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ANATOMICAL AND HISTOCHEMICAL CHARACTERISTICS OF THE LACRIMAL GLANDS IN BACTRIAN CAMELS (*Camelus bactrianus*)

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ABSTRACT

The anatomical and histochemical characteristics of the lacrimal, main lacrimal and harderian glands and superior glands of the third eyelid in 5 bactrian camels were observed with gross-anatomical, histological and histochemical methods. The main lacrimal gland was situated on the dorsolateral aspect of the eyeball and it appeared irregular triangular in shape and light brown in appearance. The gland consisted of tubuloacinar units which were PAS-positive and Alcion blue-positive. The superior gland of the third eyelid situated at antero-medial aspect of the eyeball. It surrounds the cartilage of the third eyelid. It is oval in appearance and has tubuloacinar units which mainly showed PAS-positive. The harderian gland was located distal to the superior gland of the third eyelid, which was composed of tubuloacinar units and large lumen acini lined with cuboidal epithelial cells. The cells of tubuloacinar units showed a positive PAS reaction. The position of the lacrimal glands in bactrian camel was similar to that of other reported species, but morphological differences existed among species, which may be related to the living environment of bactrian camel. Histochemical analysis showed that the main lacrimal gland of bactrian camel is a compound tubuloacinar gland with mucoserous secretions, while the acinar epithelium cells of superior gland of the third eyelid and harderian gland in bactrian camel are mainly serous.

Key words: Bactrian camel, harderian gland, lacrimal gland, superior gland of the third eyelid

In its harsh environment, the bactrian camel is subjected to lack of feed and water, and other problems such as hot and dry climate. Adaptation of the camel to this inhospitable environment has come through certain behavioural, physiological and anatomical characteristics. Since it is well known that the lacrimal gland secretes a watery secretion, the morphological study of the lacrimal gland may contribute a great deal to a better understanding of the problem of water loss and conservation in the dromedary.

Lacrimal glands are responsible for the production of tear fluid that helps to maintain cornea clear and health. In most species, the majority of tears are secreted from the main lacrimal gland. Examination of lacrimal glands of human beings, dogs, cats, horses, pigs, rabbits, sheep and goats has been described in the literature (Sinha and Calhoun, 1966; Allen and Reid, 1972; Sisson and Grossman, 1975; Gillette *et al,* 1980; Martin *et al,* 1988; Gargiulo *et al,* 2000; Vânia Pais Cabral *et al,* 2005; Klec´kowska-Nawrot and Dzie giel, 2007a,b,c). The anatomy of the dromedary lacrimal glands has been established (Abdalla *et al,* 1970), yet the histological and histochemical characteristics have not been clearly described. Moreover, there are no reports on anatomical and histochemical characteristics of the lacrimal glands in bactrian camels. The aim of this study was, therefore, to examine the anatomical, histological and histochemical characteristics in bactrian camels which are different from other domestic animals. This may provide a morphological basis for further research on the physiology of lacrimal glands of bactrian camel.

Materials and Methods

Five heads of bactrian camels were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. Post-mortem examination of the eyes revealed no apparent ocular disease. The sex and body weight of the animals were not recorded. The heads were fixed by injecting 10% formalin into the carotid artery for 48 hours and then the lacrimal glands were obtained by gross-anatomical method. Length and width to the nearest millimetre were recorded for each gland. Histological and histochemical analyses were performed on randomly

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selected samples. Fixation with 10% buffered formalin for 24–48 hr was performed prior to processing. Each gland was sectioned in a sagittal plane and paraffin-embedded. Sections (5 μm in thickness) were stained with hematoxylin and eosin stain and examined by light microscopy for histological description. Histochemical stains for identification of glycoproteins and mucosubstances included Periodic Acid-Schiff (PAS), and Alcian Blue (AB) with nuclear red counterstain. Motic Images Advanced 3.0 was used to take and process photographs.

Results

Gross anatomy

The bactrian camels possess the superior glands of the third eyelid, main lacrimal and harderian glands. The main lacrimal gland was located within a fascial sheet of the periorbita at dorsolateral aspect of the globe beneath the frontal bone. It was light brown in colour and difficult to be distinguished from the surrounding muscles of the eyeball. The shape of the gland was an irregular triangle and the inferior surface of the gland was slightly concave, fitting over the globe. The gland, 60 mm in length and 20 mm in width, consisted of two lobes, main (palpebral) and small (eyelid) lobes, connected by a connective tissue sheath (Fig 1). The lobes were all irregularly triangular in shape. The length of the orbital lobe was 40 mm, and the width 20 mm. The palpebral lobe was 20 mm in length and width. The thickness of the gland varied between 5 mm in the middle of the orbital lobe and 2 mm in the most lateral aspect of the palpebral lobe and the most medial part of the orbital lobe. The gland possessed 3-4 excretory ducts. The orbital lobe had 1-2 ducts, whereas the palpebral lobe had a single duct. The excretory ducts emerged from the ventral surface of the corresponding part of the gland, ran parallel to each other, penetrated the periorbita, and opened anterior to the fornix of the conjunctiva of the upper eyelid.

