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DEVELOPMENTAL STUDIES ON THE FOETAL HARDERIAN GLANDS OF THE ONE-HUMPED CAMEL
(Camelus dromedarius)

(With 1 Table & One Plate and 13 Figures)

By

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دراسات عن تطور غدد الهارديريان في أجنة الجمل وحيد السنام

رمضان عبدالحفيظ ، عزيزة عبدالعزيز ، جمال كامل ، محمد رشاد فتح الباب

قام الباحثون بدراسة تطور غدد الهارديريان في أربع وتسعون جنينا من الجمال وحيد السنام ووجد أن بشائر غدة الهارديريان قد ظهرت في الأجنة التى بلغت أطوالها ٦٠ مليميتر على هيئة تجمع خلوى في الزاوية بين بدائيات الغشاء الراش والطبقة الليفية للعين . بدأت النهايات المفرزة للغدة في الظهور عندما بلغ طول الأجنة ٥٢٠ مليمتراً . كما تميزت الخلايا المفرزة إلى نوعين فاتح وغامق في نهاية مدة الحمل . فاق الحجم النسبى المئوى للجزء الخلوى لغدة الهارديريان في الذكور مثيلة في الإناث ، كما أن معدل نمو الجزء الخلوى أسرع في الذكور عنه في الإناث .

SUMMARY

Ninety-four foetal harderian glands of one-humped camel (60-1350 mm. CVR length) were used in the present study. The primordium of the gland was first observed in foetuses of 60 mm. CVR length, and was represented by a cellular proliferation of the conjunctival bulbar epithlium at the angle between the primordria of the fibrous coat and the nictitating membrane of the eye. At 150 mm CVR length, the primordium was represented by four canalized tubular structures constituting the anlage of the duct system of this gland. The primary elements of the glandular end-pieces were demonstrated in foetuses of 520 mm. CVR length as various cellular proliferations from the duct analges. The rate of proliferation of the end-pieces was greatly enhanced in foetuses of 1030 mm CVR length and reached its maximal potency in foetuses of 1150 mm CVR length. In full-term foetuses the glandular cells were differentiated into few dark and abundant light cells. The rate of growth and the volume percentage of the parenchyma were relatively larger in male than in the female foetuses.

INTRODUCTION

The micromorphological features and the histochemical characteristics of the Harderian gland in different animal species have been described by several authors BJÖRKMAN, NICANDER and SCHANTZ, 1960; KANWAR, 1960 HOFFMAN, 1971; FAHMY; SHAHIEN and KANDIL, 1979 and SAKAI, 1981).

Discerning between Harderian and nictitating gland in adult animals is tedious to ascertain. This might be due to the apparent omission of investigating the developmental events of both glands. A matter which raised the question about Harderian gland-nictitating gland conflict.

Moreover, the compatability between the different animal species and birds and even within the same animal species, concerning the sex differences in the Harderian gland, was a motivation to investigate first the Harderian gland of the one-humped camel during the intrauterine life.

MATERIAL and METHODS

94 one humped camel foetuses of both sexes ranging from 60 mm to 1350 mm CVR length were freshly collected from Cairo and Bani Adi slaughter houses. The foetuses were weighed to the nearst gram (Table 1).

The Harderian glands were taken, the average length, width, thickness and weight of the glands of 25 foetuses were recorded. Materials were either fixed in calcium formol, carnoy's fluid and or Bouin's fluid. After proper fixation, the materials were dehydrated, cleared and embedded in paraffin wax. Step serial sagital, frontal and transverse sections were cut at about 5-7 um, for general histomorphological studies the following stains were adopted: Harris's haematoxylin and eosin (HARRIS, 1898); Crossman's modification (CROSSMAN, 1937), Weigert's Elastica stain (WEIGERT, 1898). For histochemical studies the following methods were employed; Periodic acid Schiff (PAS) technique (McMANUS and MOWRY, 1960); Alcian blue (pH 2.5) McMANUS and MOWRY, 1960); Alcian blue-PAS technique (McMANUS and MOWRY, 1960); Sudan black stain (LISON and DAGNELIE, 1935).

