

Normal Anatomical and Histochemical Characteristics of the Lacrimal Glands in the American Bison and Cattle

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With 10 figures

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Summary

Dorsal lacrimal glands, superior glands of the third eyelid and Harderian glands (deep gland of the third eyelid) from 19 bison and 18 cattle free of apparent ocular disease were examined to compare the normal anatomical properties of these glands. All glands were characterized and measured (length and width). The gross anatomy of the dorsal lacrimal glands was similar, with the exception of a bipartite gland in cattle. The bison's superior gland of the third eyelid and Harderian gland was longer as compared with cattle. A subset of the bison and cattle samples (five bison and five cattle) was sectioned for histological and histochemical analysis. The histology of the dorsal lacrimal and superior gland of the third eyelid revealed tubuloalveolar cells with basophilic vacuolated cytoplasm in bison and eosinophilic granular cytoplasm in cattle. The Harderian glands consisted of a tubuloalveolar anterior part combined with large lumens acini lined with cuboidal epithelium in the posterior part; the posterior part of the bison Harderian gland was more predominant than in cattle samples. Mucosubstance histochemistry revealed acidic and neutral glycoproteins with similar staining patterns in all glands of both species.

Introduction

Lacrimal glands are responsible for the production of tear fluid that helps maintain corneal health. In most species, the majority of tears are secreted from the dorsal lacrimal gland. Examination of lacrimal glands of human beings, dogs, cats, horses, pigs, rabbits, sheep, goats and guinea-pigs has been described in the literature (Prince et al., 1960; Sinha and Calhoun, 1966; Allen et al., 1972; Krochmalska, 1976; Gillette et al., 1980; Martin et al., 1988; Gargiulo et al., 2000). In general, the lacrimal and superior gland of the third eyelid are tubuloalveolar structures that produce a mucoserous secretion. Although similarities exist between the dorsal lacrimal and superior gland of the third eyelid among species, differences exist in the presence and composition of the Harderian gland. Among domesticated ungulates, cattle (*Bos taurus*) are known to possess a Harderian gland (Paule, 1957; Prince et al., 1960). Descriptions and histological analysis of the cattle lacrimal glands have been reported (Sundwall, 1907; Paule, 1957; Prince et al., 1960). The lacrimal glands of bison (*Bison bison*) have not been described in the literature to the author's knowledge.

The aim of this study was to describe and compare the normal anatomical, histological and histochemical findings of bison and cattle lacrimal glands.

Materials and Methods

The lacrimal glands of 19 bison and 18 beef cattle destined for slaughter were removed within 1 h after death. Glands from 18 bison bulls, one bison cow, six cattle steers, six cattle heifers and six cattle bulls were retrieved. Post-mortem examination of the eyes and adnexa revealed no apparent ocular disease. Length and width to the nearest millimeter were recorded for each gland and mean values were established for each group of glands. The distal end of the superior gland of the third eyelid and the proximal aspect of the Harderian gland was determined on the basis of appearance and palpation. Student's *t*-test was conducted to compare the length and width of bison and cattle lacrimal glands. Histological and histochemical analysis were performed on randomly selected samples collected from five bison (four bulls and one cow) and five cattle (two steers, two bulls, and one cow). Fixation with 10% buffered formalin for 24–48 h were performed prior to processing. Each gland was sectioned in a sagittal plane and paraffin-embedded. Sections (4 µm) were stained with hematoxylin and eosin stain and Masson Trichrome stain and examined by light microscopy for histological description. Histochemical stains for identification of glycoproteins and mucosubstances included periodic acid-Schiff (PAS), Alcian blue (AB) pH 1.0 and 2.5 with nuclear red counterstain, and high iron diamine (HID). Histochemical analyses were performed using standard protocols (Spicer and Henson, 1967) by the Kansas State University Diagnostic Laboratories.

Results

Gross anatomy

Ages were recorded for both species, the average being 2 years of age. In all glands, no differences were also noted between right and left glands (data not shown). Sex differences between species could not be established as samples from bison cows were not readily available. In cattle, no sex differences were seen between steers and heifers.

