Histological And Ultrastructural Study On The Meibomian Gland Of Camel (*Camelus dromedarius*)

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ABSTRACT

The health and integrity of the ocular surface is dependent mainly on the meibomian glands (MGs). The MGs are lipid producing holocrine glands embedded within the tarsal plate of both the upper and lower eyelids in most animals. It is well known that the camel's eyelids are devoid of MGs. Our goals of this study is to reveal the existence of MGs in the mature male camel and try to describe its structural and ultrastructural features and correlate the obtained data with its function. The present study was conducted on 10 MGs of mature male camels. The MGs were characterized, fixed, processed and 5μm paraffin sections were prepared and stained with different stains. Other MGs of $1mm^3$ thick were fixed by Glutaraldehyde and processed for transmission electron microscope. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. Macroscopically, it was an extrapalpebral gland and located in the inferior aspect of the eye with fine hairs on the palpebra tertia surface. Histologically, the MG was simple branched multilobular acinar glands that was surrounded by dense collagenous connective tissue (C.T.)..They were lined with multiple layers lipid-laden foamy meibocytes.Ultrastructurally, four types of acinar cells were observed: basal cells, moderately differentiated, fully-differentiated and degenerated meibocytes. The nuclei were flat in basal cells, discoid in moderately differentiated meibocytes, partially indented from one side by meibomian secretory vesicles(SVs) in fully-differentiated meibocytes and irregular outline with obvious indentation then fragmented in degenerated meibocytes. The moderately differentiated meibocytes were characterized by many electron dense mitochondria, and various forms of smooth endoplasmic reticulum (sER) which appeared as either grid lattice or parallel cisternae.

Conclusion: The mature male camel has MGs. It was an extrapalpebral gland and located in the inferior aspect of the eye. It was a large sebaecous gland. The structural characteristics of MGs might reflect their important physiological role in the tear film secretion and maintenance of healthy and integral ocular surface.

Key Words: meibomian glands, camel, ultrastructural,meibocytes.

Abbreviation

Ab	Alcian blue
C.T.	connective tissue
MG	meibomian gland
MGs	meibomian glands
rER	rough endoplasmic reticulum
sER	smooth endoplasmic reticulum
SVs	secretory vesicles

INTRODUCTION

The MGs are well developed as regular arrays of parallel glands in the tarsal plates of the most mammals eyelids *(1)*, as human *(2-5)*; rabbits *(2, 6)*, cats, goats, sheep, cattle *(6)*, primates, steer *(2)*, adult male albino rat *(7)*; mouse*(8)*, guinea pig *(9)* and chinchillas *(10)*. The sea lions are lacking a distinct tarsus in the eyelids *(11)* and Turtles in their lower eyelid

(12). The lemmings have only a few glands in the lid, supplemented by other extraorbital glands *(13)*. However, it is well known that the MGs are absent in both eyelids of the camel *(14-16)*, Snakes, lacertids and most sea mammals such as seals and cetaceans *(1)*. It is a series of modified sebaceous glands in cat , rabbits, goats, sheep and cattle *(6)*, rat *(17)*, bats *(18)*, human *(5, 19, 20)*. It is responsible for secretion of the outer lipid (oil) layer of the precorneal tear film *(21- 23)*. The MG is consisted of numerous branched acini, each acinus is consisted of a *basal layer* of flattened cells and a mass of rounded cells with vacuolated cytoplasm in the adult male albino rat *(7)*. There are two separate types of cells : undifferentiated basal cells and well differentiated cells of various sizes. The latter have rounded vesicular nuclei in the calf *(24)*. Three types of cells in MGs acini basal, differentiating and degenerating are encountered in rabbit , primate *(2)* and human *(25)*. Its oily secretion spreads as a surfactant over the globe and contributes as an aqueous barrier that remains functional after blinking *(26)* and maintains the vertical column of tears between the upper and lower lids *(20)*. The secretion reduces evaporation by adding a hydrophobic layer to the surface of the tear film *(3, 19, 27)*, preventing dryness and maceration of the skin of the lid margin by tears *(1)*, preventing the tears overflowing to on the lids and ensure their passage towards the *lacus lacrimalis* and the puncta *(28)*, preventing contamination of the tear film by sebum through forming a barrier *(3, 29)*, decreasing the surface tension of the tear film, thus preventing break up *(3)*, preventing an overflow of tears along the palpebral rim *(3,30)*, sealing the lid margins during prolonged closure, providing a barrier against foreign particles, providing some anti-microbial activity *(31)* and providing of a smooth surface for refraction of incoming light rays *(29)* which enhances the protective properties of tear film.

The purpose of this study was to reveal the existence of MGs in the mature male camel and try to describe its structural and ultrastructural features and correlate the obtained data with its function.

MATERIAL AND METHODS

Samples

Ten eyelids and their adnexa of adult healthy male camels were obtained from El Qurain abattoir in Sharkia. The eyelids and their adnexa were carefully examined and dissected to display and photographe the MGs. The palpebra tertia surface of the MGs was photographed by the stereo-microscope.

For light microscopy

Specimens were fixed in 10% buffered neutral formalin, and submitted for histological processing, including stains with H&E , Alcian blue (Ab) (2.5), Weigert's stains , Silver technique *(32)* and Crossmon's trichrome stain *(33)*.

For electron microscopy

Samples of 1 mm^3 thick were fixed in 2.5% glutaraldehyde in phosphate buffer. After hours the tissues were washed thoroughly in the buffer, postfixed in 1 % osmium tetroxide, rinsing with buffer then, dehydrated in ascending grades of ethanol ending with propylene oxide, and embedded in Epoxy resin. Semithin sections were cut on a MT2 Sorvall microtome and stained with toluidine blue. Ultrathin sections were cut on a RMC MT6000-XL ultramicrotome, mounted on copper grids and stained with uranyl acetate and lead citrate. The ready ultrathin sections were examined and photographed at electron Microscope Research Laboratory (EMRL), Faculty of Science, Ain Shams University by a JOEL electron microscope(JEM 1200 EX II) *(34)*.

RESULTS

Macroscopically

The MG was an extrapalpebral well-developed flattened sheet structure that located in the inferior aspect of the eye. It occupied the

area between the eyelids and third eyelid. It had two attached ends; one with third eyelids fascia and the other end was embedded in the nasal side of both upper and lower eyelids toward the medial commissures (Fig.1). It had two surfaces; the smooth whitish palpebral and the irregular slightly greyish palpebra tertia surfaces. The latter surface showed dark melanin pigments mainly towards the both attached ends, several ductal orifices, and associated fine hairs (Fig.2).