Paraffin sections 5-7 um thickness stained with haematoxylin and eosin of 28 glands of foetuses 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 glandular cells/end-piece, height of the glandular epithelium and nuclear volume of the glandular epithelium in the glands of 16 foetuses. 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).

Table 1: Materials available in the present study during the prenatal period.

Foetus No.	Crown-to Rump (CVR) length (mm)	Sex	Foetus No.	Crown-toRump (CVR) length (mm)	Sex
1	60	•	26	320	M
2	60	•	27	350	F
3	75	•	28	360	M
4	90	•	29	360	F
5	100	•	30	370	F
6	115	•	31	370	F
7	125	•	32	370	F
8	150	•	33	380	M
9	150	•	34	380	M
10	170	M	35	400	M
11	200	F	36	400	F
12	210	F	37	400	F
13	230	M	38	410	M
14	230	M	39	420	F
15	240	F	40	420	M
16	240	F	41	430	M
17	240	F	42	430	M
18	250	M	43	440	F
19	265	F	44	450	M
20	280	F	45	450	F
21	290	F	46	450	F
22	300	M	47	460	M
23	300	F	48	500	F
24	300	M	49	510	F
25	310	M	50	510	M
51	520	F	73	800	I
52	520	M	74	800	M
53	520	M	75	800	F
54	530	M	76	810	M
55	540	F	77	820	F
56	550	M	78	820	F
57	560	M	79	840	F
58	590	F	80	840	M
59	600	M	81	860	M
60	600	M	82	880	F
61	620	M	83	930	F
62	630	M	84	980	F
63	640	M	85	980	M
64	650	M	86	1010	M
65	680	M	87	1020	M
66	700	F	88	1030	F
67	720	F	89	1040	F
68	720	F	90	1100	M
69	730	M	91	1150	F
70	740	M	92	1170	F
71	750	F	93	1170	M
72	800	M	94	1350	F

• Sex could not be determind.

M = Male.

F = Female.

RESULTS

The primordium of the Harderian gland was first observed in foetuses as early as 60 mm CVR length. This primordium was represented by a cellular proliferation of the conjunctival bulbar epithelium at the angle between the primordium of the nictitating membrane and the primordium of the fibrous coat of the eye (plate 1 and Fig. 1). This proliferation was formed of about 3-4 layers of Epithelial cells which exhibited basophilic cytoplasm and large deeply-stained nuclei. The mesenchymal connective tissue cells below the previously mentioned epithelial proliferation were densely arranged.

At 100-125 mm CVR length, the epithelial portion of the primordium of the Harderian gland was represented by a single pedunculated cellular mass which showed several peripheral small out growths (Fig. 2). The cells within this mass were orientated into two distinguished regions. The peripherally situated cells were large in size and had large deeply stained nuclei. However, the centrally located cells harboured smaller lightly stained nuclei. Most of these nuclei were demonstrated undergoing several retrogressive changes leaving various cavities indicating the commencement of canalization within the cell mass (Fig. 3). At 150-310 mm CVR length the primordium of the Harderian gland was represented by four canalized tubular structures constituting the analge of the duct system of this gland. The cells of the duct analge were polyhedral in shape and arranged into two rows. Their cytoplasm was basophilic and their nuclei were oval in shape. The connective tissue portion of the primordium of the Harderian gland was represented by a densely arranged cellular formation which embrassed the previously described epithelial structures. A small cartilagenous plate was differentiated ventrally within the peritubular connective tissue (Fig. 4).

In foetuses of 320-420 mm CVR length, the epithelial primordium of the Harderian gland presented four glandular units of variable developmental rates which demonstrated various degrees of proliferation at their terminal portion (Fig. 5 a,b). several (3-4) cartilagenous plates of different sizes were demonstrated within the inter-tubular connective tissue.

The nictitating gland originated as pigmented epithelial cord-like prolfiration of the conjunctival epithelium at the angle between the nictitating membrane and the palpebral conjunctival surface of 320-360 mm CVR on camel foetuses (Fig. 6). The primordium extended deeply into the underlying connective tissue and occupied a superficial position to the Harderian gland.