The superior gland of the third eyelid was situated on anterior-medial aspect of the eyeball, surrounding the cartilage of the third eyelid. The main body was oval, pale yellow and lobulated in appearance (Fig 2). The proximal part surrounded the cartilage of the third eyelid. It contacted ventrally the anterior margin of the harderian gland and was partially covered by the ventral oblique muscle. The gland measured 30 mm in length and 20 mm in width. The thickness of the gland was 8 mm and the volume of it was approximately 2.75 ml. The superior gland of the third eyelid possessed a single excretory duct which opened to the third eyelid and the distance of the nozzle to the third eyelid was about 1-2 cm.

The harderian gland was situated in the posterior part of the eyeball, exhibited a whitish colour and was surrounded by a connective tissue capsule. Harderian glands were located distal to the superior gland of the third eyelid and were encased in a single fascial sheet with the superior gland. In bactrian camel, a triangular end-piece to the superior gland of the third eyelid was noted and determined to be the harderian gland. There was no obvious separation between the superior gland of the third eyelid and harderian gland and a slight narrowing was palpated at the junction where the cobble-stoned appearance of the superior gland changed to a more smooth appearance of the harderian gland (Fig 2). The triangular-shaped gland had a cobble-stoned proximal part and smooth-appearing distal portion. Harderian gland of bactrian camel was about 25 mm in length and 12 mm in width. The volume of it was about 1.4 ml.

Light microscopy

The main 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 (Fig 3). The diameter of the acinar varied between 15 μm and 55 μm. The acini were composed of tall pyramidal or columnar cells with small lumens. The tubules were bordered by short columnar cells with large lumens and were seen intermingled between the nests of acini. The acinar cell nuclei were from 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.

Superior gland of the third eyelid of bactrian camel also has tubuloalveolar units which completely surrounded the cartilage shaft of the third eyelid. In general, these tubuloalveolar units were less compacted compared with the main lacrimal glands. The acini were mainly composed of tall pyramidal cells and the cell nuclei were round in shape and basally located.

Harderian gland included an anterior part, that histologically resembled the superior gland of the third eyelid and a mid-to-distal part of the gland that was composed of large lumen acini lined with cuboidal epithelial cells (Fig 4). These cells contained a round or oval nucleus. The lumen often contained an acellular, finely granulated eosinophilic secretion.

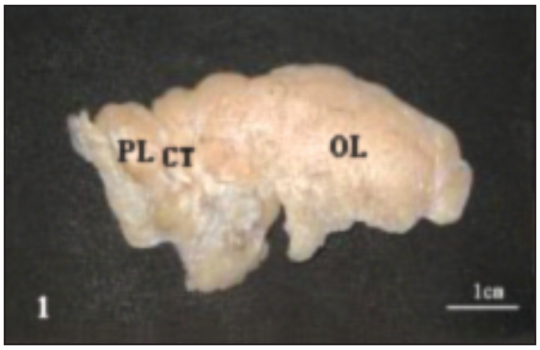

Fig 1. Main lacrimal gland of bactrian camel. PL: palpebral lobe; OL, orbital lobe; CT, connective tissue between palpebral lobe and orbital lobe.

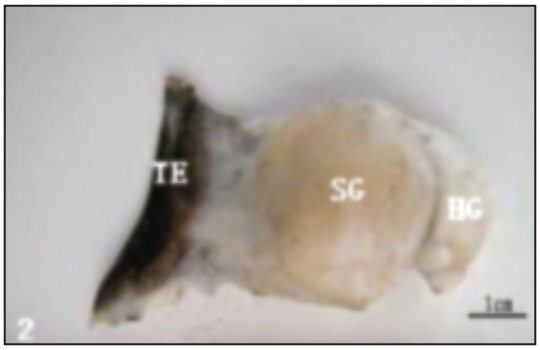

Fig 2. Superior gland of the third eyelid and harderian gland of bactrian camel. TE, the third eyelid; SG, superior gland of the third eyelid; HG, Harderian gland.