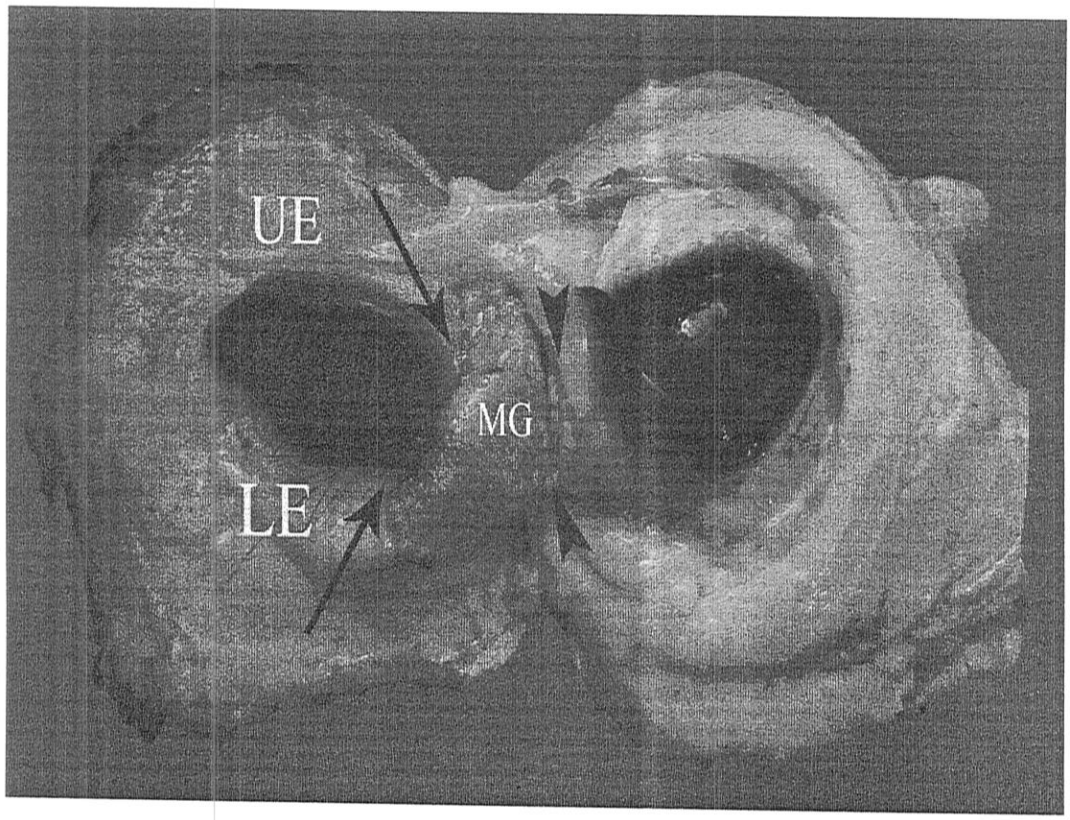

Fig.1. A photograph of back view of dissecting flap of upper "UE" and lower "LE" eyelids to illustrate the meibomian gland "MG".It appeared attached between the nasal side of the eyelids "arrow" and the third eyelid"arrowhead".

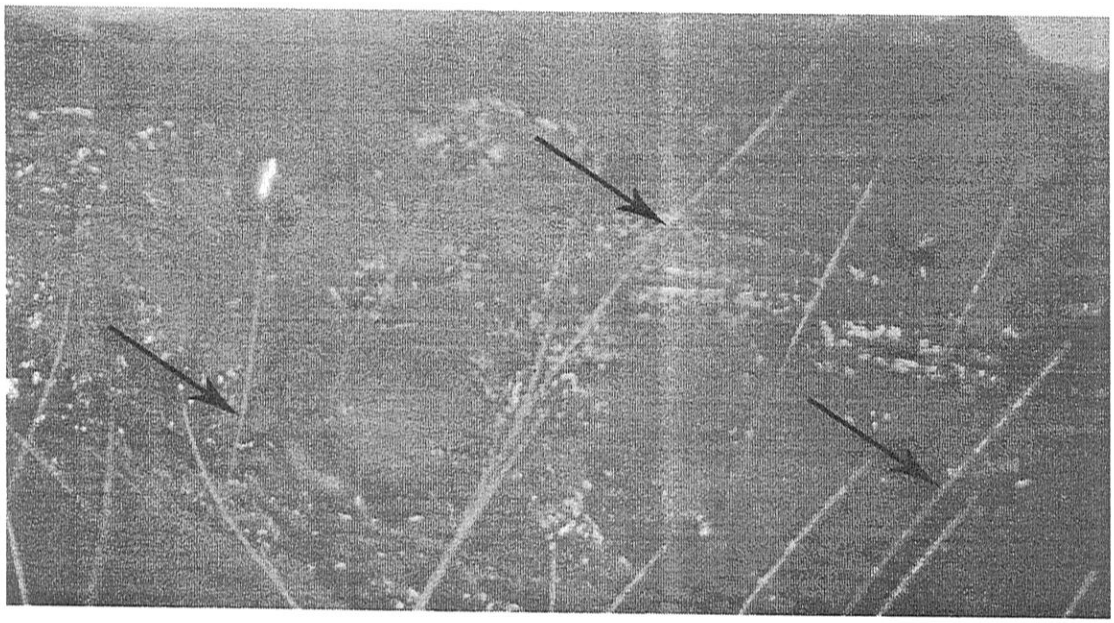

Fig.2. A photograph of the MG *palpebra tertia* surface showing the associated fine hairs "black arrows".

Light microscopic examination

Examination of the MG sections showed that, it was consisted of epithelial elements supported by C.T. elements.The epithelial elements were formed of covering (surface) and glandular epithelium. The latter was large simple branched multilobular acinar glands (Fig. 3). The proximal end of single main duct opened onto the palpebra tertia surface and had numerous fine folds that were arranged concentrically around the orifices.While,the disital end was branched into many ductules towards the saccular acini.. The proximal end of the main duct was lined with stratified cuboidal epithelium with obvious melanin pigments at the basal cell layer (Fig.4A) and stratified squamous epithelium toward the disital end (Fig.4B) .The ductules that directly connected to acinar glands were lined with flat bilayer cells (Fig.4C). Each lobule was clusters of saccular (alveolar) acini .The latter appeared honeycomb-like and lined with four types of cell layers. These cells arranged from basement membrane to the lumen;basal cells with flat nuclei , moderately differentiated meibocytes of polygonal in shape with large discoid basophilic nuclei and eosinophilic cytoplasm , well- differentiated meibocytes of polygonal in shape with slightly basophilic nuclei and pale cytoplasm , the degenerated meibocytes with pyknotic and fragmented nuclei surrounded by pale cytoplasm. The acidophilic secretions were found adjacent to the acinar lumen. The cell boundaries of moderately, well- differentiated and degenerated meibocytes were clear (Fig.5).The acini of MGs were negatively reacted with both PAS (Fig.6A) and Ab (Fig.6B).The interlobular hair follicles were detected (Fig.3, 7) and formed from outside to inside;the C.T. sheath of mainly collagenic fibers, glassy membrane that reacted to Ab (Fig.6B), external and internal root sheath that reacted to PAS (Fig.6A). The covering epithelium was covered only the palpebra tertia surface and formed of stratified cuboidal epithelium with many goblet cells (Fig.8A).The latter was reacted positively with both PAS (Fig.8B) and Ab (Fig.8C). The C.T. elements were found under the covering epithelium and around the glandular epithelium (Fig. 3).The C.T. elements composed primarily of closely packed bundles of collagen fibers (Fig. 9) .The reticular fibers were restricted around the glandular acini (Fig. 10). Very fine dispersed elastic fibers observed especially towards the palpebral surface. Examination of semithin sections showed nuclei of fibroblasts as well as large numbers of oval to fusiform mast cells were noticed in C.T. around acini (Fig. 11).