In foetuses of 430-510 mm CVR length, the primitive duct system of the developing Harderian gland reached, qualitatively a peculiar developmental stage. The ducts demonstrated several branches which possessed large irregular lumen (Fig. 7). They were lined by 2-3 cell layers. The basal cells were irregularly polyhedral in shape with distinct boundaries and large spheroidal, vesicular centrally located nuclei. The luminar layer was formed of columnar cells with clear boundaries and large, vesicular, ovoid, basally-situated nuclei. Near the openings, onto the bulbar conjunctival epithelium, the ducts were lined by 3-4 layers of polyhedral cells.

The epithelium of these ducts demonstrated several proliferative potencies at lateral and terminal sites (Fig. 8 a). The proliferative epithelial tissue was represented

by masses, clumps, cords or tubular formations. The cells of the latter strucutres were polygonal in shape and have slightly basophilic cytoplasm. Their nuclei were large, rounded vesicular and centrally located (Fig. 8 b).

The primary elements of the glandular end-pieces were demonstrated in camel foetuses of 520 mm CVR length as various proliferations from the duct anlages. Some end-pieces were differentiated and surrounded by the primary elements of the myoepithelial cells. The end-pieces were lined by pyramidal cells with truncated apices. The cell boundaries were distinct and some cells beared acidophilic apical brush border. The cytoplasm was vacuolated or contained fine acidophilic granules. The nuclei appeared vesicular large, oval or rounded in shape and basally situated.

On reaching 980-1010 mm CVR length, the amount of proliferated end pieces increased especially at the periphery of each lobule. The rate of differentiation of the end-pieces was relatively high and the amount of the vacuolated glandular cells was more than the previously described age.

The rate of proliferation of the end pieces was greatly enhanced at the periphery of the glandular lobules in foetuses of 1030 mm CVR length and reached its maximal potency when the foetuses reached a length of 1150 mm.

In full-term foetuses (1170-1350 mm CVR length) the glandular cells were differentiated into two types namely; dark and light cells. The dark cells were relatively few in amount and demonstrated coarse acidophilic granules within their cytoplasm. However, the light cells were relatively, abundant and exhibited a less granular lightly-stained cytoplasm (Fig. 9). The volume percentage of the cartilagenous plates reached its maximum in foetuses of 720-1020 mm CVR length then decreased gradually towards the end of gestation where it reached its minimum in full-term foetuses.

In foetuses of 320 mm CVR length some lymphocytes started to appear accumulating around some capillaries within the connective tissue below the bulbar conjunctival epithelium. The lymphatic tissue increased towards the end of pregnancy where it was about three folds as large as the detected in 980 mm CVR length foetuses.

The connective tissue cells began to differentiate into some lipoblasts scattering in between the glandular end-pieces in foetuses of 450 mm CVR length.

The volume percentage of the adipose tissue demonstrated a gradual increment in both sexes towards the end of gestation and reached its maximal amount in full-term foetuses (Fig. 12).

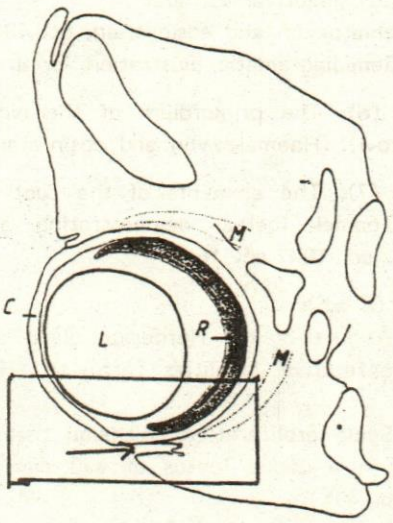
Qantitative analyses of the different structural constituents of the foetal Harderian gland revealed that the volume percentage of the parenchyma was relatively higher in male than in female foetuses. In both sexes the percentage of the parenchyma increased progressively toward the end of the gestation period. The rate of growth of the parenchyma was relatively faster in male than in female foetuses (Fig. 10). Statistical analyses revealed that the volume percentage of the connective tissue, in both male and female foetuses decreased gradually towards the last phases of gestation