Bison and cattle possess dorsal lacrimal glands, superior glands of the third eyelid and Harderian glands. The dorsal lacrimal glands of both species were similar in appearance with the exception of an accessory lobe in cattle (Fig. 1). The main

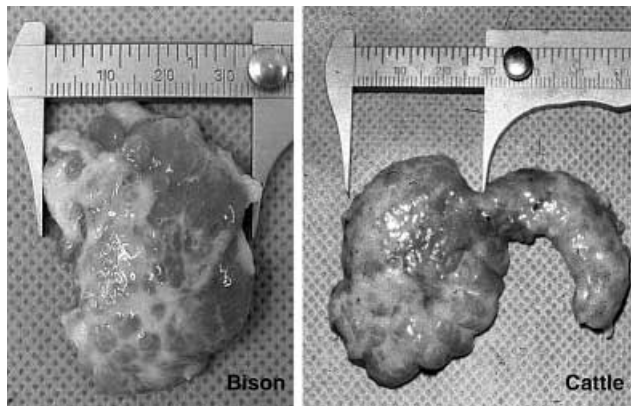


Fig. 1. Dorsal lacrimal glands of bison and cattle. The cattle dorsal lacrimal gland is a bipartite gland as compared with bison. Both glands have a lobulated appearance and are surrounded by fat.

body was flattened, oval, pale yellow and lobulated in appearance. These glands were located within a facial sheet of the periorbital at the level of the dorsolateral aspect of the globe beneath the frontal bone. The inferior surface was slightly concave, fitting over the globe. No significant differences were seen in the length and width between both species (Fig. 2).

Similarities were also seen between bison and cattle superior glands of the third eyelid. The proximal part surrounded the cartilage of the third eyelid and had a lobulated or cobblestoned appearance. In the bison samples, this gland extended significantly caudally from the distal edge of the cartilage as compared with cattle (Fig. 3). This in turn was reflected in a statistical difference in length ($t_{(35)} = 16.6$, $P < 0.0001$), but not in width, between bison and cattle samples (Fig. 2).

Both bison and cattle Harderian glands were located distally from the superior gland of the third eyelid and were encased in a single fascial sheet with the superior gland. The Harderian gland had an elongated tubular shape with smooth exterior. In both species, there was no obvious separation between the superior gland of the third eyelid and Harderian gland. In the bison, a slight narrowing was palpated at the junction where the cobblestoned appearance of the superior gland changed to a more smooth appearance of the Harderian gland (see arrow in Fig. 2). In the cattle samples, a triangular end-piece to the superior gland of the third eyelid was noted and determined to be the Harderian gland. The triangular-shaped gland had a cobblestoned proximal part and smooth-appearing distal portion (Fig. 2). Considerable differences in length were noted

with the Harderian glands (Fig. 2); bison Harderian glands were longer than cattle samples ($t_{(34)} = 12.3$, $P < 0.0001$).

Light microscopy

Histological examination of the bison and cattle lacrimal glands revealed similarities and differences between the species. The bison and cattle dorsal lacrimal glands consisted of tubuloacinar units separated by dense sheets of connective tissue into lobules. Within a lobule, single sheets of connective tissue separated acinar and tubular units from each other. The acini were composed of tall pyramidal or columnar cells with small lumens; the tubules were bordered by short columnar cells with large lumens. The tubules were seen intermingled between the nests of acini. The bison acinar cells had basophilic, granular, vacuolated cytoplasm and appeared to be arranged in a loosely irregular fashion (Fig. 4). In contrast, the cattle samples revealed a compact organized architecture of acinar cells with an eosinophilic, granular, uniform cytoplasm (Fig. 5). Cattle samples also had more cellular infiltrate between the acini. In both cattle and bison samples, the acinar cell nuclei were oval to round in shape and basally located. Inter-lobular ducts with pseudostratified lining epithelium, veins and arterioles were found in connective tissue septae that separated lobes of the glands.

The bison and cattle superior gland of the third eyelid resembled each other. In both species, tubuloalveolar units completely surrounded the cartilage shaft of the third eyelid. In general, these tubuloalveolar units were less compacted compared with the dorsal lacrimal glands of both species.