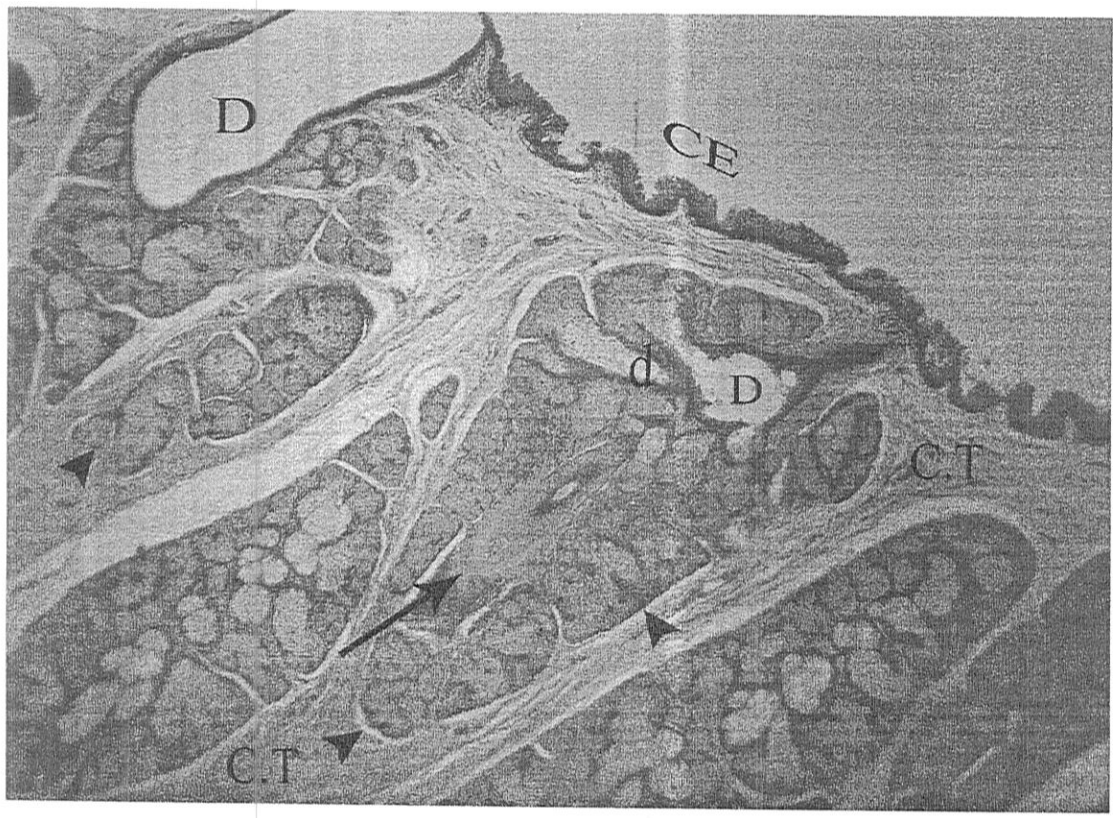

Fig.3.A photomicrograph of meibomian gland section showing covering epithelium"CE", multiple lobules"arrowheads" are connected by means of short ductules "d" to the terminal end of single main duct "D". Hair follicles "arrows" are seen inbetween the lobule. The connective tissue"C.T." are surrounded the lobules.
Stain : H& E Obj. x4 : Oc.x10

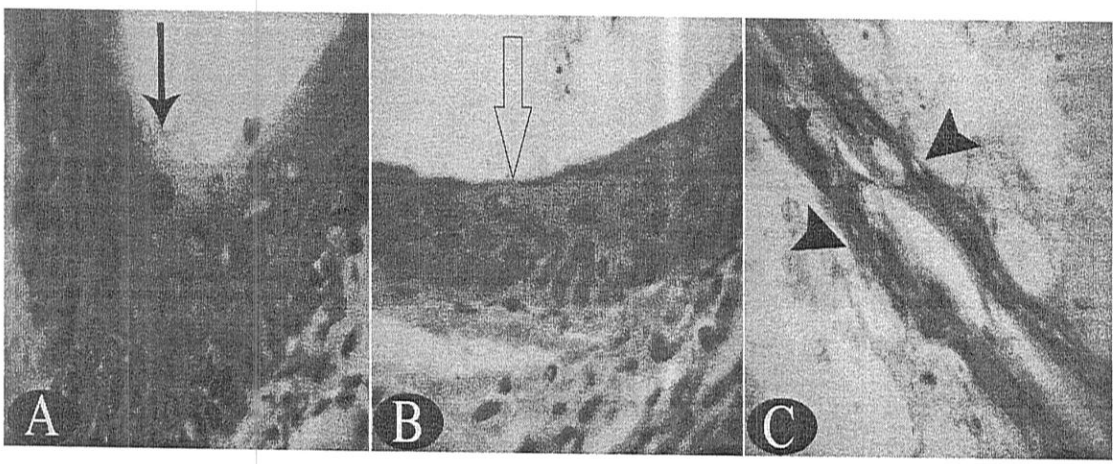

Fig.4.A higher magnification of the previous image showing(A) stratified cuboidal epithelium "arrow" of the main duct proximal end ,(B) stratified squamous epithelium "open arrow"of main duct disital end, (C) flat bilayer cells "arrowheads" of the ductules.
Stain : H& E Obj. x100 : Oc.x10

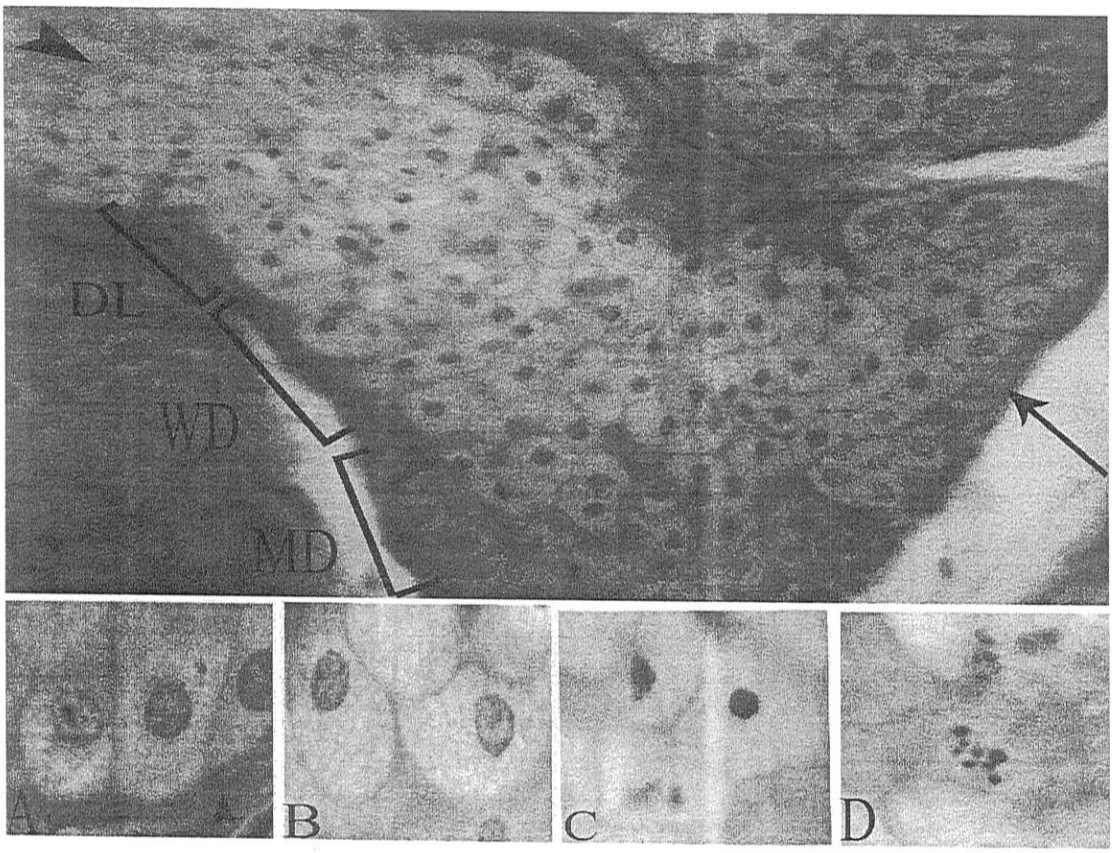

Fig.5. A higher magnification of (Fig.3) showing acini is lined with basal cell layer "arrow", moderately differentiated "MD",well-differentiated" WD" and degenerated cell layers "DL".Note acidophilic secretion"arrowhead" adjacent to the acinar lumen.
Stain : H& E Obj. x40 : Oc.x10
Inset of meibocytes in various differentiated stage; (A) basal cell" arrowhead" and moderately differentiated meibocytes, (B) well-differentiated meibocytes ,(C,D) Degenerated meibocytes with pyknotic (C) then fragmented nuclei (D).
Stain : H& E Obj. x100 : Oc.x10

