HISTOLOGIC AND HISTOCHEMICAL STUDIES OF THE PROTECTIVE APPARATUS OF THE EYES IN HEREFORD AND ABERDEEN ANGUS CATTLE

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ABSTRACT

HISTOLOGIC AND HISTOCHEMICAL STUDIES OF THE PROTECTIVE APPARATUS OF THE EYES IN HEREFORD AND ABERDEEN ANGUS CATTLE

by Steve Goldsberry

This investigation consists of histologic and histochemical studies of the eyelids, nictitating membrane, lacrimal glands, nictitans glands and Harderian glands. The purposes are to establish and compare the normal histologic architecture of the protective apparatus in the two breeds and to study the histochemical nature of these structures.

Seven Hereford and seven Aberdeen Angus cattle were used in this study and of that number, five of each breed were used for histologic studies while two of each breed were used for gross dissections. The eyelids and intraorbital contents were removed in toto and fixed in 10% formalin for 48 hours. Sections were made by the paraffin method using dioxane as a dehydrating agent.

Breed comparisons revealed only two outstanding differences; (1) Epidermal and conjunctival pigmentation reach maximal density in Aberdeen Angus cattle, especially in the deeper epithelial layers but melanophores are rarely seen in the subepithelial connective tissues. In contrast, pigmentation in the Hereford occasionally reaches moderate density but is usually very sparse or even absent. (2) The conjunctival epithelium of Aberdeen Angus is usually stratified squamous and occasionally, it becomes transitional or stratified

columnar in crypts and in the fornix but mucous intraepithelial glands and goblet cells are minimized. In Herefords, the conjunctival membrane consists of stratified columnar epithelium in which goblet cells and mucous intraepithelial glands are extremely numerous. The eyelids are formed around a core of connective tissue and covered on the outer surface by a cutaneous layer which resembles the same layer on other parts of the body. The bulbar surface of the eyelids is covered by the conjunctiva which also covers the exposed surface of the sclera. The epithelial layer of the bulboconjunctiva is similar to that of the palpabral conjunctiva in the respective breeds. The lamina propria of the bulboconjunctiva consists of very fine and loosely arranged connective tissue fibers and numerous melanophores while the lamina propria of the palpebral conjunctiva is made up of a more densely arranged connective tissue with many free cells. At the corneoscleral junction of the eyeball, the covering epithelium thickens abruptly but no utricular or saccular glands (of Manz) are present at this location in either breed.

The nictitating membrane, Harderian gland and nictitans gland form a very closely related morphological complex. Forming a rigid support for the nictitating membrane is a "T" plate of hyaline cartilage. A part of the cartilage is embedded in the soft tissues near the medial canthus. Encircling this embedded cartilage is the nictitans gland and attached to the deep end is the Harderian gland. The Harderian gland is divided into an anterior and a posterior lobe. The anterior lobe is continuous with the nictitans gland and the two are histologically and histochemically similar. They consist of very small seromucoid acini which are Alcian Blue and PAS positive. The posterior lobe consists of very large acini which are filled with a colloid-like substance.

The acini are lined by simple cuboidal to simple columnar epithelium and are PAS and Alcian Blue negative for mucopolysaccharides.

The lacrimal gland, as well as numerous accessory lacrimal glands of the eyelids consist of small seromucoid acini. The ratio between serous and mucous elements varies greatly from one lobule to the other, but usually, about 70% of the acini are Alcian Blue positive for acid mucopolysaccharides.

Six to eight large lacrimal ducts open on the lateral half of the upper eyelid. Occasionally, some open in the lateral canthus or even on the lateral third of the lower eyelid.

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TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - Continued

INTRODUCTION

The circumorbital and intraorbital structures of man and some animals have been studied extensively but this is not true of cattle, especially in reference to specific breeds. In the past decade, several investigators have studied ocular squamous cell carcinoma which is often manifested in the eyes and eyelids of Hereford cattle. An editorial (1947) and articles by Wynne and Russell (1955), Russell et al. (1956), Anderson (1960) and Anderson and Skinner (1961) are some of the more prominent references to this neoplastic condition. According to Russell et al. (1956), 95 percent of ocular cancer in cattle involves Herefords and control of this condition would have immediate economic effect on one of our country's largest and most vital industries. In addition, they noted that ocular cancer in Herefords is several times more numerous than all neoplastic conditions in man combined. They concluded that additional research is urgently needed, especially on precancerous tissues.

This investigation includes histologic and some histochemical studies on the eyelids, nictitating membrane, lacrimal gland, nictitans gland and the Harderian gland. The purposes of this investigation are to establish the normal histologic structure of the protective apparatus of the eyes of Hereford and Aberdeen Angus cattle, and to study the histochemical nature of these structures. Histochemical studies include the identification of mucopolysaccharides, lipids, metachromatic materials and the differentiation between the various types of connective tissues.

Although this study does not encompass squamous cell carcinoma of the eyes, it is indeed hoped that the findings will enhance the work of clinicians and investigators who are concerned with problems which involve the eyes and associated structures.

LITERATURE REVIEW

Eyelids

General descriptions of the eyelids were given by Trautmann and Fiebiger (1957), Sisson and Grossman (1953), Smythe (1958) and Prince et al. 1960), but it should be noted, however, that there are very few available references to specific domestic breeds. Several students in the Anatomy Department at Michigan State University have completed theses on the integument of the various domestic animals. Those which include a brief description of the eyelids are: (1) Strickland (1958) cat; (2) Smith (1960) newborn swine; (3) Fowler (1962) fetal pigs; (4) Marcarian (1962) adult Yorkshire hogs; (5) Sar (1963) goats and (6) Sinha (1964b) Holstein cattle.

It is generally agreed that the eyelids are formed around a core of connective tissues, the tarsal plate, and are covered on each surface by epithelium. A cutaneous membrane covers the outer surface and the inner surface is covered by a mucous membrane, the conjunctiva. Sinus or tactile hairs are described by Trautmann and Fiebiger (1957), those for cats are described by Strickland and Calhoun (1963) while sinus hairs of goats are described by Sar (1963) and those of hogs by Marcarian (1962). They also described sweat glands and sebaceous glands which are associated with the cilia.

Prince et al. (1960) described the conjunctiva as a mucous membrane lined by stratified epithelium covering the inner surface of the eyelid (palpebral portion), reflecting anteriorly forming a pouch (fornix portion), and covering the surfaces of the eyeball (bulbar portion). The conjunctiva also covers all free surfaces of the nictitating membrane. They also observed numerous goblet cells and "intra epithelial" mucous glands in this layer and lymphoid nodules in the underlying lamina propria.

Aureli and Kornerup (1949) examined the eyes of man and swine for the presence of Manz's glands. They found that Manz's glands are present in the conjunctiva of fetal pigs, but they regress with age and are absent in the adult. They are located in the limbus of the eye and were especially well developed on the nasal and temporal sides. They found no such structures in man at any age, however, a peculiar arrangement of the papillary pegs near the limbus was noted. Similar to the findings in the pigs, the glands of Manz were observed in the bovine eye by Prince et al. (1960). They also observed other glands in the lamina propria of the fornix. Although these are commonly known as Wolfring's glands and glands of Krause, they described them as accessory lacrimal glands.

In addition to the tarsal plate, the middle layer of the eyelid includes widespread connective tissue and muscle (Smythe 1958). He observed that the orbicularis, corrigator and levator palpebral muscles make up the major portion of the musculature, however, special smaller muscles were noted. Riolan's muscle was found around the roots of the cilia and were said to be small bundles of striated fibers which branched from the orbicularis oculi muscle. The muscle of Müller is smooth muscle which inserts into the tarsal plate. The two latter muscles were similarly described by Prince et al. (1960).

Acheson (1938) observed that the nictitating membrane of the cat retracts in response to certain stimuli, and found that this response is effected by two sheets of smooth muscles. They arise from the ventral and medial rectus muscles and inserted, by one slip, into the third eyelid while a second slip extends into the eyelids proper. He believed this to be the Müller's muscle of the upper and lower eyelids.

Stibbe (1928) compared the nictitating membrane of birds and mammals. He found the nictitating membrane of mammals cannot move independently, however, he acknowledged the presence of several small bundles of smooth muscle fibers in the nictitating membrane of calves, sheep, rabbits and cats. He also observed hyaline cartilage which consists of a large number of small chondrocytes in the third eyelid of mammals. Trautmann and Fiebiger (1957) found elastic cartilage in the nictitating membrane of the horse, pig and cat. In birds the connective tissue which surrounds the cartilage is primarily elastic (Stibbe 1928).

The Lacrimal Gland

The lacrimal gland varies greatly in size in the different species (Prince *et al*. 1960). For the ox, they gave the approximate size as 65 x 36 x 10 mm. and stated that it conforms to the space between the eyeball and the bony orbit on its dorsolateral side. According to Sisson and Grossman (1953) the lacrimal gland of the ox is embedded in the periorbital fat. They described it as a thick lobulated gland which is divided into distinct upper and lower lobes which excrete through 6-8 large and several small ducts.

This gland was classified as a serous secreting tubuloacinar gland with prominent septa between lobules (Prince *et al*. 1960). They also observed that the acini are lined with simple cuboidal epithelium and that occasional pigment cells and macrophage are seen in the connective tissue septa.

From a comparative standpoint, Trautmann and Fiebiger (1957) stated that the lacrimal gland is serous secreting in most species, but a few mucous secreting cells were found in the sheep, goat and dog. They also observed that this gland is mucous secreting in swine and that fat droplets were seen in the secretory cells of all animals.

Smythe (1958) found the lacrimal gland well developed with two distinct lobes. He noted that both lobes excrete through the same duct system and if the anterior lobe were clinically removed or obstructed by disease, the function of the whole gland would be lost.

The lacrimal secretions lubricate and clean the surface of the eyeball. The fluid contains about 1% sodium chloride, a small amount of protein and a bactericidal enzyme-lysozyme (Trautmann and Fiebiger 1957).

Harderian - Nictitans Complex

There are many references which deal with the Harderian gland and the nictitans complex of rats, mice, guinea pigs and rabbits but there are relatively few references which deal with the same structures in domestic animals. Smythe (1958), Prince *et al*. (1960), Trautmann and Fiebiger (1957) and Sisson and Grossman (1953) serve as good general references for the eyes and associated structures in the domestic animals but they rarely mention specific breed characteristics.

The Harderian gland has been studied more extensively than any other accessory structure in the orbit of the eye. Investigators such as Schnier (1950), Paule (1957) and Davis (1929) gave credit to Johannes Jakob Harder for discovering the "New lacrimal gland" while dissecting a deer in 1694. This gland is now called the Harderian gland in his honor.

For more than one hundred years following Harder's discovery there were very few reports of research on the Harderian gland. More recently an additional gland has been found which is attached to the nictitans cartilage and to the original Harder's gland. According to Davis (1929) this new gland is an independent structure in the rabbit, and Paule (1957) and Prince *et al*. (1960) made a similar observation for the pig. For many years the term "Harderian gland" was used to include both glands, but more recently, there is a tendency to separate the two glandular masses, and with this tendency a controversy of nomenclature has developed. Paule (1957) and Prince et al. (1960) consider the gland which embeds and encircles the nictitans cartilage as the nictitans gland (originally Harderian) while the more recently discovered gland is now called the gland of Harder. Based upon this newer nomenclature, Paule stated that the deer has no Harderian gland but only a nictitans gland. This implies that Harder really discovered the nictitans gland in 1694 and someone else discovered the Harderian gland about two hundred years later. Since the new nomenclature of Prince et al. (1960) appears to be favored by most recent authors, it will be used in this discussion.

According to Schnier (1950) Rolleston, as early as 1870, examined many animal species for the presence of this gland and concluded that the Harderian gland is present in all birds and all mammals except bats and primates. Glands of the third eyelids were described in the sheep, calf, horse, mouse, guinea pig and swine by Loewenthal (1892). He stated that Harder's gland is present in animals which have well developed nictitating membranes and that there is no counterpart in man. He also observed accessory serous glands associated with the excretory ducts as well as goblet cells in the walls of these ducts.

Although early reports of investigations of the Harderian gland were usually limited to Morphologic descriptions, Sundwall (1907) inclused histochemical findings in his report which resulted from studies of the Harderian gland of ox. Sundwall (1907 and 1916) not only gave gross and histologic accounts of the Harderian gland of the ox, but also described the reactions of the secretory cells and their granules to numerous biological stains. According to Sundwall (1907) this gland of the ox consists of two lobes which contain tubulo-acinar lobules.

Additional observations show a connective tissue capsule enclosing the whole gland and contributing to the formation of very strong septa between lobules.

Although several noteworthy investigations had taken place earlier, the description of the eye and orbit of rabbits by Davis (1929) included a much more comprehensive study of the Harderian gland than any other report prior to 1930. In addition to other gross anatomical descriptions of the Harderian gland, he reported that there are two lobes, one red and one white. Each is made up of large acini lined by cuboidal epithelium. He also observed that glandular cells of the red lobe contain a course reticulum, while those in the white lobe contain a fine reticulum and cells of both lobes contain many fat globules. He noted further that the Harderian secretion is a complex alkaline mixture in which fat predominates and that it is negative to osmic acid, but positive to Sudan III. Finally, Davis (1929) concluded that the secretory substances which lubricate the surfaces of the eyeballs are expelled through a single large duct which opens on the bulbar surface of the nictitating membrane.