where it reached about 17% of that observed at the first trimester of parturition (Fig. 11). On the other hand the volume percentage of the cartilagenous plates increased grandually till it reached its maximum in foetuses 720-1020 mm CVR length then decreased gradually towards the end of gestation where it reached its minimum in full term foetuses. Qantitative statistical analyses revealed that the diameter of the glandular end-pieces fluctuated between 33.30 and 42.36 um and demonstrated no significant sex-or age-related changes. On the other hand the number of the granular cells/end-piece increased gradually towards the mid-gestation period where they were about 14 cells and then decreased towards the end of pregnancy where they were fewer than at the commencement of their differentiation. The height of the glandular epithelium demonstrated a gradual increment towards the end of gestation. However, the nuclear volume demonstrated non significnt age-related changes other wise a marked increment at the commencement of the end-pieces differentiation.

Histochemically, the foetal Harderian gland of one-humped camels started to demonstrated PAS-positive granules within their duct anlage at 430 mm CVR length. These granules increases towards the end of gestation. However, the glandular cells of the end-pieces began to demonstrate moderate PAS-positive cytoplasmic granules, as well as PAS/AB postive material in foetuses of 520 mm CVR length. These granules increased gradually towards the end of pregnancy. The cells of both the glandular end-pieces as well as the ducts demonstrated various sudanophilic materials within their cytoplasm (Fig. 13 a,b).

Plate 1:

Semidiagrammatic illustration of the eye of 60 mm CVR long-camel foetus, showing the site of development of the harderian gland (arrow).
C= Cornea. L = Lens.
M= Ocular muscles. R= Retina.
Rectangle = Fig 1. (60 x).



LEGEND OF FIGURES

Fig. (1): Rectangle in Plate 1: Frontal section at the rectangle drawn in plate 1, showing the primordium of the Harderian gland in a camel foetus of 60 mm CVR length (arrow). (Haematoxylin and eosin stain. oc. 10X, ob. 6.3X).

Fig. (2): The primordium of the Harderian gland of a camel foetus at 100 mm CVR length, showing a pedunculated epithelial cellular mass (arrow), and a parallel proliferation into the underlying connective tissue (dotted arrows). (Haematoxylin and eosin stain, oc. 10X, ob. 6.3X).

Fig. (3): The epithelial portion of the Harderian gland of 100 mm CVR-long camel foetus. Showing several cells undergoing degeneration (arrows) and mitosis (dotted arrow) indicating the commoncement of canalization. (Haematoxylin and eosin stain. oc. 10X. ob. 100X).

Fig. (4): The epithelial primordium of the Harderian gland in camel foetus of 150 mm CVR length, showing the anlage of the duct system reprsented by 4 canalized tubular structures (arrows). C: cartilagenous platlet. (Haematoxylin and eosin stain, oc. 10X, ob. 6.3X).

Fig. (5): a- The epithelial primordium of the Harderian gland in camel foetus of 320 mm CVR length, showing 4 glandular units of variable developmental rates.
C= Cartilagenous plate.
B = bulbar conjunctival surface.
(Haematoxylin and eosin stain, oc. 10X, ob. 16).
b- Semidiagramatic illustration for a.

Fig. (6): The primordium of the nictitating gland of 360 mm CVR long-camel foetus (arrow). (Haematoxylin and eosin stain, oc. 10X, ob. 6.3X).

Fig. (7): The elements of the duct system of the Harderian gland of 430 mm CVR-long camel foetus, demonstrating a branching formation. (Haematoxylin and eosin stain, oc. 10X, ob. 16X).

Fig. (8 a,b):
a- A duct of the Harderian gland of 430 mm CVR long camel foetus, showing several proliferative localities (arrows). (Haematoxylin and eosin stain, oc. 10X, ob. 16X).
b- Some proliferative epithelial tissue represented by various masses or tubular formations in a camel foetus of 430 mm CVR length. (Haematoxylin and eosin stain, oc. 10X, ob. 40X).

Fig. (9): Some end-pieces of the Harderian gland of a full-term camel foetus demonstrating few dark cells (arrows) and abundant light cells. (Haematoxylin and eosin stain. oc. 10X, ob. 100X).