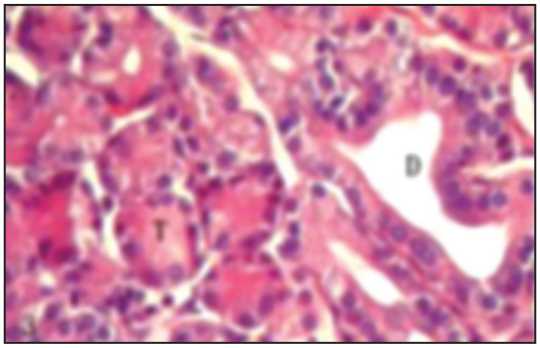

Fig 3. The tubuloalveolar units of the main lacrimal gland in bactrian camel. D, duct; T, tubuloalveolar units; H&E staining (40 x 10).

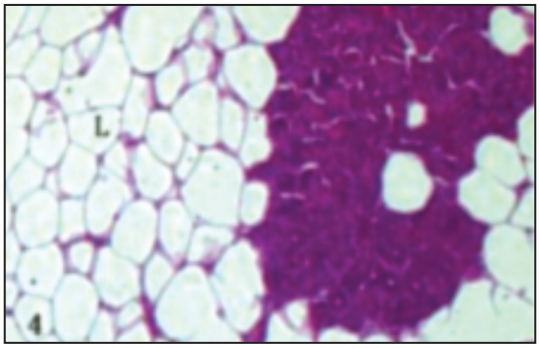

Fig 4. The mid-to-distal part of the harderian gland in bactrian camels has acini with large lumen. L, large lumen acini; H&E staining (10 x 10)

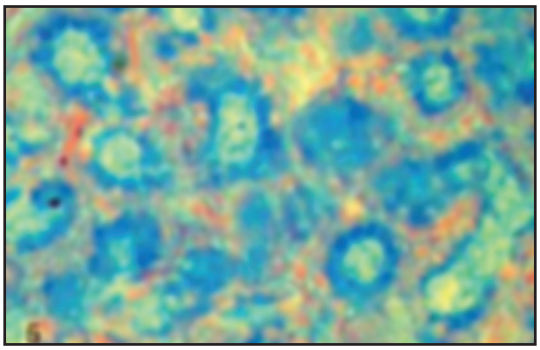

Fig 5. Alcian blue (AB) staining of the main lacrimal gland in bactrian camel (40×10).

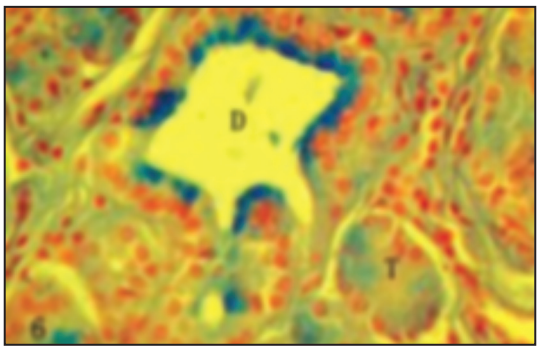

Fig 6. Superior gland of the third eyelid and harderian gland of bactrian camels with Alcian blue stain. D, duct (40×10).

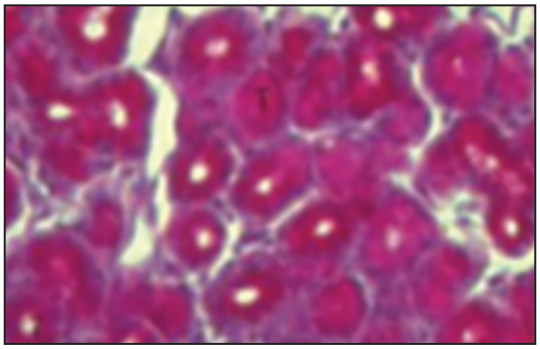

Fig 7. Main lacrimal gland of bactrian camels with PAS stain. T, tubuloalveolar units (40×10)

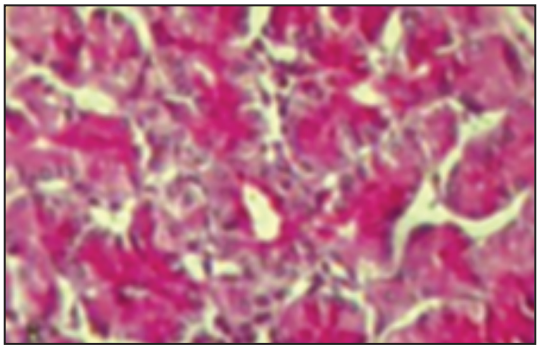

Fig 8. Superior gland of the third eyelid of bactrian camels with PAS stain (40×10)

This finding is characteristic of an apocrine gland. In bactrian camel, the proximal portion occupied a larger portion of the gland and distinct connective tissue septae separated the nest of acinar cells from the large lumen acini.