A phylogenetic study of orbital glands in vertebrates was made by Walls (1942). He reported that orbital glands are absent in lower vertebrates such as the fishes and aquatic amphibians, and that terrestrial salamanders possess a row of small glands in their lower eyelids. He observed further that frogs, toads and several species of snakes and lizards possess a single large orbital gland but it is not clear whether this is a Harderian or lacrimal gland. In addition to the definite Harderian and lacrimal glands observed in turtles and alligators, he observed a special orbital gland in some species of snakes. But it was not established, however, whether this was a Harderian or an accessory salivary gland. Finally, Walls (1942) concluded that a Harderian gland is present in all birds and all mammals except bats and primates. Although these conclusions were similar to those obtained by Schnier (1950) and Paule (1957), there was partial disagreement. For, in addition to acknowledging the absence of Harderian glands in primates and bats, Walls (1942) also noted their absence in dogs, cats, sheep and deer. Prince et al. (1960) later verified these findings for dogs, cats, and sheep, but they did not include the deer in their study.

It seems that the dispute which revolves around the presence or absence of Harderian glands in certain species is due to varied nomenclature. It is interesting to note that the presence of a Harderian gland in the deer is disputed--the species in which Harder made his original discovery. Prince et al. (1960) recognized this problem and in their description of the eye and orbit of domestic animals they considered the glandular tissues which lie immediately around the nictitating cartilage as the "nictitans" gland. When there was a distinct glandular mass lying distal to the cartilage, they referred to it as the Harderian gland. According to this nomenclature, it should be noted that Harder actually discovered the "nictitans" gland and that the Harderian gland was discovered later by someone else.

Since 1960, electron microscopy as well as histologic, histochemical and physiologic techniques have been employed in Harderian gland studies in rats, mice, hamsters, guinea pigs and rabbits. The presence of porphyrin pigments in the Harderian glands of rats, mice and golden hamsters was reported by Gafflin and Venable (1940). They established that this substance is secreted by the Harderian gland itself and that it becomes fluorescent in the presence of ultra violet light. A quantitative study of Harderian pigments in the mouse was made by Collins (1957). He observed melanin-like pigments as well as porphyrins. Further observations showed that the quantity of porphyrin increases proportionally with an increase of the environmental temperature while melanin remains unchanged.

In addition to the study of the rabbit's eye and orbit by Davis (1929), noteworthy contributions have been made by Schnier (1950) and Björkman et al. (1960). Schnier (1950) added that the cells of the red and white lobes of the Harderian gland are cytologicly and cytochemically different and that the fat droplets in the cells from the red lobe are large and those in the cells of the white lobe are small. While secretory activity is both apocrine and holocrine, no mitotic figures have been observed in actively secreting glands, therefore there is no obvious replacement for cells in holocrine secretion. He also observed alkaline phosphatase activity in the white lobe and none in the red, but the cytoplasm of cells of both lobes showed a strong oxidase reaction.

In a study of the rabbit's Harderian gland, Björkman et al. (1960) found that the cytoplasm of all glandular cells is very dense to the electron microscope, and that the mitochondria have branched, closely packed cristae and a dense matrix. He also noted that the endoplasmic reticulum is a fine mesh with many ribosomes attached, and that few lemalla but many vaculoes are present in the Golgi complex. He finally concluded that the high degree of cytoplasmic basophilia is due to the presence of RNA in the ribosomes because the substance is dispersed when treated with the enzyme ribonuclease.

Although laboratory animals were studied more frequently than domestic animals, the latter have not been ignored completely. One recent reference to studies on the Harderian gland of domestic animals contains a description of the gland of the third eyelid in sheep by Materazzi and Travaglini (1961a). They found that the secretions are oily but contain proteins and are PAS-positive. Later Materazzi and Travaglini (1961b) found a similar fatty secretion in the dog and the cat which is PAS positive. It is interesting to note this description of

Harderian activity in the dog and cat, especially, since Paule (1957) and Prince et al. (1960) had denied the existence of a Harderian gland in these species.

According to Trautmann and Fiebiger (1957), the Harderian gland of swine is a mucous gland. Aureli (1957) agreed with this observation, however, he added that the Harderian gland of the pig is completely independent and separate from the nictitans gland. The presence of generic lipids, fatty acids, lipins and PAS-positive granules were also noted. He stated further that the epithelium of excretory ducts actively secretes PAS-positive substances, however, in modest quantities.

The presence of myoepithelial cells in most glands is commonly accepted. These cells were described in the Harderian glands of rodents by Chiquoine (1958) who concluded that:

1. Myoepithelial cells are located between the secretory epithelium and the basement membrane.
2. That the long axis of the cylindrical nucleus is parallel to the basement membrane.
3. That the cytoplasm consists of a central mass which contains the nucleus and also gives rise to numerous radiating processes, and that the cytoplasm can be seen only with special techniques.

MATERIALS AND METHODS

Source and Securing of Specimens

Specimens used in this study were taken from seven Hereford and seven Aberdeen Angus cattle which ranged from 18 to 24 months of age. All cattle, from which study specimens were selected, were slaughtered at Van Alstine's Packing Plant near East Lansing, Michigan. Within ten minutes following slaughter, the eyelids and skin as far as the bony margin of the orbit, and the intraorbital contents were removed in toto and fixed in ten percent formalin for forty-eight hours. Specimens were taken from only those cattle of known ages which possessed no pathological lesions about the eyes and associated structures. As far as could be determined all specimens used in this study were selected from purebred cattle (Table 1).

Histologic and Gross Anatomical Techniques

Of the seven Hereford and seven Aberdeen Angus cattle used five of each breed were selected for histologic and histochemical studies. After proper fixation, the desired tissues were removed, and blocks were trimmed from specific areas as illustrated in figures 1 and 3. Tissues were dehydrated by the dioxane method of Bucher and Blakely (1936) which included three changes of dioxane for four hours each and a fourth change for sixteen hours. Dehydration proceeded at room temperature (approximately 23°C to 27°C) after which the dehydrated tissues were infiltrated with 56°C to 58°C paraffin. The procedure consisted of four changes of paraffin, of which the first three were four hours each, and the fourth was sixteen hours. The entire infiltration procedure was carried out in an oven which

Table 1. List of Specimens Used

Code Number	Breed	Sex	Age (Months)	Eyelid Color
A	Hereford	female	21	red
B	Hereford	female	24	white
C	Hereford	female	18	white
D	Hereford	male*	20	red and white
E	Hereford	female	19	red and white
F	Aberdeen Angus	female	24	black
G	Aberdeen Angus	male*	23	black
H	Aberdeen Angus	female	19	black
I	Aberdeen Angus	female	22	black
J	Aberdeen Angus	male*	18	black
K+	Hereford	female	23	white
L+	Aberdeen Angus	female	19	black
M+	Aberdeen Angus	male**	20	black
N+	Hereford	female	22	white

* - Castrated males
** - Bulls
\+ - Specimens used for gross dissection only

maintained 58°C to 60°C temperature. Following infiltration, specimens were embedded in paraffin and sections for general histologic and histochemical studies were cut at seven to nine microns while those for serial sections were cut at fifteen to twenty microns. All sections for general study were stained with Harris hematoxylin and eosin (Malewitz and Smith 1955). For the characterization of elastic and collagenous fibers, Weigert and Van Gieson's stain was used. Additional stains included (1) Dominici's stain (Gridley 1957) for mast cells and metachromatic materials (this stain also reacted with other tissue elements so that interrelationship as well as metachromatic substances could be evaluated); (2) Periodic Acid-Schiff (PAS) reagent for general mucopolysaccharides; (3) Alcian Blue-Nuclear Fast Red for acid mucopolysaccharides and (4) unstained sections for evaluation of pigment in the skin and conjunctiva of the eyelids.

For each breed, two specimens, one fresh and one formalin fixed, were dissected to show the relationship of the nictitating membrane to the eyelids, caruncle and eyeball. Also the structure of the nictitans and the Harderian gland complex and the relationship of the lacrimal gland and ducts to the eyelids were studied. The course of the lacrimal ducts between the gland and their conjunctival orifices was determined by serial sections and by microdissection.

Counting Tarsal (Meibomian) Glands and Eyelashes

Tarsal glands in both fresh and fixed specimens from Herefords were easily counted with the aid of the dissecting microscope. In the Aberdeen Angus, however, the glands were more difficult to see because of heavy epidermal pigmentation and for that reason the orifices of the gland ducts were used to determine the numbers.

Eyelashes were difficult to count, even with the dissecting microscope because they were intermingled with cover hairs of the eyelids. Their sizes varied so that many of those among the cover hairs could not be identified.

Measurements of Eyelids

The thickness of the eyelids was determined by employing a dissecting microscope and a stage micrometer. The stage micrometer was calibrated to 0.1 millimeter divisions and when used with the dissecting microscope, measurement of structures thicker than 0.1 millimeter was quite accurate.

The eyelids gradually increased in thickness from the free margin to their attachment. Since it is difficult to measure different lids at exactly the same points, the constant marginal arcuate artery served as a landmark and all measurements of the eyelids were taken at a plane which passed through this artery (fig. 5).

RESULTS AND DISCUSSION

Eyelids

Most authors agree that the eyelids are formed around a core of connective tissue and are divided into three layers blended together so that their boundaries are poorly defined. The cutaneous layer is outermost and continuous with the adjacent periorbital skin (fig. 1), while the conjunctival layer covers the bulbar surface of the eyelid. Lying between the cutaneous and conjunctival layers is the middle layer which consists of a thick layer of connective tissue in which the tarsal gland and the palpebral musculature are embedded. The thickness of the eyelids varies greatly (Table 2). The free margins are thinnest but there is a gradual increase until the thickest point is reached at the level of the conjunctival fornix (fig. 5). The marginal artery is adequately described by Prince et al. (1960).

The upper eyelids are thickest in the medial third and thinnest in the lateral third but the lower lids are uniform in thickness throughout. There are no significant breed differences between Hereford and Aberdeen Angus cattle so far as eyelid thicknesses are concerned. In Herefords, the upper lid averages are 10.0 mm. in the medial third, 9.2 mm. in the middle third and 8.4 in the lateral third while in the upper eyelids of Aberdeen Angus, the averages were 9.8 mm., 9.4 mm. and 9.2 mm. respectively. In both breeds, the lower eyelids were uniform in thickness--those of the Hereford averaged 7.6 mm. while those of Aberdeen Angus averaged 7.7 mm.

The Cutaneous Layer

The cutaneous layer of the eyelids consists of the epidermis

Table 2. Eyelid Thickness in Millimeters

	Upper Eyelid				Lower Eyelid		
	Code*	2**	3	4	6	7	8
Hereford	A	10.2	8.7	8.0	7.0	7.2	7.2
Hereford	B	9.5	9.0	8.2	7.2	7.0	7.2
Hereford	C	10.5	9.5	8.0	7.8	7.5	7.5
Hereford	D	10.0	9.7	9.2	7.7	7.5	7.7
Hereford	E	9.7	9.2	8.5	7.5	7.2	7.0
Average		10.0	9.2	8.4	7.4	7.3	7.3
Aberdeen Angus	F	10.0	9.7	9.5	8.2	7.2	7.7
Aberdeen Angus	G	9.5	9.2	9.2	7.5	7.7	8.0
Aberdeen Angus	H	10.2	10.0	9.2	7.5	7.5	7.7
Aberdeen Angus	I	9.5	9.2	9.0	7.5	7.5	8.0
Aberdeen Angus	J	10.3	8.8	8.5	7.9	7.6	7.2
Average		9.9	9.4	9.1	7.7	7.5	7.7

* - Specimens as described in Table 1.
** - Numbers identify the location of sections on which measurements were made--fig. 1.

and the dermis (fig. 5 and 6). The cutaneous layer is continuous with the adjacent skin at the margins of the orbit and with the palpebral conjunctiva near the margins of the eyelids. The point of transition between the conjunctiva and epidermis of the cutaneous layer is gradual and non-specific, however, an abrupt change in the surface epithelium takes place at the orifices of the tarsal glands (fig. 5). On the cutaneous side of the ducts the epithelium is thicker with few papillary pegs while that on the conjunctival side of the ducts becomes thinner but papillary pegs are numerous.

Epidermis: The epidermis of the cutaneous layer consists of stratified squamous epithelium. The structure of this layer in the eyelids is similar to the epidermis of other skin areas in Hereford and Aberdeen Angus cattle (Goldsberry and Calhoun 1959). The epidermis of the lid margins is thicker than that of the hair covered portion of the lids. On the margins it ranged from 18 to 24 cell layers while in hair covered areas, it averaged about five cell layers in thickness.

Cutaneous pigmentation is maximal in Aberdeen Angus cattle where it forms very dense masses completely camouflaging the basal layers of the epidermis. Large dense patches in the more superficial spinous layers are not unusual but in spite of the heavy pigment deposits, very few chromatophores appear in the dermis. Hereford cattle differ in that cutaneous pigmentation is frequently absent and even in areas covered by red hair, it is restricted to the basal layer and occasional patches in the spinosum.