Fig. (10): Volume percentage of the parenchyma in the male and the female foetuses in relation to the CVR length.

Fig. (11): Volume percentage of the connective tissue in the male and the female foetuses in relation to the CVR length.

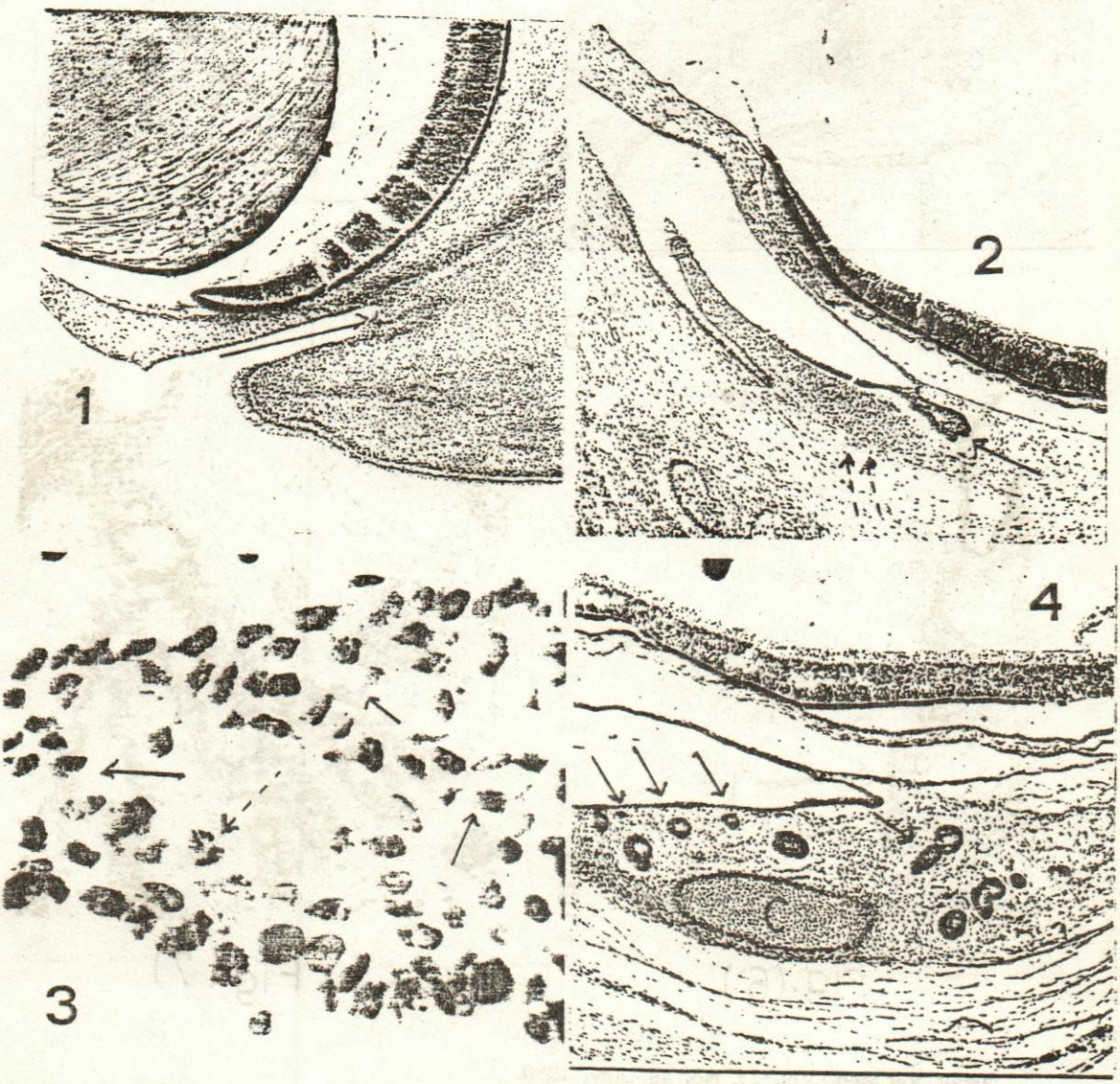
Fig. (12): Volume percentage of the adipose tissue in the foetal Harderian gland in relation to the CVR length.

