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LIGHT AND SCANNING ELECTRON MICROSCOPICAL STUDIES OF GOAT'S CORNEA

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ABSTRACT

The present investigation was conducted on the cornea of goats to point out its microscopical structure. The cornea of the goat appeared to be formed of five layers arranged from outside to inside as the corneal epithelium, Bowman's membrane, substantia propria (corneal stroma), Descemet's membrane and the corneal endothelium. The corneal epithelium was formed of stratified squamous non cornified epithelium consisted of 10-14 layers of epithelial cells. Scanning electron microscopical examination revealed polyhedral to hexagonal surface epithelial squamous cells demarcated from each other by thick tight junction. The outer cell membrane of the most apical squamous cells showed numerous microplicae. The Bowman's membrane or the external limiting membrane appeared as a thick homogenous PAS-positive intense line rich in elastic fibers. The substantia propria or corneal stroma constituted the majority of the corneal thickness. It consisted of parallel bundles with closely-packed collagen fibers and few fine interwoven fibers. The stromatocytes or corneal corpuscles were dispersed in between the lameller connective tissue. They had clear oval basophilic darkly stained nuclei. The Descemet's membrane or the internal limiting membrane was thinner than the Bowman's membrane. It appeared as a fine PAS-positive homogenous acellular layer. The inner most or posterior layer of the cornea is the corneal endothelium which appeared as a single layer of flattened squamous cells.

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INTRODUCTION

The normal vision requires a healthy transparent avascular cornea. Therefore, it is very important to understand the normal microscopic structure of the cornea in order to overcome any pathological affection of this valiable organ (Harper *et al.,* 2005). Understanding the complexity of the microanatomy of the normal cornea requires the combined use of many strategies and protocols including general and special staining techniques as well as electron microscopy.

The mammals cornea, as studied by many authors consists of five layers named from external to internal: corneal epithelium, Bowman's membrane, corneal stroma, Descemet's membrane and corneal endothelium (Konsowa and Abd-Alaziz, 1999; Hayashi *et al.,* 2002 and Joyce, 2003). On the other hand the cornea of goats received a little attention from the histologests, consequently, the present study aimed to point out the microanatomy of goat's cornea from different aspects using different histological protocols as well as scanning electron microscopy.

MATERIALS and METHODS

A total number of twenty four normal and apparently healthy goat eyes were used for the present study collected from Beni Suef slaughter houses. After aspiration of the vitrous humor of the eye ball 10% neutral buffered formalin was injected for proper fixation and to give the eye degree of hardness. After two hours, the corneas of the fixed eyes were excised and fixed in the same fixative for light microscopic studies. Then the fixed corneas were dehydrated in alcohol, cleared in xylene, impregnated in soft paraffin and blocked in hard paraffin. Sections of about 4-6 micrometers-thick were obtained to be stained with Harris's hematoxylin and eosin, Crossmon's trichrome, Weigert's elastic and PAS techniques. The above mentioned techniques were applied according to Drury and Wallington (1980) and Bancroft and Steven (1996).

For scanning electron microscopy, the excised corneas of four goats were fixed in 2.5% gluteraldhyde in 0.1M phosphate buffer then post fixed in 1% OsO4-0.1M pBS for 1 hour, dehydrated in ethanol (Hayat, 1986), substituted with 2-methyl-2-propanol and freeze dried in an Eiko ID-2 freeze

drier, the specimens were then mounted on alomenium stubs, coated with osmium using an osmium plasma coater, then viewed with a Hitachi S-800 scanning electron microscopy.

RESULTS

The cornea of the goat appeared to be formed of five layers arranged from outside to inside as the corneal epithelium, Bowman's membrane, substantia propria (corneal stroma), Descemet's membrane and the corneal endothelium (Fig.1).