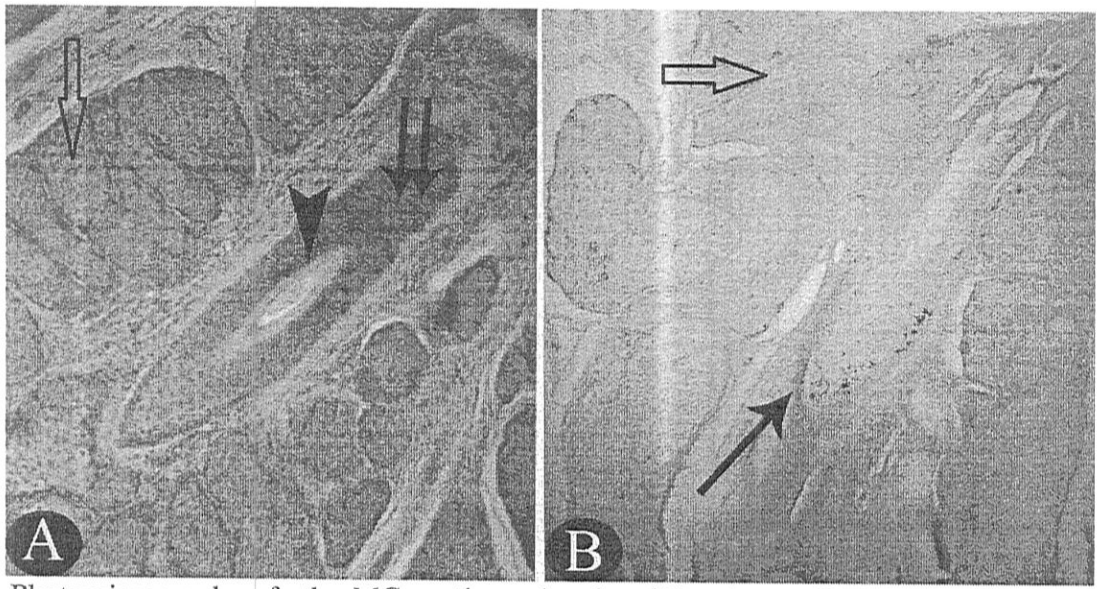

Fig.6. Photomicrographs of the MG sections showing (A) acinar cells are negatively reacted to PAS "open arrow" and positive in external"double arrow" and internal"arrowhead" root sheath of hair follicle.(B) acinar cells are negatively reacted to Ab "open arrow" and positive in the glassy membrane "arrow" of hair follicle.
Stain: (A) PAS (B) AB Obj. x10: Oc.x10

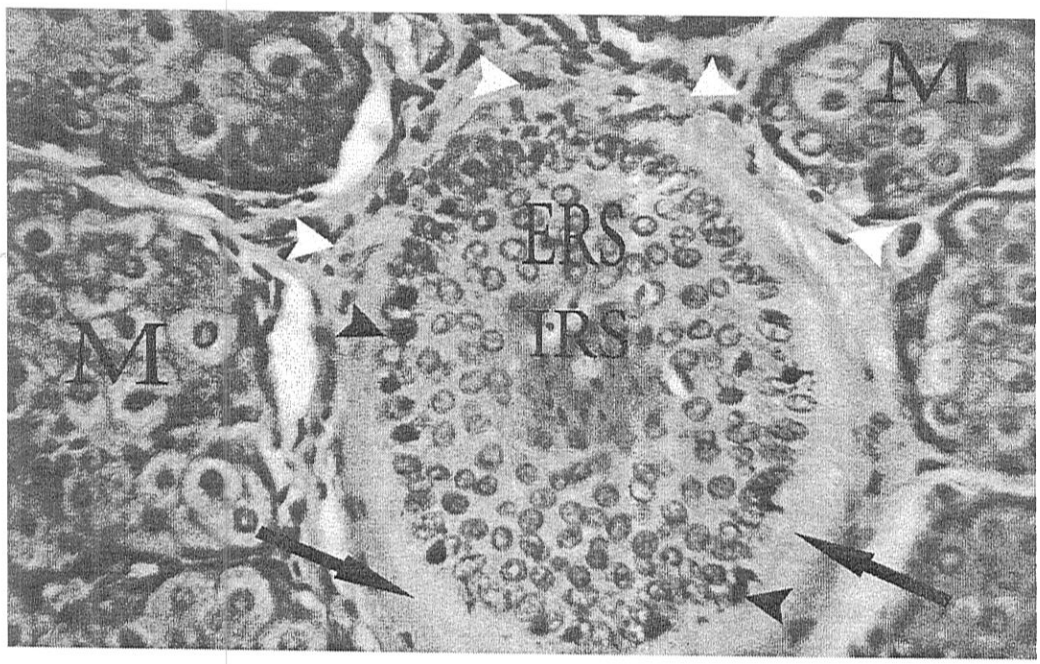

Fig.7. A photomicrograph of the MG section showing transverse section of hair follicle formed of C.T. sheath "white arrowheads", melanin pigment "black arrowheads", glassy membrane"black arrows", external "ERS" and internal"IRS" root sheaths. Note meibocytes "M"of surrounded acini.
Stain : H& E Obj. x40 : Oc.x10

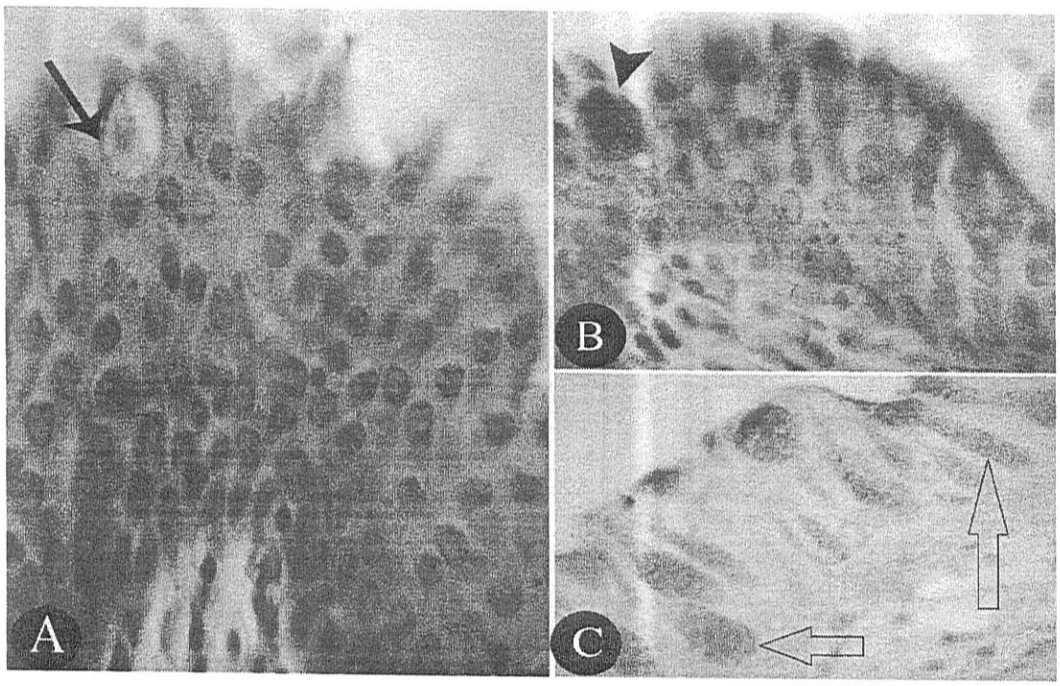

Fig.8. Photomicrographs of the covering epithelium revealing the (A) stratified cuboidal epithelium with goblet cells"arrow". (B) The goblet cells positively reacted with PAS "arrowhead" and (C) Ab "open arrow" .
Stain : (A):H& E (B):PAS (C):Ab Obj. x100 : Oc.x10

