. 16X).

Fig. (14): The bulbar conjunctival epithelium (B) demonstrating PAS-positive materials within the apical part of the cytoplasm.

E- End pieces.

A= Adipocytes.

(PAS technique oc. 10X, ob. 40X).



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Fig. (15): The Harderian gland of the one-humped camel demonstrating various sudanophilic droplets within the glandular cells.

(Sudane black, a- oc. 10X, ob. 25X. b- oc. 10X, ob. 40X).

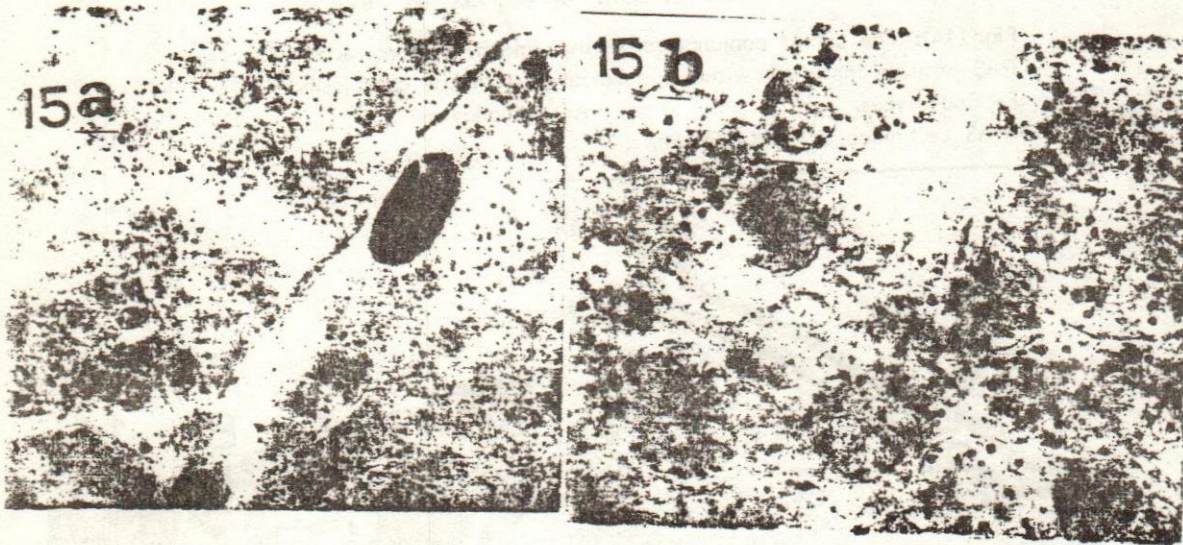


Table 2: Quantitative morphological analysis of the Harderian glands of the one-humped camel.

Animal Number	Age (year)	Sex	Volume percentage				
			Parenchyma	Connective tissue	Cartilage	Adipose tissue	Lymphatic tissue
1	1	M	67.7	4.9	11.6	5.0	10.8
2	1	M	70	6.5	9.0	8.2	6.3
3	3	M	71	5.5	9.9	6.7	6.0
4	4	F	75.7	5.1	7.0	6.6	5.8
5	4	F	78.5	9.0	6.6	4.1	5.8
6	6	F	79.3	4.9	6.6	4.9	4.3
7	7	M	75.8	7.4	6.6	4.9	5.3
8	7	M	71.4	8.5	6.5	3.9	5.7
9	8	F	80.1	5.4	6.6	3.3	4.6
10	8	F	80.1	6.4	5.6	3.3	4.6
11	9	F	80.0	6.6	5.7	4.1	4.0
12	10	M	79.4	6.8	5.8	4.2	3.8
13	10	F	80.5	6.3	5.6	4.0	3.8
14	10	F	81.8	5.1	5.3	4.2	3.6

M = Male

F = Female

Table 3: Histochemical characteristics of the Harderian gland of one-humped camel.

Histochemical method	End pieces			Duct system							
	Secretory epithelial cells.	Luminal secretion	Intralobular ducts	Mucus-secreting cells	Luminal secretion	Epithelial cells	Mucus-secreting cells	Luminal secretion	Epithelial cells	Mucus-secreting cells	Luminal secretion
PAS	+++ / ++	+++	+	+++	+	-	+++	+++	-	+++	+++
AB	(+) / + / ++	+ / ++	+ / ++	+	++ / +++	-	+++	++ / +++	-	+++	++ / +++
AB/PAS	(+) / + / ++	+ / ++	+ / ++	+++	++ / +++	-	+++	++ / +++	-	+++	++ / +++
Sudan black	++	-	++ / +	-	-	+	-	-	-	-	-

Reaction Intensity: (+) = very weak, + = weak, ++ = moderate, +++ = strong, - = no reaction visible.

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DISCUSSION

The Harderian gland in domestic animals received an incompetent work as a trial to interpretate its role in protecting the eye ball (WETTERBERG, GELLER and YAWILER, 1970) as well as its involvement in reproduction (THIESSEN, GLANCY and GOODWIN, 1976 and PAYNE, 1977).