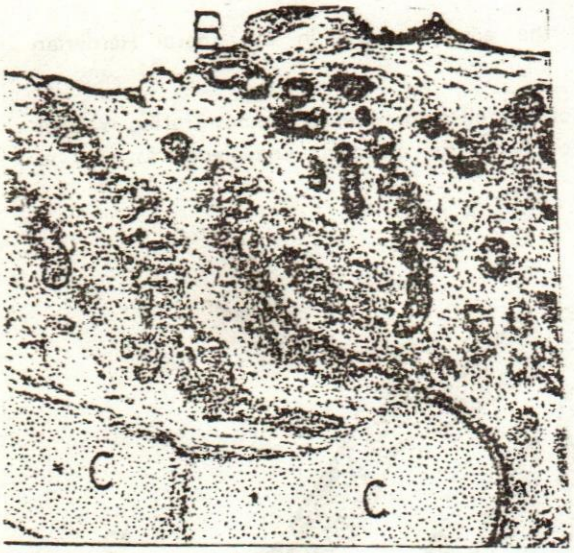
Fig. (13): The Harderian gland of a full-term foetus. Demosntrating various sudanophilic materials within the cells of both the glandular end-pieces as well as the intra and interlobular ducts.

A- Adipocytes. (Sudan black stain)

a- Oc. 10X. ob. 16X.

b- Oc. 10X. ob. 40X.





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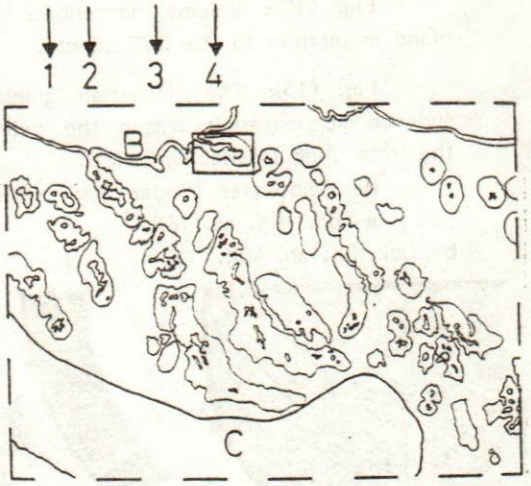

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Fig.(5)

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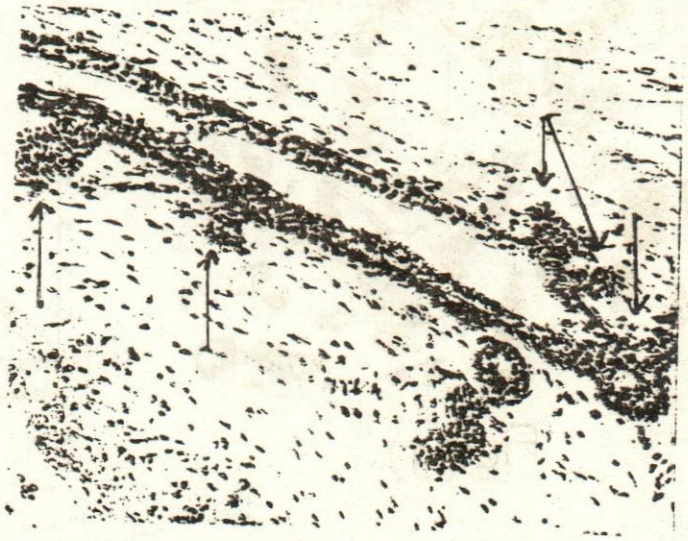