Bison Harderian gland included an anterior part that histologically resembled the superior gland of the third eyelid. The mid-to-distal part of the gland was composed of large lumen acini lined with cuboidal epithelial cells (Fig. 6). These cells contained a round to oval nucleus. The lumen often contained an acellular, finely granulated eosinophilic secretion (this was also present in cattle samples). Cytoplasmic blebs at the apical end of the cells were seen throughout the samples. This finding is characteristic of an apocrine gland. The cattle Harderian gland samples also showed a mixed gland (Fig. 7); however, the proximal portion occupied a larger portion of the gland than seen in bison. The large lumen acini lined with cuboidal epithelial cells were not as numerous or extensive in cattle compared with the bison samples. In most bison and cattle samples, distinct connective tissue septae separated the nest of acinar cells from the large lumen acini.

The Masson Trichrome-stained connective tissue septae surrounding individual acinus and tubules in bison and cattle

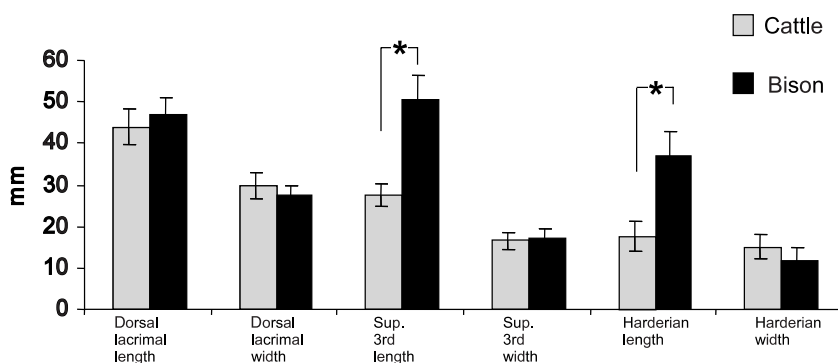


Fig. 2. Bison and cattle lacrimal gland measurements.

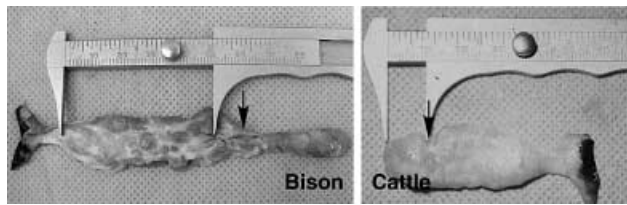


Fig. 3. The superior gland of the third eyelid and Harderian gland of bison and cattle. The margin of the third eyelid is seen in both specimens. The superior gland of the third eyelid completely surrounds the cartilage of the third eyelid and its termination is confluent with the beginnings of the Harderian gland. In the bison, the Harderian gland is a tubular-shaped gland, whereas in cattle, the Harderian gland is more triangular than tubular in shape. Slight narrowing is seen at the termination of the superior gland of the third eyelid and beginning of the Harderian gland in both species is identified by the arrow.

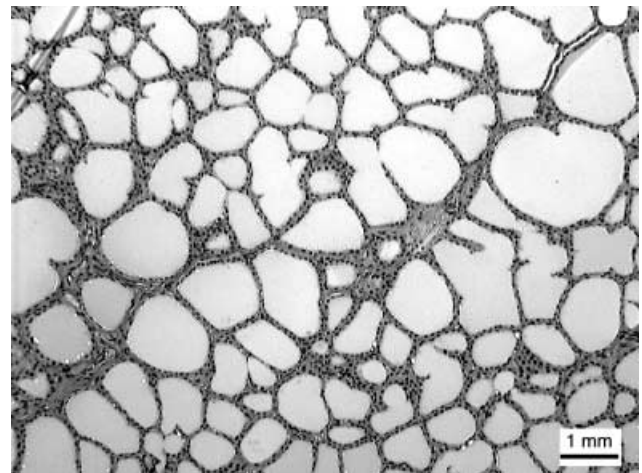


Fig. 6. The bison Harderian gland has a proximal part that resembles the superior gland of the third eyelid (not shown). The mid-to-distal part of the gland has acini with large lumen that are lined with cuboidal epithelial cells. The lumens contain an acellular eosinophilic secretion; cytoplasmic blebs can be seen originating from the cuboidal epithelium.