One interesting observation is the presence of numerous "clear" cells in the epidermis, and in the superficial layer of the dermis. They are concentrated along the dermo-epidermal junction, but occasionally a few inhabited the more superficial layers of the epidermis. These cells contain small and very compact nuclei which are completely filled with chromatin; however, nucleoli could be seen upon very careful observation. The cytoplasm contains no granules and is clear, and its ratio in proportion to nuclear mass is much greater than that of typical lymphocytes. Clear cells have no cytoplasmic affinity for PAS, Alcian Blue, Weigert-Van Gieson, Oil Red 0 or Hematoxlyn-eosin stains. Andrew and Andrew (1949) observed similar cells in the epidermis of rats and man. They noted that migrating lymphocytes undergo a transformation in the epidermis and give rise to "clear" cells. They also stated that "clear" cells are epithelial in character, and that they are located in areas where typical epithelial cells are degenerating. From these notations they concluded that lymphocytes function to replace epithelial cells of the epidermis and that this accounts for the relatively low mitotic activity usually observed in the epidermis. Thus, in addition to other functions, lymphocytes were believed to play an important role in regeneration of the epidermis as well. Initially it appeared that "clear" cells were more numerous in Hereford than in Aberdeen Angus cattle. But it is now apparent that these cells become camouflaged by heavy masses of pigment and are not easily seen under these circumstances. Except for the very obvious difference in epidermal pigmentation, there are no significant differences between the Hereford and Aberdeen Angus breeds.

Dermis: The dermis becomes continuous with the deeper connective tissues which form the central core of the eyelids (fig. 5). It consists of a connective tissue stroma upon which the epidermis rests and is subdivided into a superficial papillary layer, and a deeper reticular layer. The papillary layer consists of fine collagenous and elastic fibers while the reticular layer contains coarser collagenous fibers. At the dermo-epidermal junction the papillary layer forms dermal papillae which project into the spaces between papillary pegs of the epidermis. The papillary layer is relatively thin and at the level of the sebaceous glands its fibers become progressively coarser. This gradual transition continues into the deeper reticular layer and the points of junction can not be clearly identified. The reticular layer consists of rather coarse collagenous fibers arranged into large bundles. They lie parallel to the surface of the epidermis and extend either from one canthus toward the other, or from the margin of the eyelid toward the base, but very few are vertically arranged (fig. 6). Fine elastic fibers extend through the papillary layer and interlace with the collagenous fibers. These elastic fibers form nets about the hair follicles, especially at the point at which the arrector pili muscles attach. Dermal papillae are more highly developed near the lid margins than in the hair covered portions. They contain numerous capillary beds, and near the dermo-epidermal junction connective tissue meshes become very find and delicate. Numerous blood vessels and nerve trunks extend through the dermis and are surrounded by areolar connective tissues. They branch freely in the vicinity of hair follicles and the various skin glands.

The dermis is richly endowed with free cells scattered throughout the papillary layer and grouped along the vascular plexuses of the reticular layer (fig. 6). Approximately 60% of these cells appear to be lymphocytes and plasma cells, about 20% mast cells, 5-10% eosinophils, and 10-15% miscellaneous and unidentified cells. Included with the miscellaneous group are numerous "clear" cells which become abundant near the epidermis but are absent in deeper parts of the dermis.

Hair: On the basis of morphology and size, hairs of the eyelids fall into three categories: sinus hairs, which are very large and contain blood sinuses within the dermal sheath of the follicle, (2) cover hairs--which are typical in structure, and (3) large hairs, which are much larger than cover hairs, but are similar to them in structure.

The latter group includes the eyelashes and numerous solitary hairs scattered at random among cover hairs (fig. 8).

In this investigation, cover hairs and their follicles conform to the morphological description by Trautmann and Fiebiger (1957) and by Sinha (1964b).

Arrector pili muscles are intimately associated with the hairs and their sebaceous glands (fig. 10). These muscles attach to the follicle about midway between the sebaceous glands and the hair bulb, and at this point, elastic fibers form a network about the hair follicle. Passing from the hair follicle toward the epidermis the muscle bands form triangles with the hair follicle and the epidermis, and the lobes of respective sebaceous glands occupy the triangular spaces. Excretory ducts of sweat glands also ascend through these triangles and pass through the septa between sebaceous lobules before entering the hair follicles near the epidermal surface. A specific and unique modification of the hair follicle occurs between the distal tip of the sebaceous lobules and the attachment of the arrector pili muscles (fig. 10). In this region, the cells in the epithelial layer of the outer root sheath change in shape from cuboidal to tall columnar or cylindrical cells. The dermal root sheath, opposite this modified epithelium is interrupted by an extensive nerve plexus. On sagittal sections, the nerve plexus in the dermal root sheath is much more extensive than the epithelial modification associated with it. As in other parts of the hair follicle, the glassy membrane separates the dermal root sheath and the outer root sheath but very fine processes from the modified epithelium can be seen crossing this membrane. Numerous nerve trunks are present in the dermis near the modified hair follicles. The plexus extends as deep as the origin of the arrector pili muscle and ascends as high as the lobules of the sebaceous gland. In many examples (fig. 10), the plexus becomes wedged between the glassy membrane and the sebaceous lobules, pressing the lobules aside. From studies of this complex on cross sections, it appears definite that the modifications do not completely encircle the follicle, instead, there are usually two of these structures, one lying directly opposite the other. In cross section they appear as symmetrical swellings on opposite sides of the circular follicle and hair root. It is not established whether these structures are present on all hairs but at least representatives have been seen on cilia and the larger hairs scattered over the eyelids. Since this structure does not encircle the follicle, it is obvious that many hairs on any section will appear to be without the modification. The dermal root sheath consists of a rather dense inner circular layer covered by a comparatively loose outer longitudinal layer which becomes continuous with the surrounding connective tissue of the dermis. A similar connective tissue capsule also encircles the sebaceous gland.

In addition to general cover hairs, many larger hairs are present. They are randomly scattered and resemble the eyelashes, differing with them only in location. Except for size, they are also similar to hairs which generally cover the skin and eyelid surfaces.

The eyelashes in Hereford as well as Aberdeen Angus cattle are extremely numerous in the upper lids, and moderately numerous in the lower lids. In the upper lids they are arranged in a broad irregular band and frequently intermingle with adjacent cover hairs. They are more dense in the medial and lateral thirds of the eyelids than in the middle third. The number of cilia in the upper lids ranges from 150 to 300 and from 100 to 150 in the lower lids. Counts with a high degree of accuracy are difficult to attain because of irregular arrangements and frequent intermingling of eyelashes with cover hairs. Morphologically they appear similar to other hairs except for size (fig. 5). They are endowed with well developed sebaceous glands (of Zeis) which frequently encircle the follicle. Tubular glands (of Moll) are well developed also and their ducts ascend between sebaceous lobules to open on the epidermal surface. Glands of the eyelashes will be discussed with cutaneous glands, therefore, detailed descriptions are omitted here.

Sinus hairs are randomly scattered over the upper and lower eyelids. They can be identified grossly because of their large size and extreme length. Structurally, a single but very large hair occupies the follicle. The shaft and epithelial root sheath are similar to other hairs yet the dermal sheath offers an extreme contrast (fig. 8). The dermal sheath consists of a concentric layer of collagenous fibers between the sebaceous glands and the epidermal surface. In the vicinity of the glands, however, it becomes extremely thick and dense so that it encircles the sebaceous glands and gives rise to septa between their lobules. Immediately below the sebaceous glands, the dermal sheath bifurcates forming two layers: the inner layer which remains attached to the root sheath, and the outer layer which diverges, leaving a space between it and the inner layer. The two layers lie parallel as they descend to the bulb of the hair where they again fuse, thus forming a closed pouch or sinus. The sinus is filled with blood and traversed by numerous trabeculae which branch freely before attaching on the opposite layer.

Histologically, the two layers differ greatly. The inner layer consists of very loose and delicate areolar tissue and the delicate areolar fibers which form a thick layer between the blood sinus and the glassy membrane also form the substance of the trabeculae. Near the junction of the hair shaft and the hair bulb, this inner layer becomes dense, consisting of concentrically arranged collagenous fibers just before fusing with the outer layer. The outer layer consists of a very thick concentric layer of dense collagenous fibers. It is continuous and uniform from the sebaceous gland to the hair bulb where it fuses with the inner layer. As the trabeculae, which consist of areolar tissue, attach to the outer layer, the transition from areolar to dense collagenous tissue is abrupt. The blood sinuses extend from the tips of the sebaceous gland lobules to the expanded level of the hair bulb. Endothelium lines all of its surfaces as well as the trabeculae. Illustrations by Trautmann and Fiebiger (1957) show blood sinuses extending deeper than the hair bulb and surrounding it completely. They also show that sinus pads are present in the tactical hair follicles of carnivores but are absent in the same follicles of ungulates. In this investigation, the absence of sinus pads in this group is substantiated, however, serial sections show that the blood sinus does not extend deeper than the expanded part of the hair bulb. In the vicinity of the hair bulb, numerous large arteries and nerve trunks branch extensively. Many of the larger branches ascend through the internal as well as the external layer of the dermal root sheath, but only small branches are seen in the trabeculae. Arterioles also enter the sinuses near the sebaceous glands and some enter the dermal papilla of the hair bulb, as well, but no nerves enter these papillae. The outermost fibers of the external sheath are arranged longitudinally, however, this layer is poorly defined and fuses immediately with the dermis.

Both smooth and striated muscle fibers associate with the sinus follicle. The skeletal fibers ascend from the orbicularis oculi layer and insert along the dermal sheath between the sebaceous gland and hair bulb. The smooth muscles observed in this study are classified as intrinsic muscles because they form concentric bands between the circular and longitudinal divisions of the outer dermal sheath, and are entirely within the dermal sheath. These narrow thin bands are located about halfway between the bulb and the sebaceous glands and are observed in longitudinal as well as serial cross sections of the sinus follicle. The sebaceous glands of the sinus hairs are highly developed and completely encircle the follicle (fig. 8). Approximately twenty sebaceous ducts enter the follicle. The stratum corneum from the surface continues into the follicle as far as the orifices of the sebaceous ducts and large masses tend to accumulate around the hair from the sebaceous ducts to the surface. There are no tubular or sweat glands associated with these follicles.

Sebaceous Glands: Sebaceous glands are prominent in the hair covered areas of the eyelids (figs. 5, 8 and 10). They are always associated with the hair follicles and their development is inversely proportional to hair density. In areas with thin hair, sebaceous lobules completely encircle the follicles, but in dense areas only one or two lobules persist.

Morphologically, sebaceous glands consist of cell clusters which form the oily secretions, and excretory ducts by which they empty into the hair follicles. In highly developed glands many lobules subdivide, forming secondary lobules, but these minor lobules communicate with the lumen of the lobes. Stratified cuboidal epithelium and polyhedral cells line the functional portion of sebaceous glands. The cuboidal cells lie on the basement membrane and are covered by several layers of the larger polyhedral cells. Many of the surface cells show degenerative changes in the cytoplasm and nucleus, while others are detached and fragmented in the lumen of the gland. In agreement with Goldsberry and Calhoun's (1959) findings for Hereford and Aberdeen Angus cattle and Sinha's (1964b) findings for Holstein cattle, sebaceous ducts in the eyelids of cattle used in this study always opened between the follicular folds and the epidermis. This is in contrast to the observations of Smith (1960) and Marcarian (1962) who noted that the sebaceous ducts of swine open into the hair follicle between the follicular folds and the hair bulb. No morphological differences between the typical sebaceous glands and the glands of Zeis were observed (fig. 5 and 6).

Sweat Glands: Glands of two distinct histologic types are encompassed within the cutaneous layer of the eyelids. Saccular glands are associated with the general cover hairs and these glands vary from slightly serpentine (fig. 8) to highly coiled (fig. 5). Coiled tubular glands are associated with the eyelashes and other large hairs, especially near the medial fissure of the eyelids (figs. 6 and 7).

The saccular type sweat glands possess a thin wall and large lumen (fig. 9). They are usually located very close to the hair follicle into which their ducts terminate and as a rule the bodies of these glands extend from about midway along the hair follicle to or slightly below the hair bulb. The morphology of the saccular glands is uniform throughout and as they ascend along the hair follicles, their diameters decrease abruptly, giving rise to a single narrow excretory duct for each gland. The ducts are lined by simple cuboidal epithelium except near their terminations where they penetrate the stratified squamous epithelium of the hair follicle.

Histologically, the gland wall is made up of three layers. The outer layer consists of circularly arranged collagenous fibers which blend with the surrounding fibers of the dermis. The inner layer of the gland wall consists of simple columnar epithelium. The middle layer which is very thin encompasses myoepithelial cells which lie between the glandular epithelium and the connective tissue layers. The myoepithelial nuclei are spindle shaped and lie parallel to the basement membrane but appear to spiral around the body of the gland. The cytoplasmic processes, described by Chiquoine (1958) were not seen in this study but the central mass of cytoplasm which surrounds the nucleus is prominent, especially when myoepithelial cells are cut diagonally (fig. 9). In this type of gland, the lining cells are extremely low, and in many instances they appear to be more squamous than cuboidal. The cytoplasm stains noticeably basophilic, and the boundaries between cells are not visible. The nuclei of the lining epithelium are oval and dense with prominent nucleoli.