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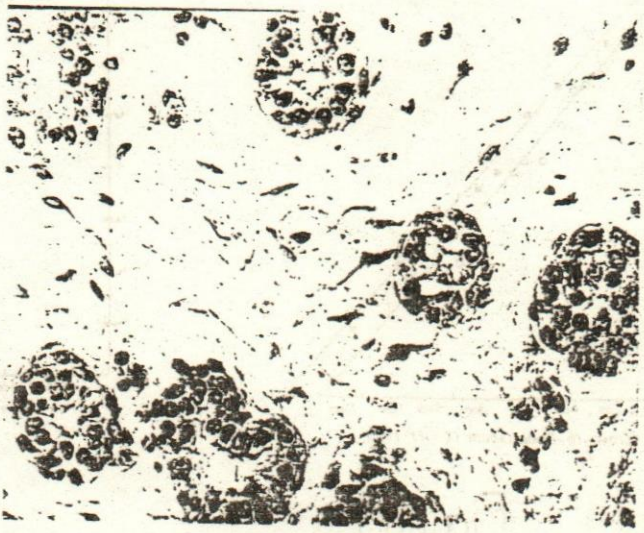
Fig.(8)



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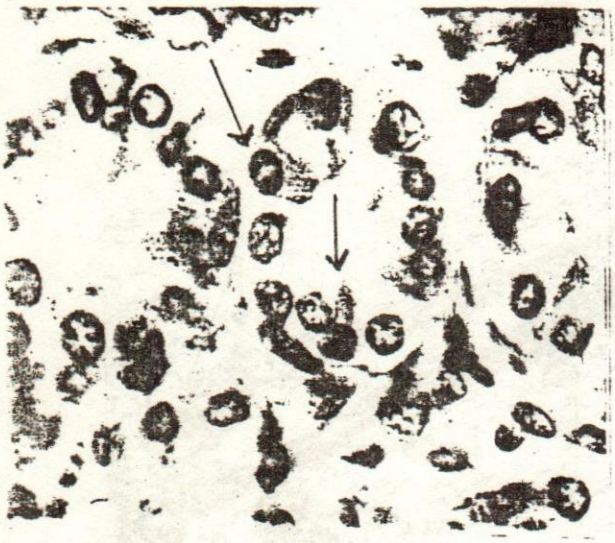

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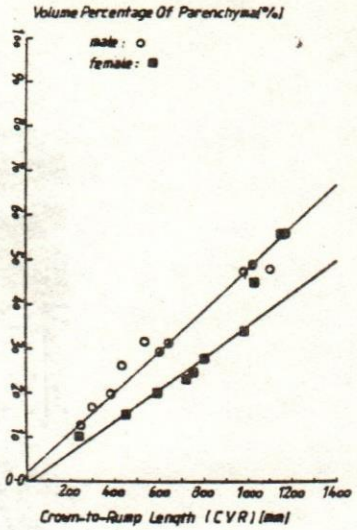

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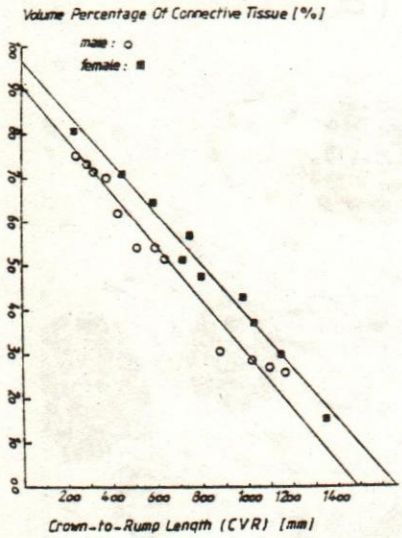

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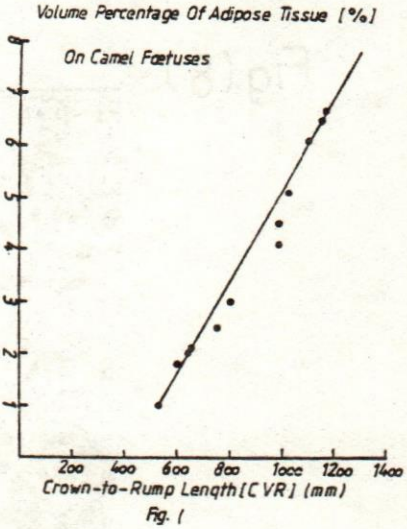

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Fig.(12)