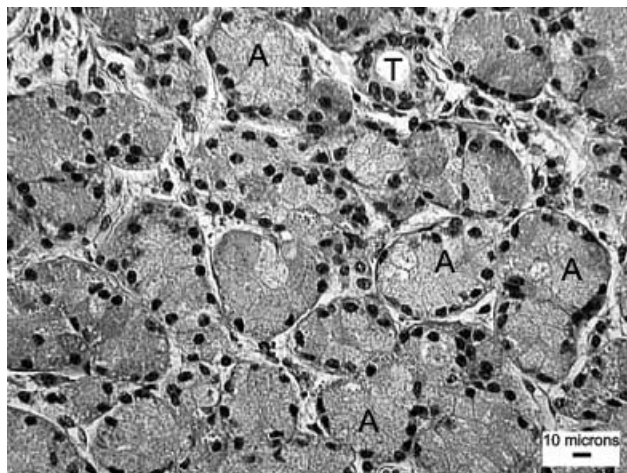


Fig. 4. The bison dorsal lacrimal gland has tubuloalveolar units that are loosely arranged throughout the gland. The acinar cells (A) have a vacuolated basophilic cytoplasm and these cells are pyramidal in shape. As shown here, the tubules have pseudostratified cells surrounding an empty lumen (T).

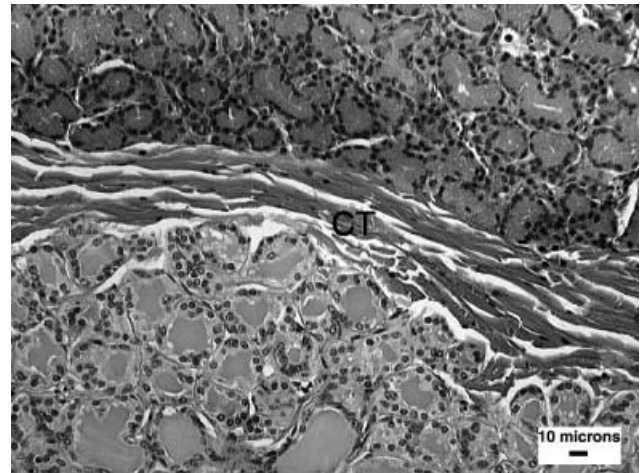


Fig. 7. The cattle Harderian gland has a proximal part containing small lumen acini and tubules and a distal part containing large lumen acini lined with cuboidal epithelial cells. Dense connective tissue septae (CT) separate the two types of acini. As seen with the bison, an acellular eosinophilic substance is found within these large lumen acini.

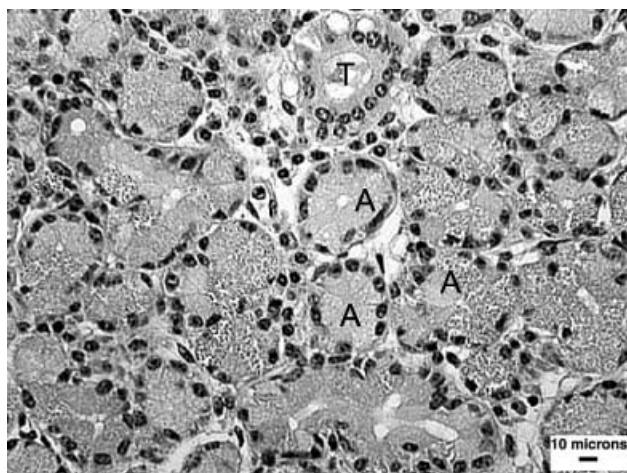


Fig. 5. Cattle dorsal lacrimal glands also have tubuloalveolar units with acini (A) lined by pyramidal cells with eosinophilic granular cytoplasm. As shown here, the tubular units (T) are less numerous than the acinar units.

dorsal lacrimal gland and superior gland of the third eyelid. Larger sheets of connective tissues were found and separated the glands into lobules. In these large sheets of connective tissue, inter- and intra-lobular ducts, as well as veins and arterioles were seen. This stain also revealed scarlet red staining of the interior acini of the Harderian glands, most prominently in the bison samples. At the distal periphery of the bison Harderian glands, central red lumens were surrounded by an unstained rim, followed by deep purple to blue staining at the gland's edge (Fig. 8).