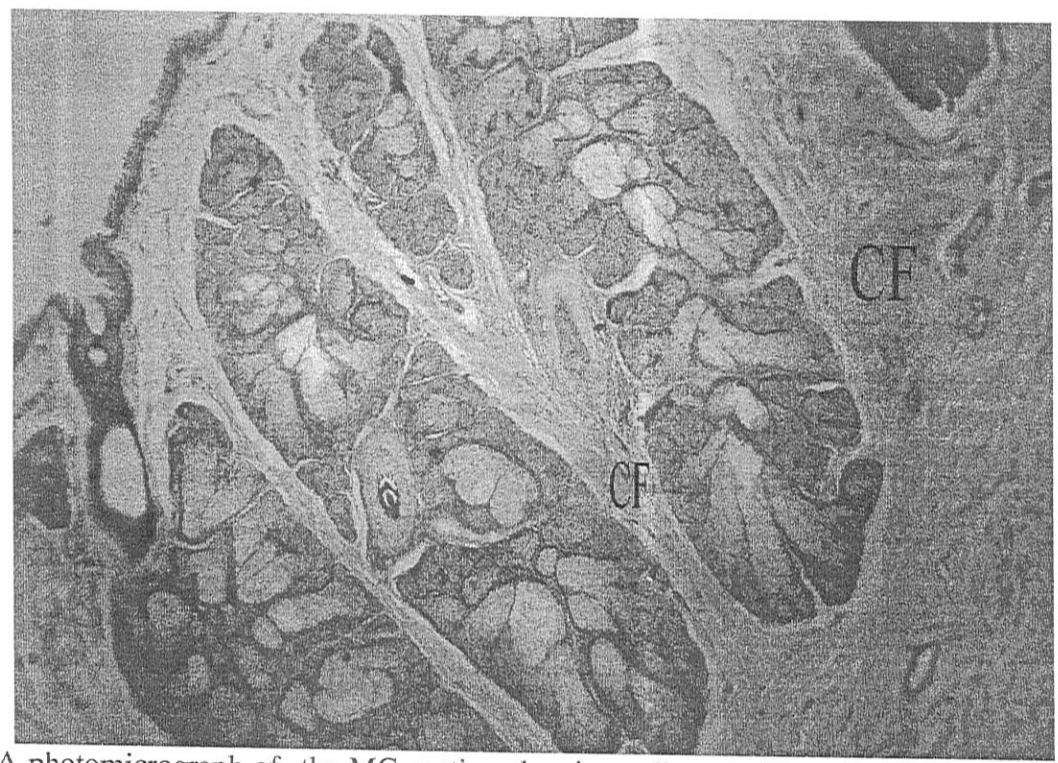

Fig.9. A photomicrograph of the MG section showing collagenic fibers "CF" distribution around the multilobular glands.
Stain : Crossmon's trichrome Obj. x4 : Oc.x10

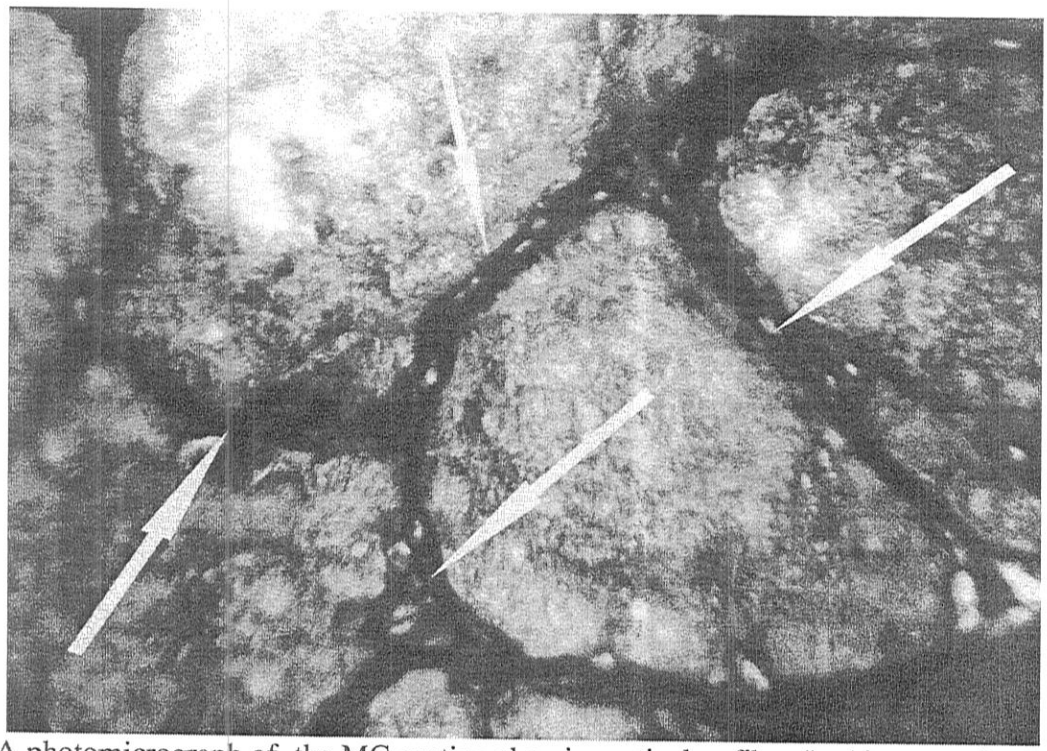

Fig.10. A photomicrograph of the MG section showing reticular fibers" white arrow"surround the glandular acini.
Stain :Silver impregnation technique . Obj. x100 : Oc.x10

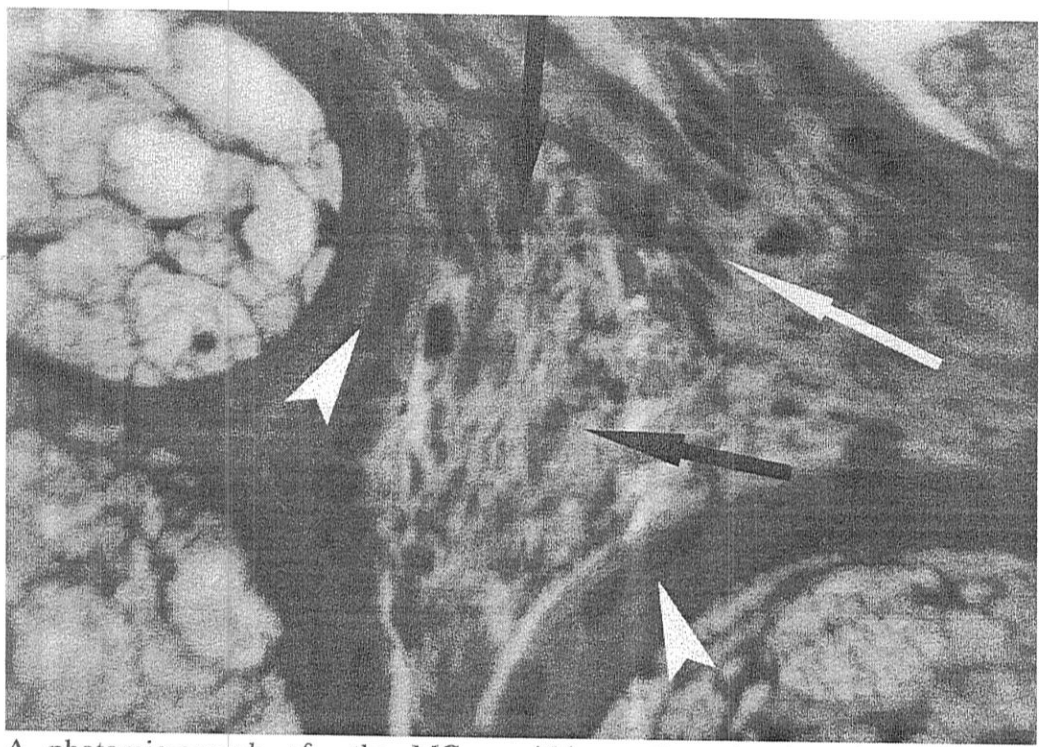

Fig.11. A photomicrograph of the MG semithin section showing the mast cells"black arrow",fibroblast cell "white arrow" and basal cells" white arrowhead".
Stain : Toluidine blue Obj. x100 : Oc.x10

Electron microscopic examination

Examination of utrathin sections revealed four types of glandular lining cells : basal cells , moderately differentiated , well- differentiated and degenerated meibocytes.The basal cells were resting on basement membrane by many hemidesmosomes and connected with the differentiating cells by interdigitations. They were flattened electron dark and contained elongated central nuclei with irregularly clustered chromatin (Fig.12). The moderately differentiated meibocytes were observed inbetween basal cells and well-differentiated meibocytes (Fig.12). They had ovoid to nearly rounded nuclei with islets of heterochromatins.In their cytoplasm,many electron dense mitochondria and moderate number of different sized SVs were appeared intermingled (Fig.13A). Two forms of sER : a grid lattice and parallel cisternae commonly associated with SVs were detected. Both the Golgi complex and rough endoplasmic reticulum (rER) were hardly seen (Fig.13B). Closely interdigitations were seen between the moderately differentiated meibocytes and neighboring cells of well- differentiated meibocytes (Fig.12).The well- differentiated meibocyte had central round electron pale nucleus that partially indented from one side by SVs.The latter was numerous , varied in size and surrounded by a disorganized cytoplasm in which many small dense bodies of lysosomes were prominent (Fig.14A). The degenerated meibocyte had irregular outline nucleus that completely indented with SVs.The latter was markedly increase in size , number and fused with each other.lysosome were observed in the obvious disorganized cytoplasm(Fig.14B). A sparse melanocytes with electron dense membrane-bounded granules were extended into the interstices between meibocytes were noticed (Fig.15).