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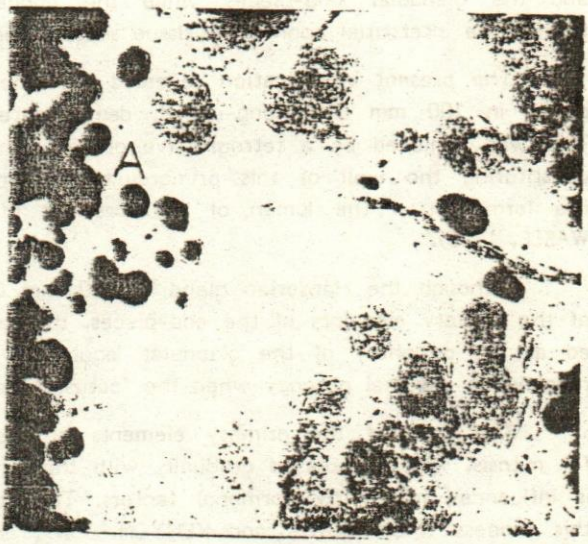

(b)

DISCUSSION

Although the micromorphological features and the histochemical characteristics of the Harderian glands are described by several authors, the questions about sex differences and Harderian-nictitating gland conflict are still encountered.

The developmental studies of the Harderian gland have received no attention from authors who have described this gland in the different animal species. This omission has misled several morphologists to hypothesize that the harderian and nictitating glands are related to one confluent. This in turn is appearently accepted as support for the various interpretations of the morphological changes resulting into several glandular disorders. Investigating the histogenesis of the Harderian gland of the one-humped camel seemed strictly indicated to clarify the questions raised about sexual differences and Harderian-nictitating gland conflict.

The origins of the Harderian gland could be recognized, viz., an epithelial (ectodermal) and a connective tissue (mesodermal) primordia. The present investigation revealed that the ectodermal primordium of the Harderian gland first appeared in camel foetuses as early as 60 mm CVR length. This primordium was represented by a cellular proliferation of the conjunctival bulbar epithelium at the angle between the primordium of the nictitating membrane and the primordium of the fibrous coat of the eye. The synchronous developmental relation between both the Harderian gland and the nictitating membrane proved true in semi and non aquatic mammals (WALLS, 1963 and KENNEDY, 1970). The epithelial (ectodermal) primordium gave rise to both of the duct system and the glandular end-pieces while the connective tissue primordium (mesodermal) formed the interstitial connective tissue septa, capsule, and cartilagenous plates.

The present investigation revealed that the epithelial primordium of the Harderian gland, in 100 mm CVR long-camel, demonstrated the commencement of canalization. This was achieved by a retrogressive process which was demonstrated among the cells constituting the wall of this primordium. This phenomenon was also described during the formation of the lumen of the fore gut of chickens (FATHEL-BAB, YOAKIM and WASEF, 1986).

Although the Harderian gland at 520 mm CVR length showed the first indication of the primary elements of the end-pieces, the rate of proliferation was greatly enhanced at the periphery of the glandular lobules in foetuses of 1030 mm CVR length and reached its maximal potency when the foetuses reached a length of 1150 mm.

The cells of the primary elements of the end-pieces exhibited a high potency for mitosis, which increased gradually with the advancement of age. This mitotic activity is influenced mainly by hormonal factors. Testosterone and Estrogen prove to enhance this process (MONTAGN'A and KENYON. 1949, and AMOROSO and EBLING, 1966). The weight of the foetal Harderian gland of the camel which was reversely related to the age of the foetus might be attributed to the previously noticed marker progressive mitotic potency.

The present study revealed that the volume percentage of the parenchyma was higher in male than in female foetuses. This may be attributed to the anabolic effect of androgens (BULLOUGH and VAN OORDT, 1950; ALLEN, 1957, 1958 and AMOROSO & EBLING, 1966).

The present finding revealed that the primary elements of the nictitating gland originated as pigmented epithelial cord-like proliferation of the conjunctival epithelium at the angle between the nictitating membrane and the palpebral conjunctival surface of 320-360 mm CVR-long camel foetuses. As it occupied a superficial position to the Harderian gland, the term superficial gland of the nictitating membrane could be reliable. This indicates the diversity of origin of both the Harderian and the nictitating glands not only regarding the site but also the stage of development.

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