Histochemical analysis

Similar staining patterns were seen in the bison and cattle dorsal lacrimal gland and superior gland of the third eyelid

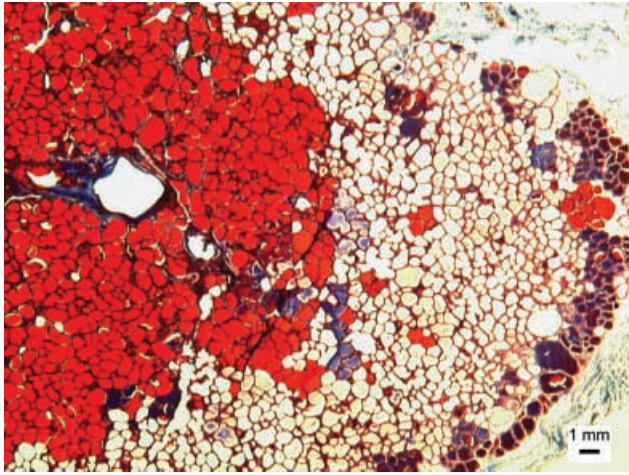


Fig. 8. The bison Harderian gland stained with Masson Trichrome revealed scarlet red-stained central acini with a rim of absence of stain, followed by dark blue-purple acini at the distal periphery.

with three different histochemical stains. However, the Harderian gland revealed staining differences between bison and cattle. PAS staining revealed the presence of positive granules in all acini of these glands (Fig. 9). No appreciable differences were seen between the species. Some acini were more heavily stained than others in both species. Stain uptake was also seen within a subset of tubules.

Alcian blue stain with pH 1.0 and 2.5 also revealed a similar staining pattern between the species. Positive granules were seen in the acini (Fig. 10). Variability of the location of the positive granules was seen between samples of both species. In some samples, the granules were only seen in the apical portion of the acinar cells. In others, the acini were completely stained with positive granules or had only one or two cells with positive staining. Grossly, more acini were stained with AB pH 2.5 than pH 1.0 in all samples.

High iron diamine staining revealed a mixture of positive (grey-blue) and negative staining acini; in the positive staining acini, some acini had only individual cells being positive,

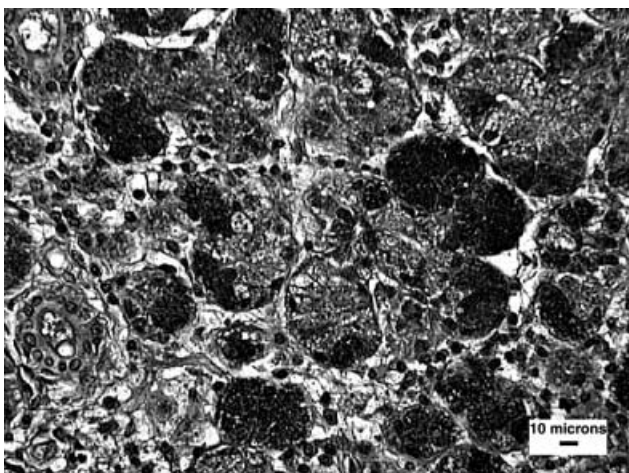


Fig. 9. The bison dorsal lacrimal gland with PAS stain. Staining variability is seen between acini and cells within an acini. Positive staining is seen also in the tubules.