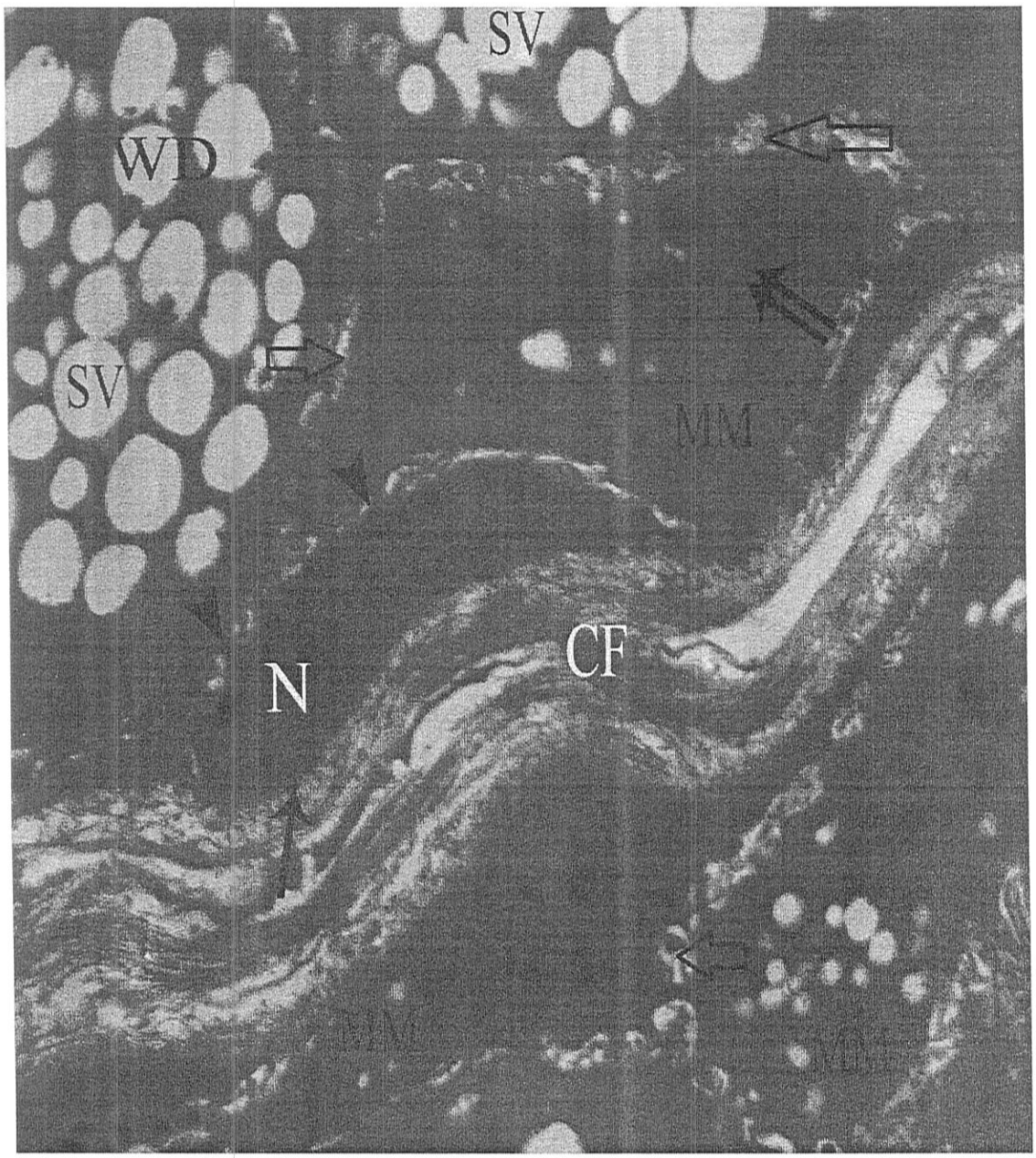

Fig.12. An electron micrograph showing three types of meibocytes;*basal cell* with elongated central nucleus "N" and is resting on basement membrane by many hemidesmosomes "arrow" and connected with the differentiating cells by interdigitations "arrowheads . The *moderately differentiated meibocytes* "MM" with many electron dense mitochondria "double arrows".The *well-differentiated meibocytes* "WM" with many secretory vesicles "SV". Closely interdigitations are seen between the moderately and well-differentiated meibocytes "open arrows". Note collagen fibers"CF" inbetween acini..
Print Mag. X 8100 : Direct Mag.x 1500

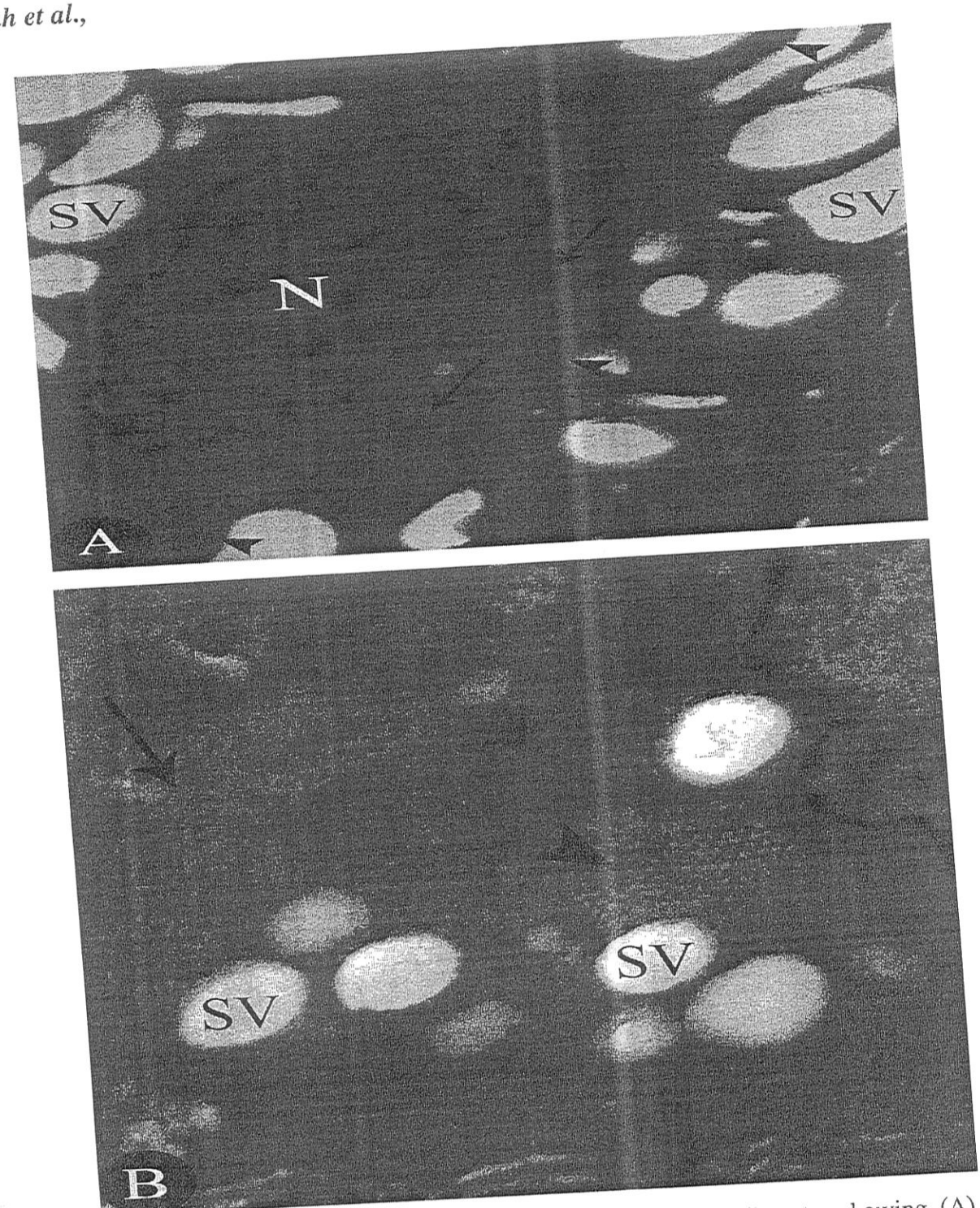

Fig. 13. An electron micrographs of the moderately differentiated meibocyte showing (A) ovoid nucleus "N", many electron dense mitochondria"arrows" and moderate number of different sized secretory vesicles "SV" .some secretory vesicles intermingled with mitochondria "arrowheads". (B) Two forms of smooth endoplasmic reticulum : a grid lattice "arrowhead" and parallel cisternae " arrow" commonly associated with secretory vesicles "SV" were seen.
(A) Print Mag. X 16200 : Direct Mag.x 3000
(B) Print Mag. X 32400 : Direct Mag.x 6000