Coiled tubular sweat glands are restricted to the eyelashes (Moll glands) and to a few large hairs near the medial and lateral fissures of the eyelids (figs. 6 and 7). They differ from saccular glands in that the epithelium is tall columnar in contrast to the flat cells of saccular glands. Further, their bodies are highly coiled; they are more deeply placed, and their walls are thicker. Histologically, the walls of these glands, also consist of three layers: the outer connective tissue layer, the middle myoepithelial layer, and the inner epithelial layer. The outer connective tissue layer and the middle myoepithelial layers are similar to the same layers in the saccular glands, but the inner epithelial layer consists of simple cuboidal to tall columnar epithelium. In some cases the epithelium appears dome-like, while in others it appears to have brush borders. The cytoplasm is basophilic, and contains no granules or droplets. The nuclei are oval with densely arranged chromatin granules and prominent nucleoli. The transition from the gland to its duct is abrupt, and as the duct ascends, its simple cuboidal epithelium becomes stratified squamous after entering the epidermis.

Histochemically, the gland cells and the substances within the lumina are positive for neutral mucopolysaccharides, but negative for acid mucopolysaccharides. Although small quantities of lipoidal substances are in the luminal contents, the lining epithelial cells are negative for lipids, and the cytoplasm is completely agranular.

The Conjunctional Layer

Many authors (Price et al. 1960; Smythe 1958; Trautmann and Fiebiger 1957), agree, generally, that the conjunctiva is a mucous membrane which consists of a superficial layer of epithelium and a supporting layer of connective tissue--the lamina propria. The conjunctiva begins on the bulbar side of the lid margins and covers the bulbar surface of the eyelids, all free surfaces of the nictitating membrane (fig. 4), as well as the surfaces of the eyeball. According to the above authors, that part of the conjunctiva which lines the eyelid is the palpebral division and that over the eyeball is referred to as the bulbar conjunctiva (figs. 2 and 5). As the palpebral conjunctiva reflects over the eyeball a pouch is formed and this pouch is known as the conjunctival fornix.

The Conjunctival Epithelium: Histologically, the conjunctival epithelium of Hereford and Aberdeen Angus cattle offers extreme breed contrasts. In Herefords, the type of epithelium varies greatly in different locations on the eyelids. Near the margins of the eyelids and immediately below the tarsal glands, stratified squamous epithelium predominates but between the tarsal gland and the conjunctival fornix, the epithelium changes first to transitional then to stratified columnar types. In contrast the conjunctiva of Aberdeen Angus cattle consists of stratified squamous epithelium (figs. 13 and 14) primarily, but in a few folds and crypts, small patches of transitional epithelium can be found. An additional contrast in the conjunctiva is the difference in the quantity of glandular elements of this membrane. In the Herefords, the conjunctiva is richly endowed with goblet cells and intraepithelial mucous alveoli (figs. 11 and 12). Goblet cells are randomly scattered in the stratified squamous epithelium near the lid margins, but they increase, finally forming a solid layer near the fornix and in the crypts. Frequently, the deeper layers of epithelium, especially in crypts and folds, contain mucous alveoli. A similar arrangement is present also in the fornix. In contrast, a few goblet cells are seen in the conjunctiva of Aberdeen Angus cattle but these are scattered and do not form large masses or a continuous layer. Even the crypts and fornices are free of large glandular collections.

Histochemically the goblet cells, including those of stratified squamous epithelium, the intraepithelial alveoli and secreted substances along the surface of the membrane are PAS and Alcian Blue positive (fig. 12), but are negative for lipids.

As may be expected, pigment deposits in the epithelium are much heavier in Aberdeen Angus than in Hereford cattle. In Aberdeen Angus, it is heaviest near the lid margin, but gradually decreases toward the fornix, however, small deposits are seen throughout, even in the fornix. In the Herefords, only those sections taken from the lid with red cover hairs show a measurable quantity of pigment. The deposits are usually sparse and disappear near the deep end of the tarsal gland. In the conjunctival fornix, glandular elements are maximal and with few exceptions, the epithelium is stratified squamous in the Aberdeen Angus, but is either transitional or stratified columnar in the Herefords. Since the fornix and the bulboconjunctiva are continuous, it seems logical that whatever histologic characteristics are found in one region should also be found in the other. This is indeed true in both breeds, however, the same breed differences, as described for the palpebral conjunctiva, persist over the eyeball. In Aberdeen Angus, goblet cells are few and the epithelium is stratified squamous with a thin stratum corneum becoming prominent near the limbus. In Herefords, stratified squamous epithelium predominates near the limbus and even in this area, an occasional goblet cell is seen.

The junction of the corneal and bulboconjunctival epithelium of the eyeball was studied with extreme interest because some investigators

(Prince et al. 1960) reported that "utricular and saccular glands" (of Manz) are found in the limbal region of the eyeball of cattle. Further interest was stimulated by the observation that 75% of ocular cancer in Hereford cattle begins at the limbus (Russell et al. 1956). Although this investigation encompasses only the normal, it became a challenge to try and find some breed differences in this region that might have been suspected as predisposing to ocular squamous cell carcinoma in Hereford cattle. Although Prince et al. (1960) stated that numerous "utricular and saccular glands" (Manz) are found at the limbus in the eyes of cattle, the ages and breeds of cattle used in their study were not given. In contrast, no glands were found at the limbus of cattle used in this study. It is established that in swine (Aureli and Kornerup 1949), glands of Manz are present in very young pigs. These glands are said to reach their greatest development in late prenatal life, then become atresic and disappear completely in early postnatal life. It is probable that a similar situation may exist in Hereford and Aberdeen Angus cattle, although no proof is offered in this investigation because only adult animals were studied. Although no glands were found at the corneoscleral junction, the surface epithelium in this narrow zone is much thicker than that over the sclera or the cornea. This is true of both breeds. The corneal epithelium was typical of general textbook descriptions (Bloom and Fawcett 1962; Copenhaver 1964; Trautmann and Fiebiger 1957).

Conjunctival Lamina Propria: The lamina propria in the bulboconjunctiva is very loose and contains very few free cells, however, pigment bearing cells are common. The pigment-bearing cells are more numerous near the dense collagenous layers of the sclera and this is especially noteworthy in that these melanocytes are also prominent in Herefords, even in those with white eyelids. Organized lymphoid tissue is absent as well as smooth and skeletal muscle and the only glands found in the lamina propria of the bulboconjunctiva are the accessory lacrimal glands located very close to the fornices. The lamina propria of the palpebral conjunctiva consists of loose areolar connective tissue but relatively coarse collagenous fibers are frequently seen (fig. 12). Unlike the lamina propria of the bulbar conjunctiva, that of the palpebral membrane has a large number of free cells (figs. 11, 12, and 13), dense organized lymphoid tissue and in places, lobules and ducts of the accessory lacrimal glands (fig. 14). Melanocytes are absent in the lamina propria, although pigment granules are very prominent in the epithelial lining. The lymphoid tissue frequently invades the lining epithelium so that only one or two surface layers of cells can be identified. The distribution and percentages of free cells in the palpebral conjunctiva resemble those given earlier for the cutaneous layer of the eyelids. Adipose tissue is absent except for a small amount near the fornix.

Middle Layer of the Eyelids

The term "middle layer," as used in this investigation is meant to encompass all structures in the eyelid that lie between the cutaneous and the conjunctival membranes. It consists of a very expansive stroma of connective tissues, a large quantity of skeletal and smooth muscle and the tarsal apparatus (fig. 5). The various elements of this layer are embedded in the expansive connective tissue which fuses with the dermis of the cutaneous layer and with the lamina propria of the conjunctiva. In the middle layer, coarse collagenous fibers predominate and are arranged in large bundles, which are encircled by finer fibers. Most of the bundles lie parallel to the epithelial surfaces and some of them extend from one side of the eyelid to the other, while others are directed from the lid's margin to its base, but there are no vertically arranged bundles. The large bundles are frequently separated by areolar connective tissue septa in which numerous nerve trunks and blood vessels lie. Branches from the nerves and arteries communicate with plexuses in the dermis and conjunctival lamina. Near the bony margin of the orbit, the connective tissue of the middle layer bifurcates, one branch continuing with the skin and the other with the intra-orbital fascia. Numerous lobules of adipose tissue are seen in the intra-orbital branch of the connective tissue, but rarely appear in other parts of the eyelids.

Bloom and Fawcett (1962) described a well defined tarsal plate in man which separates the conjunctiva from the rest of the eyelid but no welll formed tarsal plate was evident in this investigation of the eyelids of Hereford and Aberdeen Angus cattle. The connective tissue which encircles the tarsal (Meibomian) gland however, is more compact than in other areas, but is very restrictive in distribution. In areas which are usually labelled as "tarsal plate" by most authors, collagenous fibers maintain a pattern which resembles other parts of the eyelid. Immediately distal to the deep end of the tarsal gland a layer of coarse elastic fibers is present. These fibers are arranged parallel to the conjunctiva and extend toward the bony attachment of the eyelid. It is interesting to note that this elastic membrane persists in the middle-third of the eyelid but is absent in the medial and lateral thirds. Many bundles of smooth (arrectores ciliorum) muscles attach to this elastic membrane and become especially prominent near the tarsal glands around which most of the bundles arch as they ascend toward the cilia. Some of the muscles insert on the follicles of the cilia while others continue toward the epidermis and terminate in the papillary layer of the dermis.

The tarsal (Meibomian) gland is histologically and histochemically similar to the sebaceous glands of the cover hairs and the eyelashes (fig. 5). They differ, however, in that they excrete directly on the surface of the margins of the eyelids. There is a single large excretory duct for each gland, and all lobes of the gland communicate with this large duct by means of smaller secondary ducts. The orifices of the ducts are grossly visible along the margins of the upper and lower eyelids. In this investigation, the average number of tarsal glands in Hereford cattle is 35 in the upper lid, and 29 in the lower lid, while in Aberdeen Angus cattle, the average is 35 and 30 respectively. In both breeds, the range was from a maximum of 37 in the upper lid to a minimum of 27 in the lower (Table 3).

Acheson (1938) described a large smooth muscle in the eyelid and nictitating membrane of cats. He found that one smooth muscle arises from the fascia of the ventral rectus muscle, bifurcates and sends one branch into the lower eyelid and the other branch into the nictitating membrane. The second muscle arises from the fascia of the medial rectus muscle, bifurcates and sends one branch to the upper eyelid and the other to the nictitating membrane. In this investigation those muscle branches which enter the eyelids lie parallel to the conjunctiva and separate the lamina propria from other connective tissues of the eyelids. This broad sheet of muscle (Müller's) terminates among the connective tissue fibers of the "so-called" tarsal plate. It appears that the smooth muscle in the eyelids corresponds to the superior palpebral muscle described by Copenhaver (1964). From observations of the location of this muscle in relationship to other structures, it appears that contraction of the superior palpebral muscle should retract the eyelids and enlarge the palpebral fissure. Since smooth muscle is under the control of the autonomic nervous system, it seems logical that the superior palpebral muscle should be synchronized with constrictor and dilator muscles of the pupil which are controlled by autonomic innervation. Hall (1959) showed that stimulation with adrenalin causes immediate dilation of the pupil and retraction of the eyelids,

Table 3. Tarsal (Meibomian) Gland Count

	Code	Right Eyelids Upper	Right Eyelids Lower	Left Eyelids Upper	Left Eyelids Lower
Hereford	A	33	29	34	29
Hereford	B	36	28	35	26
Hereford	C	35	29	37	32
Hereford	D	37	29	36	32
Hereford	E	34	30	34	29
Hereford	K	35	29	35	30
Hereford	N	35	29	34	29
Average		34.5	29.0	34.5	29.5
Aberdeen Angus	F	36	30	36	31
Aberdeen Angus	G	35	30	35	29
Aberdeen Angus	H	38	31	37	30
Aberdeen Angus	I	32	30	33	33
Aberdeen Angus	J	34	28	34	29
Aberdeen Angus	L	36	30	34	29
Aberdeen Angus	M	34	28	36	31
Average		34.5	29.5	34.5	30.3

probably by contraction of the superior palpebral muscle, thus, placing it under the control of the sympathetic system.

In addition to the smooth muscle of the eyelids, large quantities of skeletal muscle persist. Although several muscles contribute to the skeletal musculature of the eyelids, the orbicularis oculi muscle makes up most of the skeletal muscle mass. The fibers of the orbicularis oculi muscles are arranged in small bundles which form a ring or sphincter around the palpebral fissure (fig. 5). In the middle third of the eyelids, the muscle bundles spread out, and their ratio to connective tissue is very low but in the lateral and medial thirds of the lids, the ratio is reversed. The orbicularis muscle fibers converge at the medial canthus and insert on the medial palpebral tendon which attaches to the lacrimal bone. White fibrocartilage is prominent at the point where the medial palpebral tendon attaches to the lacrimal bone. Numerous small bundles of muscles interdigitate with the cilia and the ducts of the tarsal glands. The fibers are small in diameter and appear to lie parallel to the margins of the eyelids. According to Prince *et al.* (1960), these small muscle bundles are known as the muscle of Riolan.