The corneal epithelium was formed of stratified squamous non cornified epithelium consisted of 10-14 layers of epithelial cells. The basal cell layer was represented by tall columnar cells containing vesicular oval basally located nuclei in a lightly acidophilic cytoplasm. The intermediate layers ranged from 6-8 layers of polyhedral cells with central rounded darkly stained nuclei and deeply stained acidophilic cytoplasm. The outer most layers were represented by 4-6 layers of flattened attenualtd squamous cells with darkly stained elongated nuclei and deeply stained cytoplasm (Fig.2). Peripherally, the corneal epithelium contained few brownish melanin granules. Scanning electron microscopical examination revealed polyhedral to hexagonal surface epithelial squamous cells demarcated from each other by thick tight junction (Fig.3). The most apical epithelial cells appeared highly flattened as the nucleus clearly bulged at the center of the cell (Fig.4). The outer cell membrane of the most apical squamous cells showed numerous microplicae or even stuppy microvilli (Fig.5).

The Bowman's membrane or the external limiting membrane appeared as a thick homogenous PAS-positive intense line rich in elastic fibers. It measured about 9-10 micrometers-thick. It was located between the corneal epithelium and the corneal stroma (Fig.6).

The substantia propria or corneal stroma constituted the majority of the corneal thickness. It was consisted of parallel bundles with closely-packed collagen fibers and few fine interwoven fibers (Fig.7). Electron microscopically the collagen fibers appeared as thick rough longitudinal parallel bundles separated by fine fibers connecting the bundles with each other (Fig.8). The stromatocytes or corneal corpuscles as shown in figure (7) were dispersed in between the lameller connective tissue. They had clear oval basophilic darkly stained nuclei of regular pattern while the cytoplasm appeared clear and lightly stained. There was an absolute absence of any blood vessels in the goat's cornea.

The Descemt's membrane or the internal limiting membrane was thinner than the Bowman's membrane. It appeared as a fine PAS-positive homogenous acellular layer separating the corneal stroma from the endothelium (Fig.9).

The inner most or posterior layer of the cornea is the corneal endothelium which appeared as a single layer of flattened squamous cells with elongated darkly stained nuclei parallel to the internal limiting membrane. It was in direct contact with the aquous humor of the anterior chamber (Fig. 10). There was no evidence about the presence of elastic fibers in the goat cornea.

DISCUSSION

There is a general agreement that the cornea in most mammalian species including goats formed of five layers which are the corneal epithelium, Bowman's membrane, corneal stroma, Descemet's membrane and the corneal endothelium (Konsowa and Abd-Alaziz, 1999; Hyashi *et al.*, 2002 and Joyce, 2003). On the other hand Derbalah (2001), Ahmed and Karciogiu (1997) as well as Konsowa and Abd-Alaziz (1999) affirmed that camel cornea lacks the Bowman's membrane.

Our study revealed that the corneal epithelium appeared very smooth stratified squamous epithelium which could be met with in all domestic animals, fishes and birds (Meek and Boote, 2003). The most apical layer as revealed in our study showed flattened cells with nuclei bulged at the center of the cell as mentioned by Derbalah (2001). Hayashi *et al.* (2002) as well as Meek and Boote (2003) augment our study that the cells of outer most layer of the corneal epithelium showed numerous microplicae. The periphery of the corneal epithelium in our study showed few melanin granules as well as melanocytes ás also recorded by Rahi *et al.* (1980) and Derbalah (2001). The former reported that melanin pigments had the function of absorbing stray light and protect the body against harmful effects of too much penetrating ultraviolet sunlight.

The Bowman's membrane of goat appeared thick PAS-positive homogenous membrane rich in elastic fibers. It was located between the corneal epithelium and the corneal strom. These results are in accordance with Konsowa and Abd-Alaziz (1999), Hyashi *et al.* (2002) and Joyce (2003). The latter author added that the Bowman's membrane help in attachment of the corneal epithelium to the substantia propria. Moreover, Marrin *et al.* (1982) as well as Casey and Mayer (1984) stated that the Bowman's membrane provides an effective barrier for bacterial and neoplastic invasion and explain the less resistance of primates and beagles which lack this membrane to trauma and bacterial invasions. The authors added that the PAS-positive reactivity of such membrane indicates the considerable content of mucopolysaccharide.