Similar to what was observed in camel by FAHMY, SHAHIEN and KANDIL (1979) the Harderian gland of one humped camel was represented by large and well developed elongated pear shaped structure situated, ventrally within the orbit, postero-medial to the eye ball.

The Harderian gland of the one-humped camel was of the compound tubulo-alveolar variety. KUHNEL (1974) in pigs, FAHMY, SHAHIEN and KANDIL (1979) in camels and SAKAI and YOHRO (1981) in mongolian gerbils, described the Harderian gland to be of the tubulo-acinar variety. On the other hand JOHNSTON, McGADEY, THOMPSON, MOORE and PAYNE (1983) and JOHNSTON, McGADEY, THOMPSON, MOORE, BREED and PAYNE (1985) mentioned that the Harderian gland is of the tubular variety in both Mongolian gerbils and mice; respectively.

Concerning the staining affinity of the glandular cells of the Harderian gland of one humped camel, light and dark cells could be recognized in the present investigation. In this regard the Harderian gland of the one-humped camel exhibited a sex difference, the proportion of light cells was relatively more abundant in she-camels.

Several light microscopic studies have described two cell types in the glandular epithelium of the mammalian Harderian gland (KANWAR, 1960; MULLER, 1969 a,b; BROWNSCHIEDLE and NIEWENHUIS, 1978). MULLER (1969 a) reported the existence of two secretory cells types which he designated A and B cells. The A cells contained irregularly shaped large cytoplasmic vacuoles, where as B cells were characterized by the presence of many cytoplasmic vesicles.

Ultrastructural investigations of the Hamster's Harderian gland have demonstrated two types of cells, dark cells with numerous ribosomes, mitochondria and large vacuoles than those of the light cells. BROWNSCHIEDLE and NIEWENHUIS (1978) suggested that the dark cells have more secretory activity than the light cells.

The present investigation revealed myoepithelial cells around the glandular end-pieces. The myoepithelial cells were also demonstrated in the Harderian gland of various mammals (KUHNEL, 1974; FAHMY, SHAHIEN and KANDIL, 1979; SAKAI and YOHRO, 1981; JOHNSTON, McGADY, THOMPSON, MOORE, BREED and PAYNE, 1985). The physiological significance of myoepithelial cells in the Harderian gland is that they are contractile elements, facilitating extrusion of the secretory products.

The finding of the present study, from the morphological stand point, may play a significant immunological role of the Harderian gland in the one humped camel.

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In addition to the occurrence of the three types of lymphoid cells viz. macrophages, lymphocytes, plasma cells; organized lymphatic nodules resembling the splenic corpuscles or the aggregated lymphatic nodules in subepithelial layer of conjunctival membrane also indicated a direct participation of this gland in the immune response. Moreover, the morphological study of the Harderian gland of the one-humped camel revealed that this gland may be component of the peripheral lymphoid system.

The exposed surface of this gland appeared to have in the immune responses. BURNS (1977) discussed this possibility in the fowl.

Regarding the histochemical characteristics of the Harderian gland of the one-humped camel, the present investigation revealed neutral and or acid mucopolysaccharide materials within both the glandular cells and the cells of the duct system. SHAHIEN and EL MOUGY (1975) described PAS-Positive material within the epithelium and lumina of the ducts of the Harderian glands of the camel. The latter authors added that this material are infrequent within the acinar epithelial cells.

The present study supports that the Harderian glands of one humped camel produce mucus containing both neutral and acid mucopolysaccharides. It seems important to note that this mucus is distributed as a distinct layer on the surface of nictitating membrane as well as the conjunctiva.

Here, it probably functions to prevent drying of the peculiarly hairy conjunctiva of the camel and eventually opposes the proliferation of pathogenic micro-organisms (JARRETT, 1980).

The present investigation revealed various sudanophilic materials within the cells of both the glandular end pieces; the intra and interlobular ducts. SHAHIEN and EL-MOUGY (1975) mentioned that there was neither osmiophilic lipid and sudan black material within the acinar epithelium nor the ducts of the Harderian glands of the camel. Several authors described various lipid granules within the cells of both the end-pieces and ducts of the Harderian gland of several mammals (WOODHOUSE and RHODIN, 1963, HOFFMAN, 1971; JOST, KUHNEL, SCHIMASSEK, 1974 and WOODING, 1980) considering that the Harderian gland secretes its lipid into the conjunctival sac and thereby moistens the cornea.

All these properties could be as great importance for the protection of the eye of the camel specially during the adversed climatic conditions.

Ontological studies typified the secretion of the Harderian gland into different vertebrates of five categories namely: mucous (avians and cattle), serous (reptiles and pigs), seromucous (reptiles), mixed (armadille), and lipoidal (mammals), (PRINCE, DIESEM, EGLITIS and RUSKILL, 1960; BANG and BAND, 1968; WIGHT, BURNS, ROTHWELL and MACKENZIE, 1971 and BROBBY, 1972). As a result of the present study it is suggested that the Harderian gland of the camel produces a lipoidal mucopolysaccharide secretion.

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SUMMARY