Accessory Glands of the Eyelids

All the accessory glands of the eyelids are restricted to the lamina propria of the conjunctiva, to the deeper connective tissue of the eyelids and to the lacrimal caruncle (figs. 14 and 17). Gland sizes vary considerably, but the morphology is constant. Usually, there are 4-8 small lobules made up of acini and several intralobular and interlobular ducts. From each group of lobules, many excretory ducts open into the conjunctival crypts (fig. 14). The acini are lined by simple columnar epithelium and simple cuboidal epithelium lines the ducts. Histologically, the glands appear to be serous, but histochemically some segments of the acini are PAS and Alcian Blue positive for mucopolysaccharides, while other segments are negative. Thus, it appears that the accessory glands of the eyelids are seromucoid. Large excretory ducts from the accessory glands open into the crypts and folds of the conjunctiva, while those from glands in the caruncle opened on the surface of that structure. Accessory glands appear to be randomly scattered, but are more frequently found near the fornix of the conjunctiva. A few are located in the lamina propria of the palpebral conjunctiva.

Medial Canthus and Related Structures

The medial canthus is a space formed by the converging and fusing of the upper and lower eyelids on the nasal side of the eye (fig. 1). As the eyelids fuse, they thicken and form a hairless groove which extends 10 to 15 millimeters medially. The epidermis over the groove becomes thick and cornified. Immediately beneath the groove and paralleling it is the medial palpebral tendon on which the orbicularis oculi muscle inserts. Other structures related to the medial canthus are: nictitating membrane, the lacrimal caruncle and the lacrimal punctae.

The Lacrimal Punctae: The lacrimal punctae are the orifices of the lacrimal canaliculi through which tears reach the lacrimal sac and the nasolacrimal duct. There is one punctum on each eyelid and the one on the upper lid lies opposite that of the lower eyelid. They are about 2-3 mm. from the angle of the medial canthus and are located at the junction of the palpebral conjunctiva and epidermis of the lid margins. The orifices appear slit-like and measure from 0.5 to 1.0 millimeter. The canaliculi are lined by stratified squamous epithelium and the lamina propria consists of fine collagenous and elastic fibers.

There are many free cells in the lamina propria but organized lymphoid nodules are absent.

The Lacrimal Caruncle: Projecting into the medial canthus of the eye is the lacrimal caruncle. Grossly, this structure is a rounded elevation measuring 2 to 3 mm. in diameter and located in the angle formed by the upper and lower eyelids (fig. 1). As expected, the caruncle is heavily pigmented in Aberdeen Angus cattle but in the Hereford pigment is absent in the caruncle of eyes with white lids and is light to moderate in the caruncle of eyes with red eyelids. As a rule, from five to ten fine hairs are located on the caruncle but with the dissecting microscope one could see a large number of even smaller hairs.

Microscopically the covering epithelium of the caruncle is continuous with the conjunctiva. It is characteristically stratified squamous and a few goblet cells are present in the surface layers but in the fornix formed between the eyelid and the caruncle, goblet cells seem more numerous. Coarse collagenous fibers make up the main mass of the caruncle and only a few elastic fiber persist. The structure of the hairs and follicles is typical but the sebaceous glands are poorly developed. The sebaceous glands consist of one or two small lobes and always attach to the hair follicle. There are no sweat glands associated with the hairs of the caruncle, however, several tubulo-alveolar glands are present. They consist of several small lobules and are located deep in the connective tissue of the caruncle. The epithelium of the functional units is simple cuboidal and that lining the excretory ducts is simple cuboidal in smaller ducts but becomes stratified cuboidal in larger ducts. Frequently, lymphoid nodules invade the glands and in some instances the epithelium is obliterated. These glands and their contents, as well as many of the cells which line the larger ducts, are positive for acid mucopolysaccharides. The ducts open on the caruncular surface and are independent of the hair follicles. Another noteworthy feature of the caruncle is the absence of both smooth and skeletal muscle tissue. The deep connective tissue of the caruncle is continuous with that which fills the conjunctival fornix at the medial canthus.

Nictitans-Harderian Complex

The nictitating membrane, Harderian gland and nictitans gland are being considered as one topic because they are so closely related morphologically that it is difficult to discuss one structure without discussing the others.

The Nictitating Membrane

The nictitating membrane in Hereford and Aberdeen Angus cattle is a thin "spade-like" structure located at the medial canthus of the eye (figs. 1, 3, and 4). It extends as far as the junction of the medial and middle thirds of the upper eyelid and to the middle third of the lower eyelid. The free margin arches, forming a crescent which conforms to the corneoscleral junction so that the membrane does not cover the cornea when in its normal position (fig. 2). The nictitating membrane is concave on its bulbar surface and conforms to the contour of the eyeball. The soft tissues of the nictitating membrane are formed by the conjunctiva which reflects from the eyelids anteriorly and from the sclera of the eyeball posteriorly. The nictitating membrane appears as an evagination between the eyelids and eyeball which carries a fold of conjunctiva with it, thus forming two fornices: one fornix is located between the membrane and the eyelids and the other between the membrane and the eyeball.

Histologically, the conjunctival epithelium of the nictitating membrane varies from stratified columnar to stratified squamous and transitional types and is usually four to six cell layers thick. In the

Aberdeen Angus the nictitating membrane is heavily pigmented near its free margin (figs. 3 and 4), but in the Hereford pigments deposits are light and sparse. The conjunctiva of the nictitating membrane is similar in structure to that of the eyelids. The lamina propria consists of fine elastic and collagenous fibers; capillary and nerve plexuses are prominent and a few bundles of smooth muscle fibers are located near the fornix.

Extending through the center of the membrane is a plate of "T-shaped" hyaline cartilage (the nictitans cartilage). The horizontal segment of this "T-shaped" cartilage forms a rigid support for the nictitating membrane and lies parallel to its free margin. This segment is very thin, averaging from 0.2 to 0.4 millimeters in thickness. The vertical segment of the "T-shaped" hyaline cartilage extends from the center of the horizontal bar to the medial canthus of the eye (figs. 3 and 4). Upon reaching the canthus, the cartilage continues into the orbit of the eye for one to two centimeters where it lies between the medial rectus muscle and the lacrimal bone and is embedded in the intraorbital connective tissues. This vertical segment thickens posteriorly, finally reaching 1.5 to 2.0 millimeters at its intraorbital termination. The perichondrium of the cartilage is continuous with the lamina propria of the conjunctiva anteriorly and with the septa of the nictitans gland on its intraorbital portion. Lymphoid tissue with active germinal centers and numerous free cells are very prominent, and the lymphoid elements frequently invade the conjunctival epithelium of the nictitating membrane. Scattered throughout this epithelial lining are numerous goblet cells and isolated mucous alveoli. Prince et al. (1960) referred to such mucous lobules in the dog as intraepithelial glands. Isolated tubulo-alveolar lobules are located in the deeper connective tissues and their ducts open on the surface of the membrane. Glandular elements are much more prominent in Hereford than in Aberdeen Angus cattle and are similar to those described in the conjunctiva. All goblet cells and glandular elements, even the goblet cells of the excretory ducts are Alcian Blue positive for acid mucopolysaccharides.

The Nictitans Gland

The gland which is now known as the nictitans gland was formerly labelled Harderian gland. Soon thereafter, this structure took the name of its discoverer.

The nictitans gland in Hereford and Aberdeen Angus cattle encircles and embeds the intraorbital shaft of the nictitating cartilage, and is thinner on the surface toward the eyeball and medial rectus muscle against which it rests than it is on the nasal side (figs. 2, 3, 4, and 18). Beginning at the apex of the nictitating membrane near the fornix, the nictitans gland extends a short distance beyond the deep end of the cartilage where it becomes continuous with Harderian gland.

The nictitans gland consist of tubulo-alveolar secretory units which are arranged into lobules. Encircling the entire gland is a thick, dense, collagenous capsule which gives rise to numerous interlobular septa that pass through the gland and attach to the perichondrium. Many large bundles of smooth muscle intermingle with the fibers of the capsule, but these muscle fibers are restrcited to that part of the capsule which rests against the medial rectus muscle of the eyeball. These smooth muscle fibers enter the eyelids and attach to the tarsal plate, thus forming Müller's muscle. The tubulo-alveolar units of the lobules consist of pyramidal epithelial cells which contain many cytoplasmic granules and globules. The cytoplasm is eosinophilic and in most cases the free ends of the cells completely fill the lumina. But, when the lumina are not completely closed, only a small space remains. The nuclei are spherical, very dense and basally placed. Although nucleoli are present, they are difficult to observe because of the density of the nuclear chromatin. In cross sections of alveoli the number of transected cells range from eight to twelve but those averaging ten transected cells are more common.

Upon routine histologic observation, it is difficult to determine if the nictitans gland is mucous or serous because it possesses some characteristics of both types. On the basis of staining qualities it is concluded that the nictitans gland is seromucoid. Some nuclei are typical of serous glands and some are typical of mucous glands but the cytoplasm appears to be characteristic of mucous glands in most acini. Approximately one-fourth of the acini show at least some positive reaction for mucopolysaccharides, but very few are totally positive. Most of the partly positive acini contain only a few reactive cells and in some cells only the distal half is positive.

Within the lobules the smaller ducts are lined by simple cuboidal epithelium while interlobular ducts are lined by simple columnar epithelium. Large excretory ducts possess stratified columnar epithelium in which mucin positive cells are common. The nictitans ducts terminate in large excretory ducts which are common to both the anterior and posterior lobes of the Harderian gland as well as the nictitans gland. Frequently, the acini are separated by adipose tissue but the dense septa and capsule are not invaded by fat (fig. 18).

The Harderian Gland

The gland of Harder is not a separate and independent unit in Hereford and Aberdeen Angus cattle as it is in the pig and rabbit. Harderian gland is attached to the deep or intraorbital end of the nictitans cartilage and is enclosed by the same connective tissue sheath that encloses the nictitans gland (figs. 2, 3, and 4). The anterior end of Harder's gland is attached to the nictitans cartilage by the common connective tissue capsule while a narrow band of glandular tissue connects it to the nictitans gland. This connecting band lies on the bulbar side of the cartilage and is so arranged that the exact point of transition between the two glands cannot be determined. Prince et al. (1960) considered the groove formed by the ventral oblique muscle as the point of junction between the two glands.

Davis (1929) observed that in the rabbit two lobes of the Harderian gland (one red and one white) can be identified grossly. Although two lobes of Harder's gland can be identified microscopically in Hereford and Aberdeen Angus cattle, gross identification of the two lobes in these breeds could not be made. The larger anterior lobe of Harder's gland forms the base and major portion of the gland and attaches directly to the nictitans gland. The posterior lobe forms a small mass near the apex, partly covering the anterior lobe.

Histologically, and histochemically the larger anterior lobe is similar to the nictitans gland with which its glandular substance is continuous. Because of this close similarity, the anterior lobe will not be discussed here. It appears to this investigator that the anterior lobe of Harder's gland and the nictitans gland should be considered as a single gland because of their histologic and histochemical similarity, and the continuity of their glandular substances. The posterior lobe of Harder's gland is obviously different and should be considered as such. A similar observation was made by Sinha (1964a) in the sheep. In contrast to the anterior lobe of Harder's gland as well as the nictitans gland, the posterior lobe of Harder's gland is Alcian Blue and PAS negative. The acini of the posterior lobe consist of simple cuboidal epithelium which forms very large lumina. The lumina vary greatly in size, but none are as small as those in the anterior lobe of Harder's gland or those in the nictitans gland (fig. 19). The cytoplasm stains basophilic and secretory granules are absent, but the distal ends of the cells form very large globular processes which detach into the lumina (apocrine secretion). Some of the lumina and ducts contain large quantities of the detached globules (fig. 19) while others are filled with homogenous materials and it appears that the globules eventually lose their individual identity and coalesce, forming the amorphous masses which fill a large number of the lumina (fig. 20). The acini which contain the homogenous materials are quite similar to colloidal filled follicles of the thyroid gland. The nuclei are spherical and contain dense chromatin and several nucleoli. In inactive acini, the epithelial cells are very low cuboidal and occasionally, some cells appear to be squamous.

The excretory ducts of the Harderian gland converge toward the center of the gland with the septa and anastomose forming two or three large ducts. These large ducts lie in a dense core of connective tissue as they extend from Harder's gland towards the nictitans cartilage. Finally, the excretory ducts open into the folds of the third eyelid on its bulbar surface after passing through the nictitans gland (fig. 4). These major excretory ducts receive smaller ducts from the nictitans gland as well as from both lobes of Harder's gland. Stratified columnar epithelium lines the larger excretory ducts and dense patches of lymphoid tissue infiltrate their lamina propria especially near their orifices. Many of the cells which line the ducts are positive for mucopolysaccharides. No breed differences were observed.

The Lacrimal Gland

The lacrimal gland of Hereford and Aberdeen Angus cattle is located dorsolateral to the eyeball and occupies the space between the periorbital membrane and the extrinsic muscles of the eyeball (fig. 2). In about fifty percent of the animals studied, the lacrimal gland extended around the eyeball as far as the lateral canthus and in a few specimens the narrow tail of the gland (fig. 3) continued as far as the ventral rectus muscle. The size is quite variable, averaging 60 x 10 x 35 millimeters, but in one Hereford female a maximal size of 68 x 12 x 40 millimeters was reached. Immediately surrounding the lacrimal gland is a relatively dense capsule and the encapsulated gland is embedded in the intraorbital connective tissue and fat. Although the gland is usually compact its peripheral lobules intermix with fat so that distinguishing between peripheral lobules and the surrounding adipose tissue becomes difficult. In man (Bloom and Fawcett 1962), the lacrimal gland is serous, in swine it is mucous, while in goats, sheep and cats, it is basically serous but contains a few mucous cells (Trautmann and Fiebiger 1957). According to Prince et al. (1960) the lacrimal glands of cattle are serous secreting structures, however, no specific breeds were mentioned. In contrast to the observations of Prince et al. (1960) the lacrimal glands of Hereford and Aberdeen Angus cattle are seromucoid (fig. 16).