The substantia propria or corneal stroma in our study represented the bulck of corneal thickness and formed of closely-packed parallel bundles of collagen fibers. Such results are in full agreement with those recorded in all mammalian species (Konsowa and Abd-Alaziz, 1999; derbalah, 2001 and Harper *et al.*, 2005). Kessel and Kardon (1970) as well as Harper *et al.* (2005) affirmed that such arrangement of collagen fibers in the corneal stroma has a great role in corneal transparency. Moreover Meek and Boote (2003) recorded that the substantia propria gives the cornea its strength, attachment and stability.

The Descemet's membrane of goat differs from Bowman's membrane as it formed purely of mucopolysaccharides without any evidence of elastic fibers (Konsowa and Abd-Alaziz, 1999). The authors added that the Descemet's membrane is recorded in all animal species.

The corneal endothelium of goat appeared as a single layer of flattened attenuated endothelial cells. similar findings are also recorded in all mammalian species (Rahi *et al.*, 1980; Konsowa and Abd-Alaziz, 1999 as well as Derbalah, 2001).

In the opinion of most histologists who studied the cornea in different animal species attributed the corneal transparency to several factors including the smooth surface of the corneal epithelium, regular arrangement of collagen fibers in the substantia propria as well as the lack of blood vessels in the cornea in addition to the chemical composition and state of metabolism and hydration of the corneal constituents. In this context, the goats cornea in the present study showed a total absence of any blood vessels except in the most peripheral areas where few blood capillaries could be observed. Similar results are also recorded in camel cornea (Konsowa and Abd-Alaziz, 1999; Derbalah, 2001 as well as Harper *et al.*, 2005).

LIST OF FIGURES

Figure (1):- A section through the goat's cornea showing three layers could be detected by H&E stain: corneal epithelium (EP), corneal stroma (p), and corneal endoithelium (En). H&E stain X100.

Figure (2):- A photomicrograph of goat's cornea showing the corneal epithelium formed of stratified squamous epithelium. H&E stain X400.

Figure (3):- A scanning electron micrograph of goat's cornea showing the outer surface of corneal epithelium formed of polyhedral cells connected by tight junctions (arrow). X 2000.

Figure (4):- A scanning electron micrograph of goat's cornea showing the outer surface of corneal epithelial cell become bulged at the center of the cell where is the site of the nucleus (arrow). X 5000.

Figure (5):- A scanning electron micrograph of goat's cornea showing the outer surface of corneal epithelium studded with numerous microplicae or stuppy microvilli (arrow). X 15000.