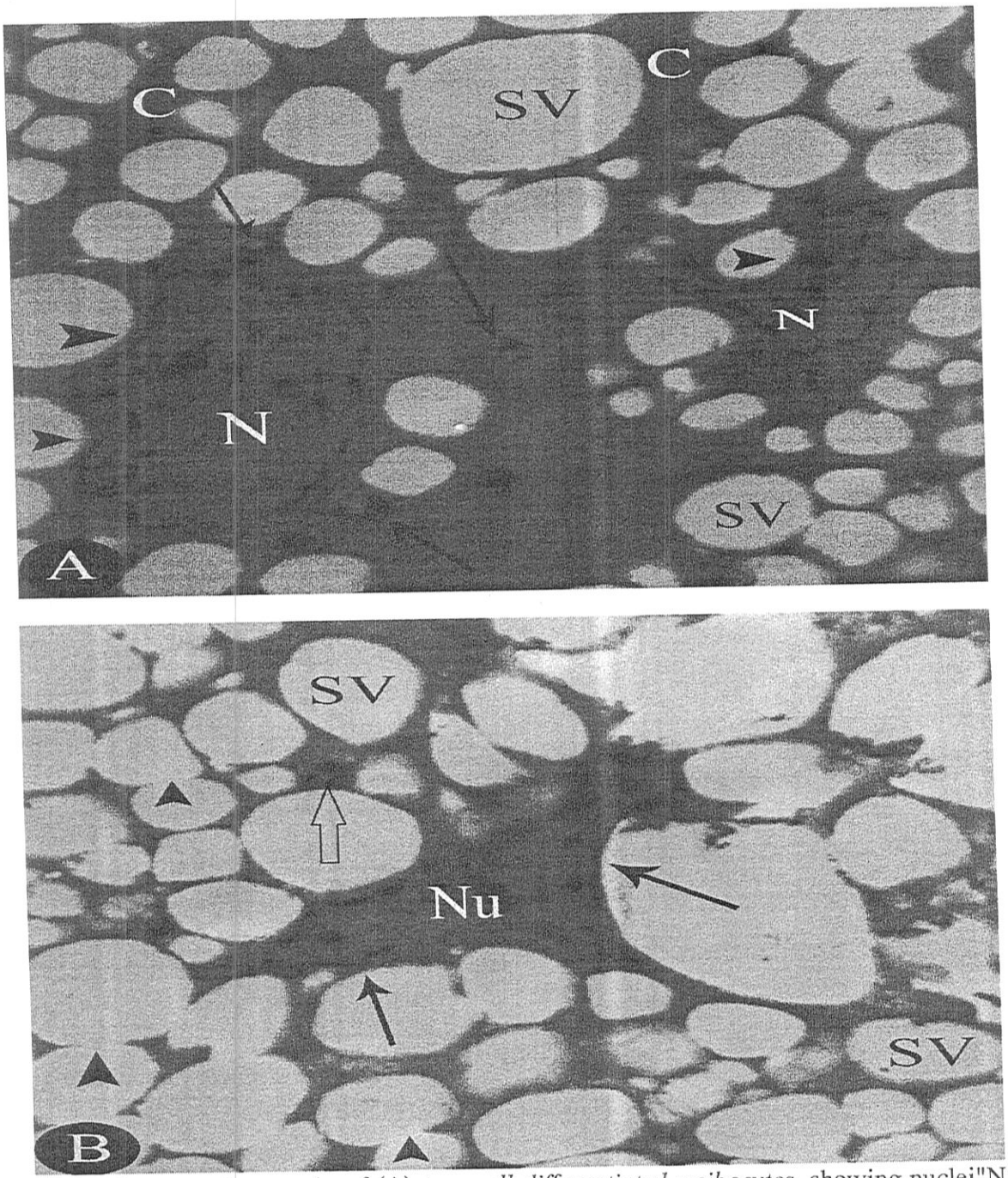

Fig.14. An electron micrographs of (A) *two well-differentiated meibocytes* showing nuclei"N" are partially indented by secretory vesicles "arrowheads" . The secretory vesicles "SV"are numerous , varied in size and surrounded by a disorganized cytoplasm"C" that housing prominent lysosomes"arrows".(B) *Degenerated meibocytes* showing nucleus "Nu" are completely indented with multiple secretory vesicles" arrows".The secretory vesicles"SV" are completely filled the cytoplasm"C" and fused with each other"arrowheads".Lysosome is observed"open arrow".
Print Mag. X 10800 : Direct Mag.x 2000

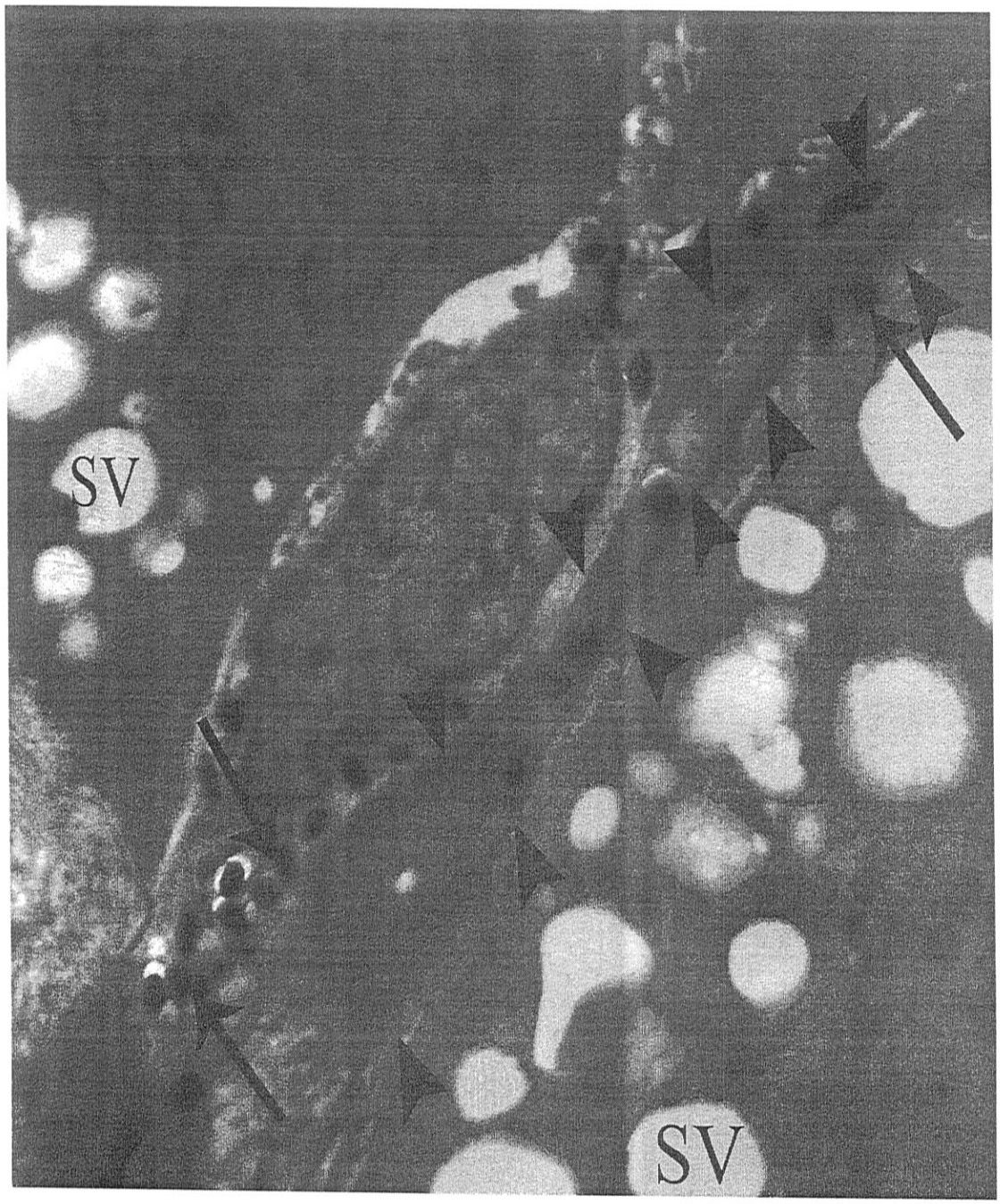

Fig.15. An electron micrograph of the interstices between meibocytes showing the melanin granules "arrows" and melanocyte processe"arrowheads" .The secretory vesicles "SV" of meibocytes were seen.
Print Mag. X 13500 : Direct Mag.x 2500