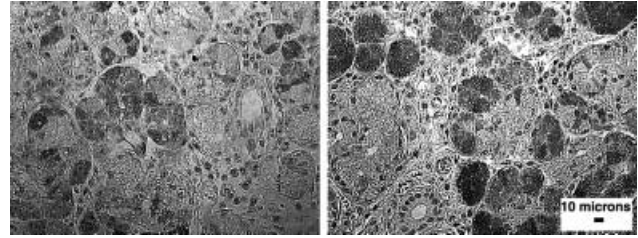


Fig. 10. Alcian blue (AB) staining of the bison dorsal lacrimal gland. AB pH 1 (left) and AB pH 2.5 (right) show a similar staining pattern in both species. The tubules did not stain with either AB stains.

whereas others were completely stained. No HID-positive granules were seen in the tubules. Staining characteristics with the superior gland of the third eyelid was identical to the dorsal lacrimal gland.

The proximal part of the bison and cattle Harderian gland had similar staining properties to the dorsal lacrimal and superior glands (described above). In the bison Harderian gland samples, neither the large lumen acini nor the lining cells contain positive staining granules for any of the aforementioned stains. In contrast, the cattle Harderian glands showed few scattered granules among the acinar cuboidal cells did reveal stain uptake for PAS, AB pH 1.0, and AB pH 2.5. No stain uptake was seen with HID in the distal portion of the bison and cattle Harderian glands.

Discussion

The lacrimal glands of bison and cattle were basically similar in appearance. Both species of animals had a lobulated appearance of the dorsal lacrimal gland and superior gland of the third eyelid, as well as a smooth appearance of the Harderian gland. It was interesting to find that the bison did not possess a bipartite dorsal lacrimal gland as seen in cattle. Other species differences included the length of the superior gland of the third eyelid and Harderian gland. In both cases, the bison samples had longer glands as compared with cattle. The bison lacrimal glands appeared more developed, especially with the Harderian gland. The cattle measurements in this study differ from that of those previously reported (Prince et al., 1960). For example, Prince et al. (1960) reported the cattle's dorsal lacrimal gland to be approximately 65 mm in length and 36 mm in width. They reported that the cattle Harderian glands to be 41 mm in length and 26 mm in width. In our study, the cattle lacrimal glands were 44 mm in length and 30 mm in width (Fig. 2). Age, breed and gender were not specified in Prince et al.'s study. In our study, our cattle samples came from a mixture of beef bulls, steers and cows with an average age of 2 years.

There is an effect of ageing on the lacrimal system in humans (e.g. Draper et al., 1999) and rats (Bromberg and Welch, 1985; Draper et al., 1998). To examine properly for ageing effects in bison or cattle would require a longitudinal study that was beyond the scope of the present study. In our study, we had too small a sample to determine age differences in bison.

We did not observe gender differences anatomically or histologically in cattle samples. In contrast to our observations, gender-related differences have been reported in rats, mice, guinea-pigs, rabbits and humans (Cornell-Bell et al., 1985; Sullivan et al., 1990; see review by Van Haeringen, 1997).

Specifically, acinar lumens were reported to be larger in males than females. Whether the reported differences observed are due to sex hormones (McClellan et al., 2001), is beyond the scope of the current study.

The most notable anatomical difference between bison and cattle was the appearance of the Harderian gland. In the bison, a clearer distinction could be made between the superior gland of the third eyelid and the Harderian gland. Although a common fascial sheet encased both glands in both species, in the bison samples, the Harderian gland appeared more developed. Grossly, the cattle superior and deep (Harderian) gland of the third eyelid grossly appeared confluent. Our histological findings of the cattle Harderian gland confirm previous reports (Sundwall, 1907; Paule, 1957). The anterior portion of the gland resembled the dorsal lacrimal gland and superior gland of the third eyelid in histological architecture and histochemical properties. The posterior aspect of the gland is characterized by large lumen acini lined by a single layer of cuboidal epithelial cells. There was no obvious distinction between the superior gland of the third eyelid and the Harderian gland. Both the anterior and posterior portions of the bison Harderian glands were similar to cattle. Grossly, the lumens of the acini were larger in the bison samples. The intense staining of the central lumens of both cattle and bison Harderian glands with Masson Trichrome was unexpected. Differences in the pH of the acinar luminal secretions, or the effects of fixation combined with the pH of the tissues/secretions are theories that could explain this unexpected staining pattern.