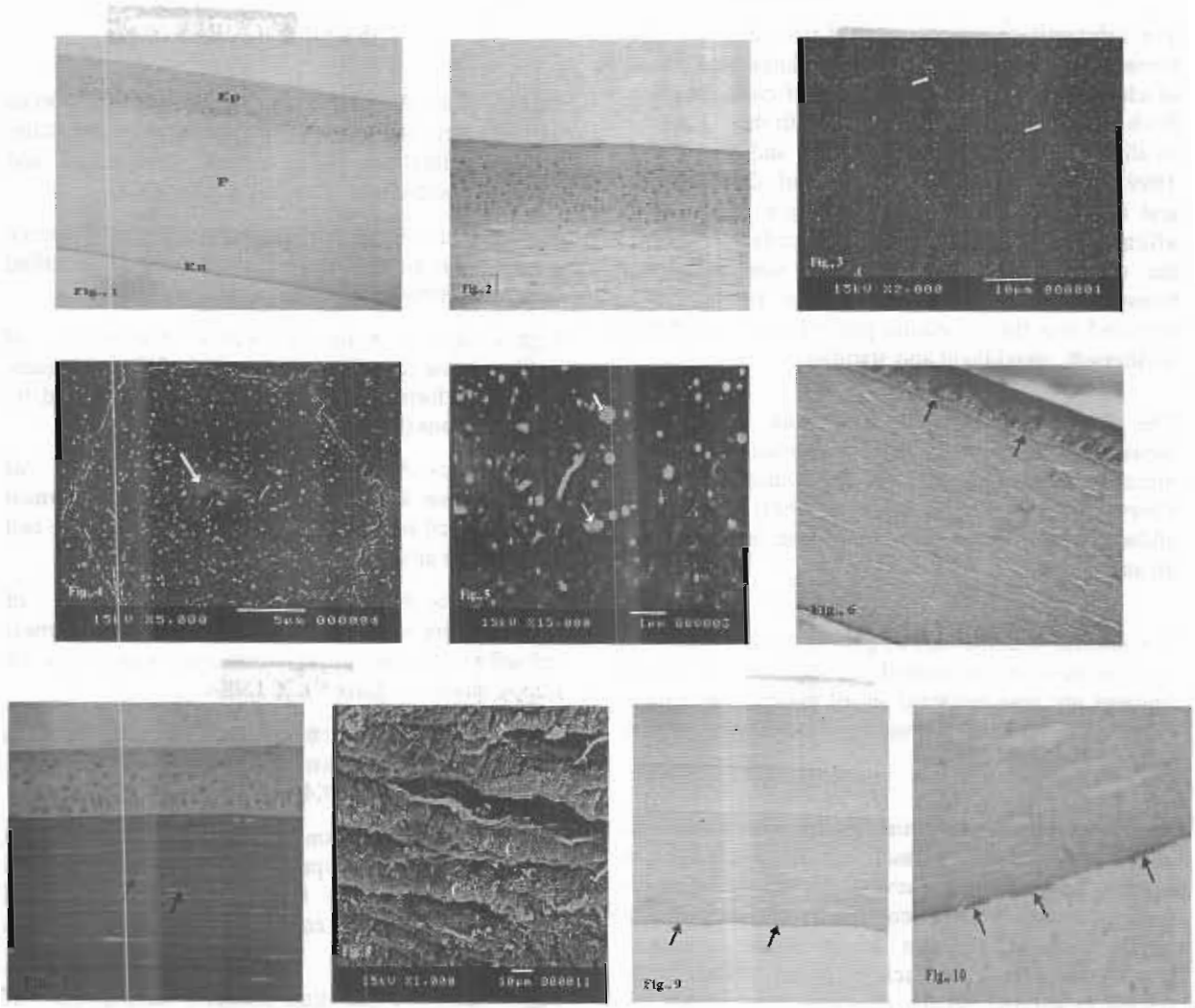
Figure (6):- A photomicrograph of goat's cornea showing thick Bowman's membrane (Arrow). Weigert's elastic stain X400.

Figure (7):- A section through the goat's cornea showing substantia propria formed of regular parallel bundles of collagen fibers housing compressed fibroblasts or corneal corpuscle (arrow). Crossmon's trichrome stain X400.

Figure (8):- A scanning electron micrograph of goat's cornea showing the substantia propria formed of thjick parallel bundles of collagfen fibers (C) separated by fine fibers.X 1000.

Figure (9):- A photomicrograph of goat's cornea showing PAS positive Descenet's membrane (arrow). PAS technique X400.

Figure (10):- A high magnification of corneal endothelium of goat showing flattened squamous cells with elongated nuclei (arrow). H&E stain X1000.



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دراسة بالمجهر الضوئى والالكترونى الماسح على قرنية الماعز

خالد محمد مظهر

أجريت هذه الدراسة على عدد أربعة وعشرين من أعين الماعز حيث عزلت القرنية وجهزت للفحص بالمجهر الضوئى والالكترونى الماسح وأظهرت النتائج ما يلى: ظهرت قرنية الماعز بالمجهر الضوئى مكونة من خمس طبقات هى:

١- طبقة النسيج الطلائى

٢- غشاء باومن (الغشاء المحدد الخارجى)

٣- طبقة السدى

٤- طبقة ديسيمت (الغشاء المحدد الداخلى)

٥- طبقة البطانة الداخلية

درست كل من هذه الطبقات ووصفت توصيفا مجهريا دقيقا وقورنت النتائج بمثيلاتها فى الحيوانات المختلفة.