The lobules of the lacrimal glands consist of tubulo-alveolar secretory units of which mucous units predominate. In some lobules, approximately 90 percent of the alveoli are mucous while in others only 70 to 75 percent are mucous. In many alveoli, both mucous and serous elements are present. Although mucous and mixed elements predominate, some independent serous units are present. Most of the serous units are poorly organized and appear as incomplete acini or even as clusters of cells. These units were Alcian Blue and PAS negative and from this observation it is concluded that these glandular units are serous elements (figs. 15 and 16). Serous alveoli in the lacrimal glands of Hereford and Aberdeen Angus cattle consist of cuboidal cells with granular cytoplasm but the nuclei are spherical and are not pressed against the basal ends of the cells. In the mucous units the cells are tall pyramidal with flattened and basally placed nuclei. Intercellular canaliculi communicate with the lumen of each unit, and on the periphery of the lobules many tubules bifurcate forming primary, secondary and occasionally tertiary branches, the terminal units of which are functional. The cytoplasm contains numerous secretion granules but vacuoles are rare. In cross section of alveoli, the number of transected cells ranges from ten to eighteen, but those containing twelve or thirteen transected cells are more common.

Smaller excretory ducts are lined by simple cuboidal epithelium, but the larger ducts frequently contain stratified columnar epithelium. The connective tissue capsule of the lacrimal gland gives rise to the interlobular septa which extend toward the center of the gland. By the converging and anastomosing of septa, an extensive core of connective tissue is formed through the center of the lacrimal gland. The duct system anastomoses along the connective tissue septa, and upon reaching the center they enter the very large excretory ducts which extend through the connective tissue core from the caudal lobules to the anterior end of the gland. From six to eight large ducts and several smaller ducts continue from the lacrimal gland into the eyelid where they terminate by opening on the palpebral conjunctiva. Along their course the lacrimal ducts penetrate the levator palpebral superioris muscle as well as Müller's muscle before reaching the conjunctiva. Although several authors (Prince et al. 1960 and Smythe 1958) state that these ducts open into the superior conjunctival fornix, those of Hereford and Aberdeen Angus cattle vary considerably. Most orifices are found on the lateral half of the upper eyelid about halfway between the fornix and lid margin, but in two specimens a few of the ducts opened in the fornix of the lateral canthus as well as in the fornix of the lower eyelid. It should be noted, however, that orifices on the ventral lids are rare. Most of the orifices appear singly, but occasionally some lie side by side. Many of the excretory ducts contain large numbers of goblet cells.

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From serial sections of the excretory ducts, small islets and lobules of lacrimal gland located along the ducts, even near the fornix were observed. These isolated glands may be equivalent to the glands of Kraus and Wolfring that are described by Prince et al. (1960) and Bloom and Fawcett (1962) (fig. 17). The ducts of these small isolated lobules open into the conjunctival fornix like those of Kraus' glands. It is interesting to note that the lacrimal glands, and the accessory glands along the conjunctival membrane are quite similar histologically and histochemically, therefore to designate them as accessory lacrimal glands instead of Kraus' glands or Wolfring's glands appears to be more appropriate. As far as could be determined by this study, there are no breed differences in the lacrimal glands of Hereford and Aberdeen Angus cattle.

SUMMARY AND CONCLUSIONS

This investigation includes histologic and some histochemical studies of the eyelids, nictitating membrane, nictitans glands, Harderian glands and lacrimal glands of Hereford and Aberdeen Angus cattle. Animals from which specimens were taken for general studies included seven Hereford and seven Aberdeen Angus cattle which ranged from eighteen to twenty-four months of age. Additional animals of the same breed and age range were used for special studies of the duct systems, sinus hair follicles and glands of Manz.

Breed Comparisons

Breed comparisons were made and two outstanding breed differences were noted. 1) As expected, epidermal and conjunctival pigmentation reach maximal density in Aberdeen Angus cattle especially in the deeper epithelial layers. In spite of heavy pigmentation, melanophores are rarely seen in the subepithelial tissues. In contrast, pigmentation in the Herefords occasionally reaches moderate density in areas covered by red hair but is usually very sparse or even absent in areas covered by white hair. 2) The epithelial layer of the conjunctiva exhibits an extreme contrast between the two breeds. In Hereford cattle, the conjunctival epithelium is stratified squamous near the lid margins but soon changes to transitional epithelium about midway between the eyelid margin and fornix. From this point to the fornix and especially in the crypts and folds, stratified columnar epithelium predominates. Glandular elements are extremely prominent in the conjunctival epithelium of Herefords consisting of a few scattered goblet cells near the lid margins, but these increase in numbers, finally forming a continuous surface layer of goblet cells near and in the fornix. In addition, clusters of mucous cells become embedded in the deeper layers of the conjunctival epithelium and form intraepithelial glands. In contrast, the conjunctival epithelium of Aberdeen Angus cattle, for the most part, is a continuation of stratified squamous epithelium from the eyelid margins. The epithelium does become interrupted by patches of transitional epithelium about midway between the margins and fornix, however, the change is much less pronounced than that in Herefords. Near and in the fornix, stratified columnar epithelium is found in the crypts and folds but when present, relatively few goblet cells and intraepithelial glands persist. In both Hereford and Aberdeen Angus breeds, the goblet cells and intraepithelial glands are PAS and Alcian Blue positive, however, glandular elements were much more numerous in Hereford than in Aberdeen Angus cattle.

The Eyelids

The eylids are covered on all surfaces by an epithelial membrane. The covering of the outermost surface resembles a cutaneous membrane and the bulbar surface is covered by the conjunctival membrane. All structures which lie between these two membranes are encompassed by the middle layer of the eyelids. Structurally, the cutaneous layer of the eyelids resembles the skin of Hereford and Aberdeen Angus cattle as described by Goldsberry and Calhoun (1959). The middle layer of the eyelids consists of a thick layer of connective tissue in which the skeletal muscles, smooth muscles (Müller's muscle), the tarsal apparatus and the accessory lacrimal glands (glands of Krans and glands of Wolfring) are embedded. Most of the skeletal muscle bundles of the eyelids belong to the orbicularis oculi muscle, however, a few branches of the levator palpebral superioris were seen in the upper eyelid. Smooth muscle constitutes three separate groups.

A relatively large sheet of muscle enters the medial half of the eyelids and lies parallel to the conjunctiva separating this membrane from the middle layer of the eyelid. The fibers insert on the dense connective tissue in the tarsal plate region. The second group of smooth muscle (arrectores ciliorum) fibers consists of many bands which arise from elastic fibers at the deep end of the tarsal gland. These fibers arch around the glands and insert on the dermal root sheaths of the eyelashes. The third muscle group consists of the arrectores pilorum muscles which are associated with the hair of the cutaneous layer.

The Eyelids--Thickness

The thickness of the eyelids varies considerably. They are thinnest at their margins and gradually increase, reaching their thickest dimensions at the level of the conjunctival fornix. The upper lids are thickest medially and thinnest at the lateral third but the thickness of the medial and lateral sides of the lower lids is uniform.

The Eyelids--Coverings

The point of transition from cutaneous epithelium to conjunctival epithelium, at the margins of the eyelids, is difficult to determine; however a unique and characteristic difference persists at the orifices of the tarsal ducts. The epithelium on the cutaneous side of the ducts is considerably thicker than that on the conjunctival side; however, the epithelium is characteristically stratified squamous in both sides of the ducts. In addition, the papillary pegs on the cutaneous side are blunt and broad while those pegs on the conjunctival side appear narrow and pointed, resembling a "saw-tooth" arrangement.

Concentrated along the dermo-epidermal junction are numerous "clear cells." The nuclei are small and dense and the cytoplasm is clear and agranular. Although the nuclei react typically with most stains, the cytoplasm shows no affinity for the various stains used in this investigation. These clear cells are present in Aberdeen Angus as well as Hereford cattle, but they tend to be camouflaged by the dense epidermal pigment in the Aberdeen Angus breed.

The lamina propria of the conjunctiva and the dermis of the cutaneous layer of the eyelids are well endowed with free cells. Lymphocytes and plasma cells constitute about sixty percent of these free cells, mast cells about twenty percent and eosinophiles make up about five to ten percent. The remaining ten or fifteen percent include miscellaneous and unidentified cells.

Hair and Associated Structures

The eyelashes and their follicles possess the structural characteristics of typical cover hairs except for their extremely large size and the arrangement of the associated muscles. The muscles of cilia arise from the connective tissue near the deep end of the tarsal glands and insert on the dermal sheath of the ciliary follicles or in the papillary layer of the dermis near them.

Tactile or sinus hairs are common on the eyelids. They are similar to the descriptions of sinus follicles by Trautmann and Fiebiger (1957); however, the extent of the blood sinuses differs from that in the horse and cat. In Hereford and Aberdeen Angus cattle, the blood sinus does not penetrate deeper than the expanded level of the sinus hair bulb while those in the horse and cat encircle the entire hair bulb. Sebaceous glands of sinus hair follicles are highly developed and are encapsulated by the dense dermal sheath layer, but tubular or sweat glands are absent. Both smooth and skeletal muscle fibers are associated with the sinus follicles. Intrinsic bundles of smooth muscle encircle the sinus follicle while bundles of skeletal muscle ascend from deeper parts of the eyelid to insert on the dermal sheath of the sinus follicle.

The Eyelids--Accessory Glands

The eyelids are richly endowed with glands of different types. Included are sweat and sebaceous glands, the tarsal glands and numerous accessory lacrimal glands. The accessory lacrimal glands vary from a few scattered tubulo-alveolar lobules to relatively large isolated glands. For the most part, these glands are embedded in the connective tissue of the eyelids and conjunctival fornix and the relatively large ducts open on the conjunctival surfaces. These accessory glands are similar to the lacrimal glands. The so-called "Manz" glands are absent in Hereford and Aberdeen Angus cattle and no accessory glands are found in the bulboconjunctival connective tissue.

The Lacrimal Caruncle

The lacrimal caruncle which is located in the medial canthus of the eye contains numerous small hairs. The structure of the hairs and their follicles is typical, although they are much smaller than usual. Sebaceous glands are present but poorly developed and sweat glands are lacking completely. Accessory lacrimal glands occupy the deeper part of the caruncle and the large ducts open directly on its surface. It is interesting to note that both smooth and striated muscle fibers are absent in the caruncle and that dense connective tissue forms the greater part of the caruncle mass.

The Nictitating Membrane

The nictitating membrane is formed around a "T" shaped plate of hyaline cartilage. The covering epithelium resembles that of the palpebral conjunctiva and the lamina propria contains numerous mucous glands and gland ducts. On the bulbar surface of the nictitating membrane the large ducts of the nictitans and Harderian glands open into a large and prominent fold. Large lymphoid nodules as well as large concentrations of lymphocytes are found in the connective tissue of the nictitating membrane.

The Nictitans Gland

The nictitans gland surrounds and attaches to the deep segment of the nictitating cartilage. The connective tissue capsule forms strong interlobular septa which attach to the perichondrium. The lobules consist of tubulo-alveolar units. Some of these units consist of mucous cells, and some of serous cells while other acini contain both mucous and serous cells. The glandular substance of the nictitans gland is continuous with the anterior lobe of the Harderian gland.

The Harderian Gland

The Harderian gland is divided into a larger anterior lobe and a smaller posterior lobe. The point of demarcation between lobes is not seen grossly, however, histologic and histochemical differences are outstanding. Like the nictitans gland, the anterior lobe of the Harderian gland consists of seromucoid alveoli which are PAS and Alcian Blue positive. In contrast, the posterior lobe of Harder's gland contains acini with large lumina and cuboidal epithelium. The lumina are filled with a homogeneous substance which stains basophilic and appears to arise from the apocrine cells which line the lumina. The posterior lobe of Harder's gland is PAS and Alcian Blue negative.

The nictitans gland as well as both lobes of Harder's gland communicate with a pair of very large excretory ducts which open on the bulbar surface of the nictitating membrane.

The Lacrimal Gland

The lacrimal gland in Hereford and Aberdeen Angus cattle is a relatively large, lobulated gland consisting of tubulo-alveolar secretory units. Although the ratio between, serous, mixed, and mucous units varies, the mucous and mixed units predominate. And, a noticeable variation between lobules can be seen, even on the same section. Associated with the lacrimal gland are six or eight large excretory ducts and a few smaller ducts. Most of the duct orifices are located on the bulbar surface of the lateral half of the upper eyelids about midway between the lid margins and the conjunctival fornix. Occasionally duct orifices open into the fornix near the lateral canthus but this is the exception rather than the rule. The lacrimal gland is Alcian Blue and PAS positive for mucopolysaccharides.