DISCUSSION

In mature male camel, morphology and position of the MG was different from other animals. The position variations of the MG were thought to be desirable in order to life nature of the camel (environmental desert habits). An apparent discrepancy between our results of the MG as it was an extrapalpebral well-developed flattened sheet structure that located in the inferior aspect of the eye and it has been reported absence of MGs in camel *(14-16)* . It is well known that, the MGs extend equally along the entire length of both eyelids with visible orifices in the lid margin as described in voles and lemmings *(13)*,rats *(7,35)*,primates , steer, rabbit, and human *(2)* ,bats *(18)* and guinea pigs *(9)*. In chinchillas, it was temporal broadened glandular complex *(10)* and in some subfamilies of microtinae and lemmings have only a few glands in the lid, supplemented by other extraorbital glands delivering their secretion at the temporal canthus *(13)*.

The investigated MG was associated with the fine hair. Results are differ from that reported in Egyptian Water Buffalo *(36)* and human *(37)* where MG are not associated with hair follicles and are embedded within the tarsal plate of both eyelids. We depicted the presence of hair to remove and prevent dust particles from entering the *palpebra tertia* surface that contained opening of ducts to avoid their closure .

The present study revealed that the MGs of the camels were simple branched acinar glands and were surrounded within dense collagenous C.T. These features were in agreement with several investigations *(6)* in rabbits, cats, goats, sheep and cattle; *(7)* in adult male albino rat; *(18)* in bats and in human *(5 , 19, 20)*.

The examined MGs were characterized by a single main duct .The disital end of the duct branched into multiple ductules connecting to alveolar acini. These findings are endorsed by the described results in steer *(2)*, and mouse *(8)*. On the contrary, acini were connected by means of short ductules along the length of the main duct, as in the primate *(2)*, rabbit *(2, 38)* and human *(5, 2, 19, 29)*.

Regarding the mode of camel MGs secretion and presence of four types of cells lining its MGs: basal cells, moderately differentiated, well- differentiated and degenerated meibocytes suggesting that the MG in camel was a holocrine gland, in which the cell ruptures and both the cell and its contents are released. This results was similar to that obtained in human *(3, 39, 40)*. The acidophilic secretions were found adjacent to the acinar lumen in the current work.These data was interpreted in rat *(17)* , rabbit and primate *(2)* as degenerating brusting cells.

The ultrastructure of examined MG acinar cells was varied.The basal cells were flattened and contained elongated central nuclei and their cytoplasm was devoid of SVs. These results are similar to the findings in human *(39)*. However, it has been reported that the lipid vesicles are occasionally seen in these cells of rat *(17)*. In the rabbit and primate *(2)* and human *(39)* added that the basal cell cytoplasm is scanty and contains a large number of free ribosomes, mitochondria, tonofilaments, scarce rER and small Golgi apparatus. The basal cells were resting on basement membrane by many hemidesmosomes and connected with the differentiating cells by interdigitation. These results were partially similar to that reported in rabbit , primate *(2)* and human *(40)* where the desmosomes linking basal stem cells to the differentiating cells.

The saturated fatty acids do not react with osmium tetroxide and so they may washed out by solvents used in dehydration and embedding *(41)*.These data were interpret the appearance and contents of the SVs in the examined MGs.

This study cleared a close interdependence between SVs, mitochondria and sER. These results were partially in accord with that *(40)* which showed that the cells initiate their differentiation, the sER and the Golgi apparatus become prominent. The moderately differentiated meibocytes of this study showed ovoid to nearly rounded nuclei, many electron dense mitochondria and

moderate number of different sized SVs appeared in their cytoplasm. The same results were described in human *(40)*.The sER was prominent and had various forms include grid lattice and parallel cisternae. The SVs varied greatly in size and number between different maturing meibocytes and within the same meibocyte. Some of these SVs fused with each other, others were intermingled with the mitochondria and they appeared to indent the nucleus.These results were mostly as those mentioned in the camel sebaceous glands in the skin *(42)*.

The lysosomes were prominent in well-differentiated meibocytes and few in degenerated meibocytes. These results were mostly similar to those mentioned in sebaceous gland of subhuman primates *(43)*.It has been suggested that lysosomes are involved in the autolytic phase of holocrine secretion *(44)*.

We could suggest that in this desert animal the melanocytes by their melanin pigment, may play a key role in the protection of the MGs from solar radiations, a similar function for the melanocytes was stated *(37)*.

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الملخص العربي

دراسات نسجية وتركيبية فائقة الدقة على غدة ميبوميان فى ذكور الجمال البالغة

أحمد مصطفى بلح ، وحيد عبدالعظيم عبدالرحيم ، أحمد محمد الباز ، نسمة إبراهيم النصيري
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ان صحة وسلامة سطح العين تعتمدعلى غدة ميبوميان. وتعتبر من الغدد الدهنية المتحورة.تقع غدة ميبوميان فى أجفان البشر والحيوانات. من المعروف ان جفون الجمل خاليه من غدة ميبوميان . لذا استهدف من هذة الدراسة الكشف عن وجود غدد ميبوميان في ذكور الإبل البالغة ومحاولة لوصف معالمها التشريحية والتركيبية وربط النتائج التي تم الحصول عليها مع وظيفتها.وقد أجرى هذا البحث على عشرة غدد ميبوميان من ذكور الجمال البالغة. تم توصيف الغدة تشريحيا وتثبيتها وتمريرها وصباغة القطاعات البارفينية منها بالصباغات المختلفة.وتم أيضا تثبيت عينات سمك ١ملم^{٣} من الغدة بغمسها فى محلول الجلوترالدهيد ثم تمريرها حتى الحصول على قطاعات فائقة الدقة وصباغتها باسيتات اليورانيل وسترات الرصاص وفحصها بالمجهرالالكتروني النافذ.وقد أظهرت نتائج الفحص التشريحى أنها غدة جفنية زائدة سفلية ولها سطح مواجه للجفن الثالث به شعر ناعم .وقد أسفرت نتائج الدراسة النسجية لغدة ميبوميان عن أنها تتكون من قناة رئيسية واحدة تصب بها قنوات صغيرة لعدد من الحويصلات المتفرعة و تدعم بالنسيج الضام المكون من الألياف الكولاجينية. تبطن الحويصلات بعدد من خلايا ميبوميان المحملة بالدهون.أما بالنسبة لنتائج المجهر الالكتروني النافذ فقد كانت الحويصلات مبطنة بأربعة أنواع من الخلايا: خلايا قاعديه،وخلايا ميبوميان نصف المتمايزة والمتمايزة كاملا والمتحللة . تتشكل النواة لتصبح مسطحة فى الخلايا القاعديه ،قرصية فى خلايا ميبوميان النصف متمايزة ،مسننة جزئيا بحويصلات مفرزة فى خلايا ميبوميان المتمايزة كاملا و غير منتظمة الشكل ومتحللة فى الخلايا المتحللة . تتميز خلايا ميبوميان النصف متمايزة بتواجد العديد من الميتوكندريا و الشبكة الاندوبلازمية الملساء وتظهر كشبكة شعرية و صهاريج متوازية .