Not all animal species possess a Harderian gland. Terrestrial carnivores, non-human primates and human beings do not have such a lacrimal gland (Seely, 1987). This gland, however, is well developed in most laboratory animals, amphibians, reptiles and birds (Seely, 1987). The Harderian gland is also found in the Dhub lizard (Sabry and Al-Ghaith, 2000). The light microscopy acinar features of the pink lobe of rabbit Harderian glands are similar to those seen in the bison Harderian gland and posterior part of the cattle Harderian gland (Janssens et al., 1999).

It was interesting to note in this study, the subtle differences in the architecture and cytoplasmic characteristics between the acini of bison and cattle dorsal lacrimal glands and superior gland of the third eyelid. The bison samples had a loose arrangement and less conformed architecture of the lobules and individual acini as compared with cattle. The bison acinar cell cytoplasm differed as compared with cattle; the basophilic vacuolated cytoplasm seen in bison samples must be a species variance from the eosinophilic granulated cytoplasm seen in cattle. In both species, the single sheets of connective tissue septae that encircled each acinar unit multiplied and became thicker around the effluent ducts and blood vessels. This is a common finding among lacrimal glands of other species (Krochmalska, 1976).

The dorsal lacrimal gland of most species has been described as a compound tubuloacinar gland with mucoserous secretions. The lacrimal gland secretory components, such as mucosubstances derived from glycoproteins, can be revealed by histochemical analysis. According to Spicer and Henson (1967), glycoprotein secretory products can be identified with PAS, AB pH 2.5 and 1.0 and HID. Neutral glycoproteins are PAS-positive and AB pH 2.5-negative and acid glycoproteins are PAS- and AB pH 2.5-positive.

Furthermore, acidic glycoproteins can be differentiated as sialylated (AB pH 2.5+, AB pH 1.0-, HID-) or sulphated (AB pH 2.5+, AB pH 1.0+, HID+) (Spicer and Henson, 1967). In this study, the dorsal lacrimal glands, superior glands of the third eyelid, as well as the proximal part of the Harderian glands of both species revealed acini and/or cells within an acinus that contained both neutral and acidic glycoproteins, which included sialylated and sulphated acidic glycoproteins. The tubular cells contained mainly PAS granules. It has been previously defined that neutral glycoproteins are contained within a serous cells and acidic glycoproteins are contained within mucous cells (Shackelford and Kapper, 1962). Therefore, our results suggest that the acinar cells are a mixture of serous and mucous secretory cells and that the tubules are serous. This is similar to the canine dorsal lacrimal gland, where the acinar endpieces are mainly mucous and tubular cells are serous secretory cells (Martin et al., 1988). The histochemical analysis of the large lumen acini seen in the bison and cattle Harderian gland did not react with PAS, AB or HID. Paule reported similar findings with PAS staining in cattle glands. He stated that the posterior endpieces of the gland had typical cytological appearance of mucous cells (Paule, 1957). Sundwall reported absence of staining in these large lumen acini with mucicarmine, a stain demonstrating epithelial mucins. Mucins and mucosubstances, which stain positively with PAS (Sheehan and Hrapchak, 1980), were not detected in our Harderian gland samples. Further studies with additional histochemical stains may elude the cellular content of these cuboidal cells.

Variability in staining intensity and distribution between samples can be due to several factors. Individual sample variation, fixation, sectioning, freshness of staining substances and technician processing can play a role in staining variability. The differences in the appearance of granules in lacrimal cells between samples could be attributed to the various secretory phases of the same cells (Mollendorff, 1936; Krochmalska, 1976).

Although anatomical and histological disparities were seen between the bison and cattle lacrimal glands, histochemical analysis did not reveal significant differences. Histochemical properties of the secretory cells could be further investigated with the use of ultrastructural analysis. This analysis would complement ultrastructural studies of lacrimal gland reported in other species (Scott and Pease, 1959; Egeberg and Jensen, 1969; Orlandini and Bacchi, 1977; Kuhnel and Scheele, 1979; Martin et al., 1988).

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