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ILLUSTRATIONS

1. medial canthus
2, 3, 4. upper eyelid
5. lateral canthus
6, 7, 8. lower eyelid
9. nictitating membrane
10. cornea
11. sclera
12. red spots around the eye

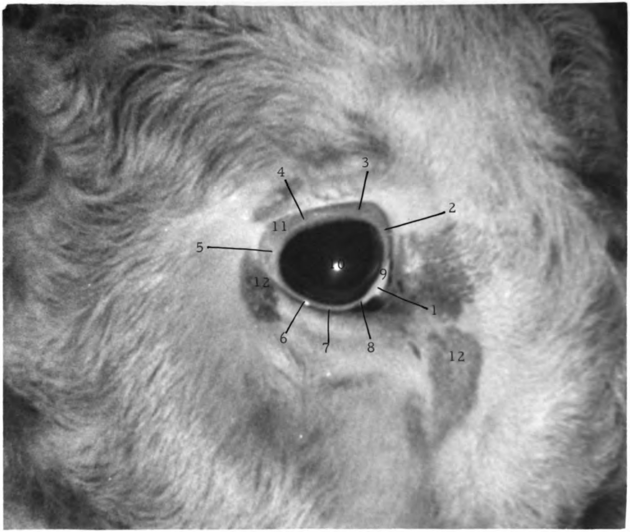

Fig. 1. Eye of Hereford bull. Lines from numbers 1-8 show planes through which sections were made.

Fig. 2. Dissected eye and associated glands.

1. lacrimal gland
2. nictitans gland around nictitans cartilage
3. Harderian gland
4. nictitating membrane, heavily pigmented
5. sclera with conjunctival covering (bulbar conjunctiva)
6. cornea
7. corneoscleral junction

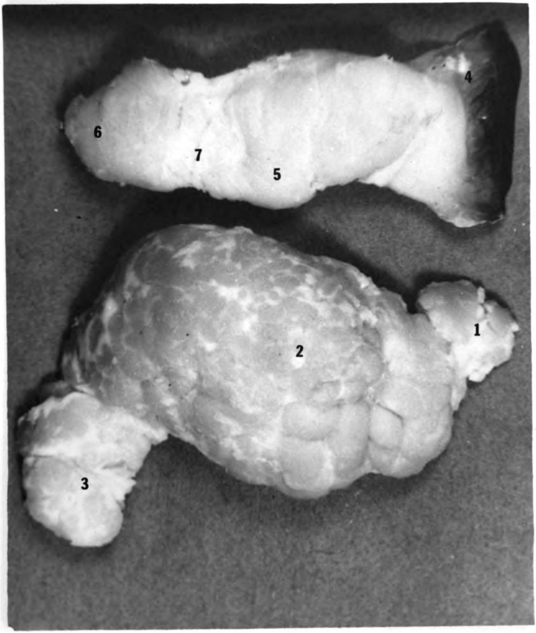

Fig. 3. Dissected lacrimal gland and nictitating--Harderian complex.

Bottom: Lacrimal gland (dorsal view)
1. anterior end
2. body
3. caudolateral end (tail)

Top: Nictitans-Harderian complex (nasal view)
4. nictitans membrane
5. nictitans gland around cartilage
6. Harderian gland
7. junction of nictitans and Harderian gland (approximate)

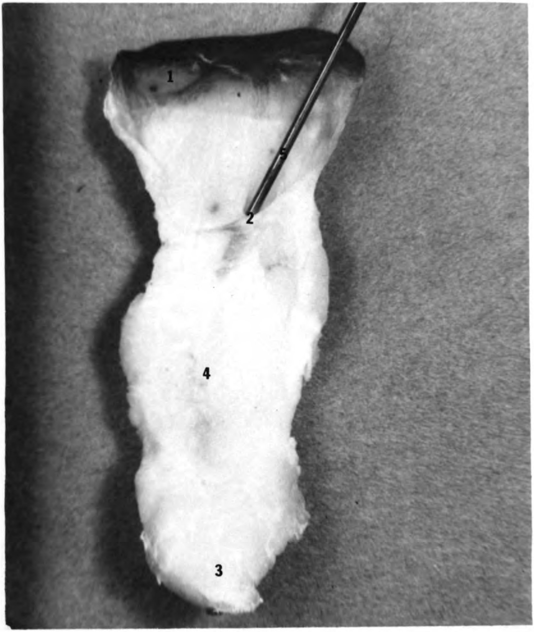

Fig. 4. Dissected Nictitans--Harderian Complex--bulbar surface.

1. nictitating membrane
2. orifice of large ducts in fold of conjunctiva on bulbar surface
3. Harderian gland
4. nictitans gland
5. probe passing through the excretory duct orifice

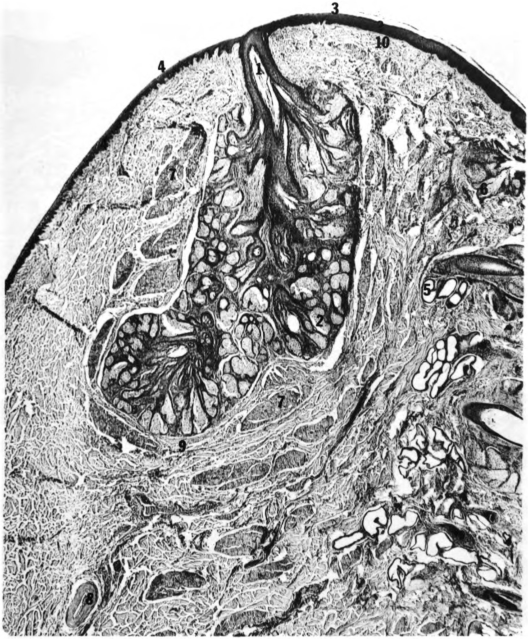

Fig. 5. Vertical section of lower eyelid of Aberdeen Angus female.

1. duct of tarsal gland
2. tarsal (Meibomian) gland
3. cutaneous epithelium of lid margin (epidermis)
4. conjunctival epithelium of lid margin
5. sweat glands of cilia (Moll)
6. sebaceous glands of cilia (Zeis)
7. skeletal muscle
8. marginal arcuate artery
9. connective tissue encircling the tarsal gland
10. dermis

Hematoxylin-eosin. 18X.

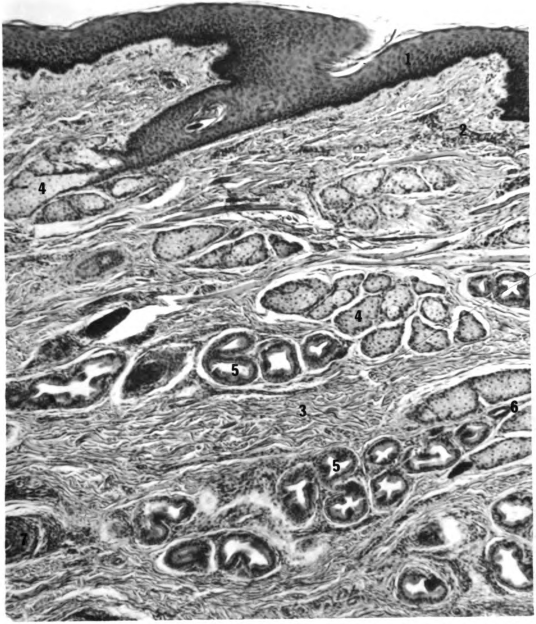

Fig. 6. Vertical section from the medial angle of the eyelid (Aberdeen Angus female).

1. epidermis with pigmented basal layers
2. free cells in papillary layer of dermis
3. reticular layer of dermis
4. sebaceous glands
5. coiled sweat gland
6. duct of sweat gland between sebaceous lobules
7. hair bulb

Hematoxylin-eosin. 60X

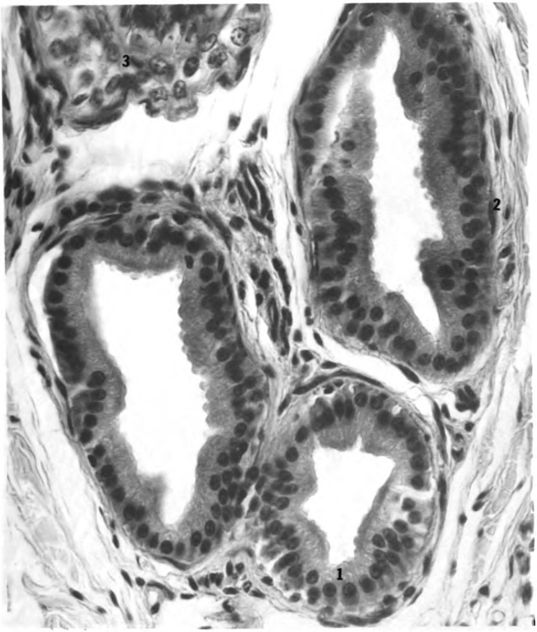

Fig. 7. Highly developed coiled tubular sweat glands from medial part of the eyelid.

1. glandular epithelium
2. myoepithelial cell
3. hair bulb

Hematoxylin-eosin. 415X

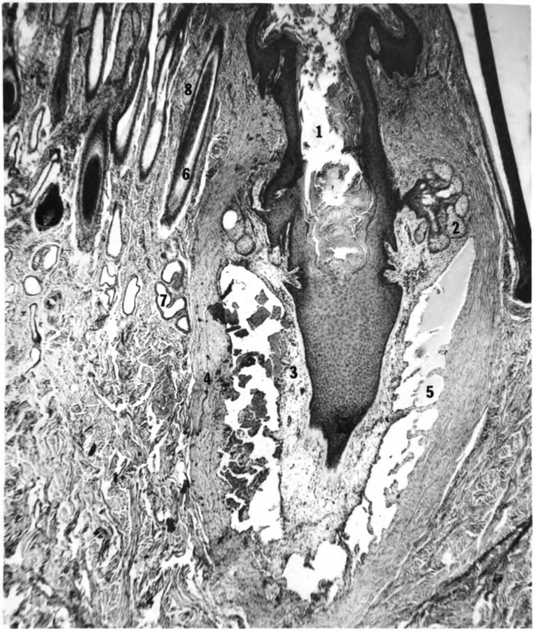

Fig. 8. Sinus hair follicle from upper eyelid (diagonal section)--Aberdeen Angus female.

1. lumen of follicle with accumulation of the stratum corneum
2. sebaceous gland of sinus hair
3. inner layer of dermal sheath
4. outer layer of dermal sheath
5. blood sinuses with trabeculae and blood
6. typical hair and follicle
7. saccular sweat glands with low cuboidal epithelium
8. sebaceous glands of regular hair follicle

Hematoxylin-eosin. 35X

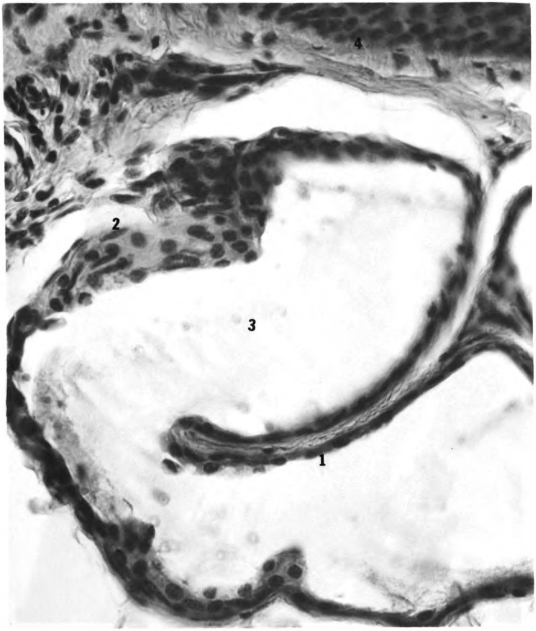

Fig. 9. Higher magnification of sweat gland from fig. 8.

1. low cuboidal epithelium
2. myoepithelial cells
3. lumen of sweat gland
4. hair root

Hematoxylin-eosin. 395X

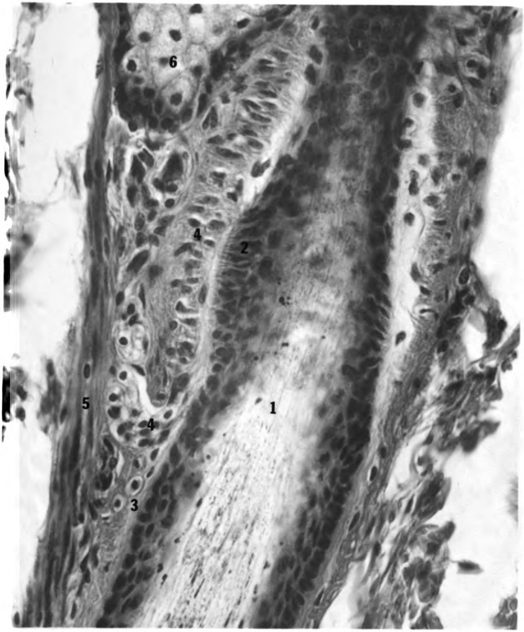

Fig. 10. Nerve plexus--hair follicle relationship.

1. hair root
2. modification of root sheath cells
3. glossy membrane
4. nerve plexus in dermal root sheath
5. arrector pili muscle
6. sebaceous gland

Hematoxylin-eosin. 395X

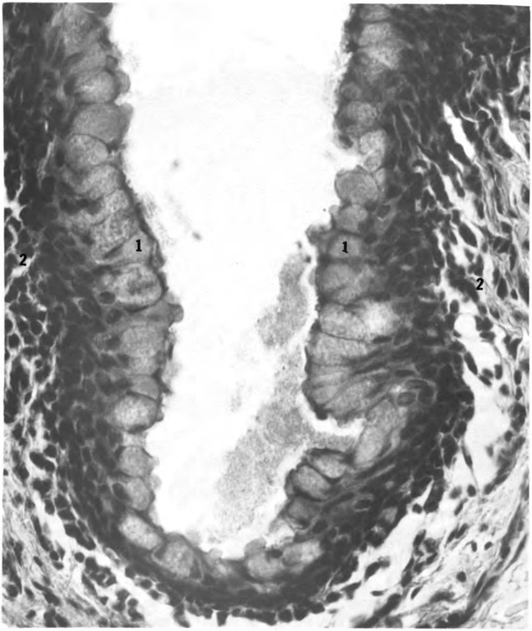

Fig. 11. Conjunctival fold on lower eyelid of Hereford female.

1. stratified columnar epithelium with surface layer of mucous cells
2. numerous free cells in lamina propria
   Hematoxylin-eosin. 420X

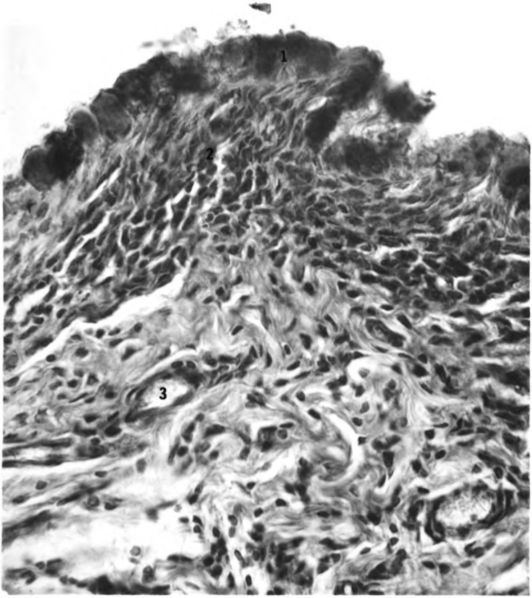

Fig. 12. Palpebral conjunctiva of Hereford female.

1. Alcian Blue positive cells (acid mucopolysaccharides)
2. lamina propria
3. blood vessels

Alcian Blue-Nuclear fast red. 420X

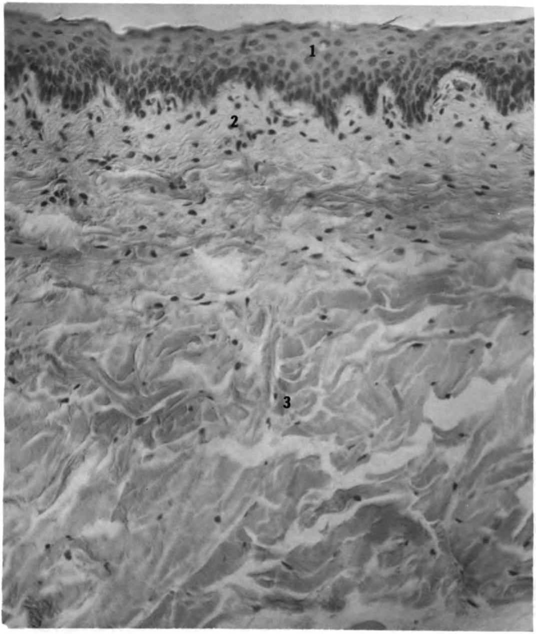

Fig. 13. Palpebral conjunctiva of Aberdeen Angus female.

1. stratified squamous epithelium
2. lamina propria
3. coarse collagenous fibers of the middle layer
   Hematoxylin-eosin. 195X

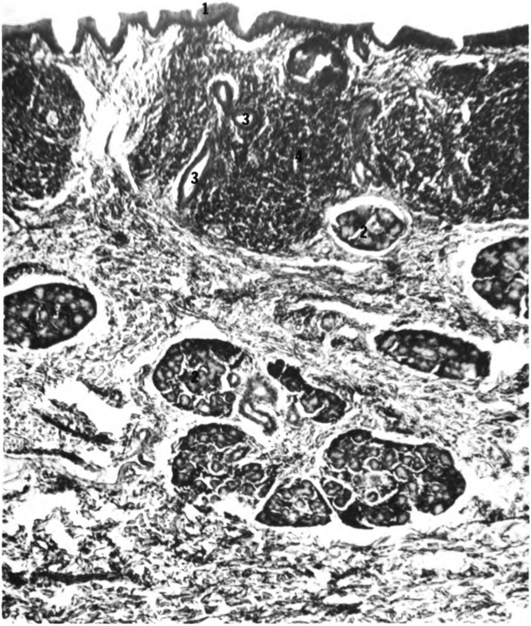

Fig. 14. Palpebral conjunctiva of Aberdeen Angus castrated male.

1. stratified squamous epithelium
2. lobules of accessory lacrimal glands
3. ducts of accessory glands
4. dense lymphoid tissue

Hematoxylin-eosin. 45X

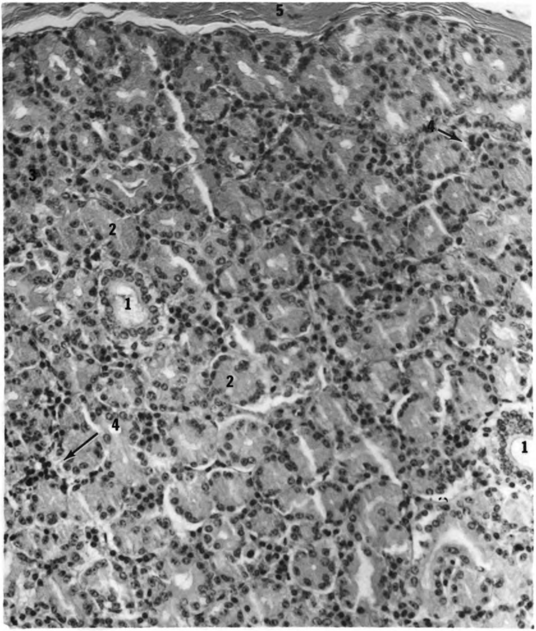

Fig. 15. Lacrimal gland.

1. intralobular ducts
2. mucous acini
3. poorly serous acini
4. clusters of plasma cells commonly seen between acini
5. interlobular connective tissue

Hematoxylin-eosin. 195X

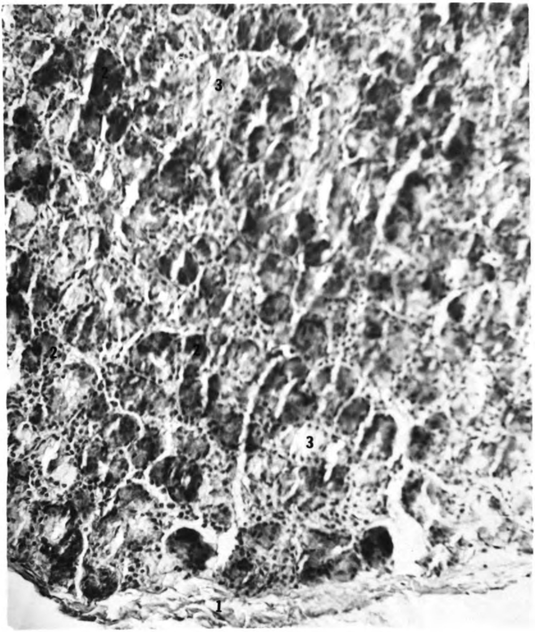

Fig. 16. Lacrimal gland showing Alcian Blue positive and negative secretory units.

1. connective tissue capsule
2. positive alveoli
3. negative alveoli

Alcian Blue-nuclear fast red. 195X

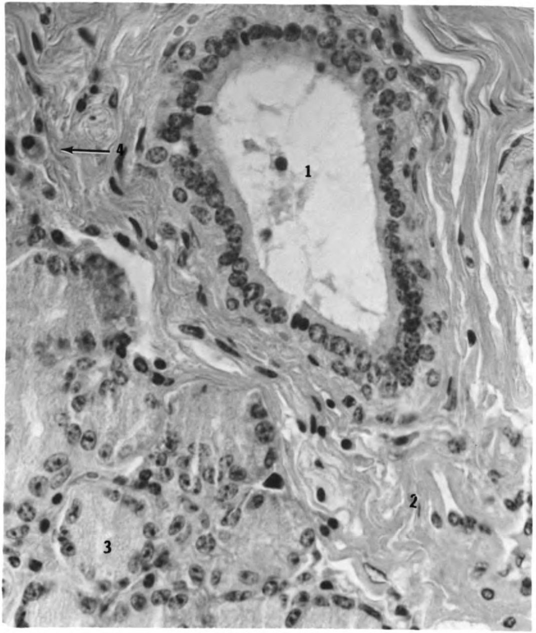

Fig. 17. Large lacrimal duct and lacrimal lobule near conjunctival orifice of duct. Hereford female.

1. lacrimal duct
2. connective tissue
3. acinus of accessory lacrimal lobule
4. plasma cell

Hematoxylin-eosin. 395X

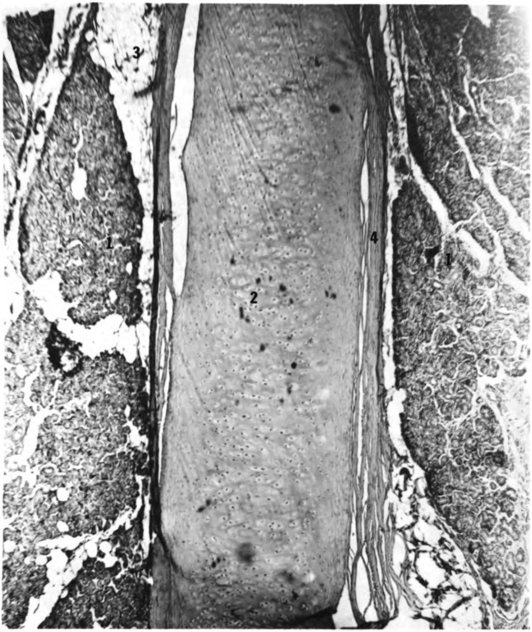

Fig. 18. Nictitans gland and cartilage.

1. nictitans gland
2. hyaline cartilage
3. adipose tissue
4. perichondrium
   Hematoxylin-eosin. 35X

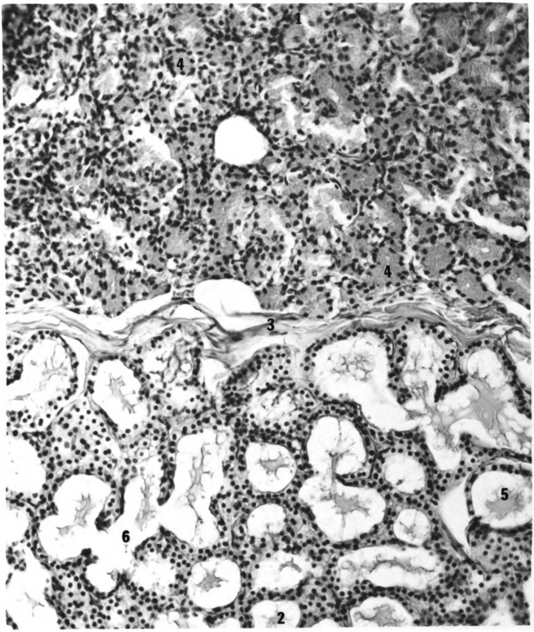

Fig. 19. Harderian gland.

1. anterior lobe (seromucoid)
2. posterior lobe (mucopolysaccharide--negative)
3. interlobar connective tissue septum
4. small acinus of anterior lobe with mucous cells
5. large acinus of posterior lobe with low cuboidal epithelium and homogeneous substance in lumen
6. several communicating acini

Hematoxylin-eosin. 195X

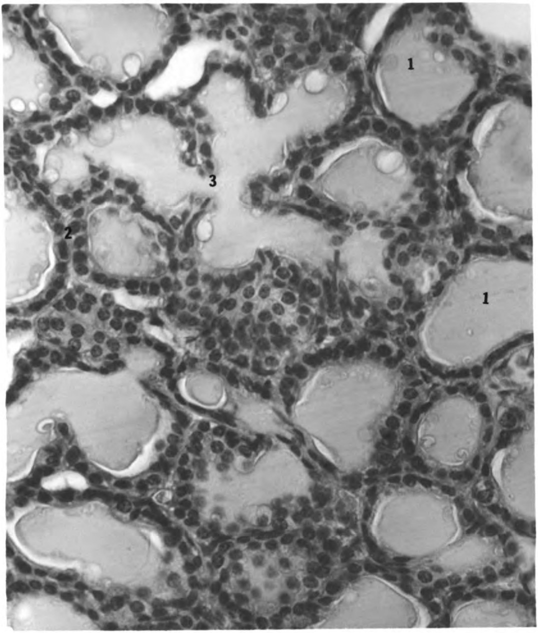

Fig. 20. Posterior lobe of Harder's gland.

1. homogeneous substance in lumen of acini
2. cuboidal epithelium with narrow interacinar septa
3. interconnecting lumina

Hematoxylin-eosin. 395X