Histochemical analysis

Alcian blue (AB) stain revealed a similar staining pattern between the glands. Positive granules were seen in the acini and epithelium of the tubules (Fig 5). However, AB granules in acinar cells of the main lacrimal gland were more than that of in superior gland of the third eyelid and in superior gland of the third eyelid. There were more AB granules in acinar cells than in epithelium of the tubules (Fig 6). Variability of the location of the positive granules was seen between samples. In some samples, the granules were seen only 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.

Similar staining patterns of PAS were seen in main lacrimal gland and superior gland of the third eyelid. PAS staining revealed the presence of positive granules in all acini of these glands (Fig 7). Some acini were more heavily stained than others and acini in main lacrimal gland were more heavily stained than that in superior gland of the third eyelid in bactrian camels (Fig 8). Stain uptake was also seen within a subset of tubules.

The proximal part of harderian gland in bactrian camels had similar staining properties to the dorsal lacrimal and superior glands (described above). In the bactrian camel harderian gland samples, neither the large lumen acini nor the lining cells contain positive staining granules for any of the aforementioned stains.

Discussion

It appears from the present study that the bactrian camel possessed main lacrimal gland and superior gland of the third eyelid which is similar to that reported in the same species. In other domestic mammals the position of the lacrimal gland is similar to that in bactrian camel. For example, in the pig (Sisson and Grossman, 1975), horse (Sisson and Grossman, 1975), ox (Sisson and Grossman, 1975), cattle (Pinard *et al,* 2003) and dog (Martin *et al,*1988), the main lacrimal gland is situated on the dorsolateral aspect of the eyeball, covered by the zygomatic process of the frontal bone. The colour of the main lacrimal gland of bactrian camel is light brown in the fresh state. This agrees with the findings of Al-Ani (1997) in the dromedary. However, the colour varies between red in the dog (Martin *et al,* 1988) and pink in the small ruminants (Sinha and Calhoun, 1966). It is probable that the variation in the colour depends on the degree of bleeding. The shape of the gland is evidently determined by its position. Thus, in bactrian camel, the main lacrimal gland is convex dorsally in conformity with the convexity of the bony orbit and is irregular in shape. These findings agree with those of Abdalla *et al* (1970). In other domestic mammals the main lacrimal gland varies in shape between species. It is triangular in pig, and bipartite in ox, sheep and goat (Sisson and Grossman, 1975). It seems that the shape of the gland depends not only on the relationships of the gland but also on the degree of development of the gland itself. The colour and shape of superior gland of the third eyelid in bactrian camel are similar to that of other domestic mammals. The present study reveals that the number of excretory ducts of the main lacrimal gland is 3–4 in bactrian camel. This confirms the findings of Abdalla *et al* (1970). In other domestic mammals there are also some variation in the number of excretory ducts. For example in the horse (Sisson and Grossman, 1975), ox (Sisson and Grossman, 1975) and small ruminants (Sinha and Calhoun, 1966) the number of excretory ducts is 12–16, 6–8, and 2–5, respectively. It is interesting that there was little difference in the size of main lacrimal gland but much difference in the number of excretory ducts between bactrian camel and cattle. We guess that this difference may relate to the special living environment of bactrian camel. Living in desert and semi-desert regions, bactrian camel is subjected to lack of water, and other problems of hot and dry climate. Bactrian camel possesses 3-4 excretory ducts that may not only ensure the outflow of tears but also prevent superfluous water loss for maintaining normal physiological function of eye.

There is an effect of ageing on the lacrimal system in humans (Obata *et al,* 1995; Draper *et al,* 1999; Van Haeringen, 1997) and rats (Draper *et al,* 1998). In present study, we had too small samples to determine age differences in bactrian camel.

All animal species donot 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 and Yasmina, 1996). The harderian gland is also found in the Gecko (Gabriella Chieffi Baccari, 2000). In this study, bactrian camels also possess harderian gland. One of the most extraordinary features of the harderian gland

is the function of providing lubrication between the nictitating membrane and the cornea to reduce friction between them (Payne, 1994) which may explain its adaption to the desert climate full of sandstorms.

As other domestic mammals, the main lacrimal gland and superior gland of the third eyelid of bactrian camel consisted of tubuloacinar units. Moreover, there was no great difference on the acinar diameter between cattle and bactrian camel. The acinar diameter in bactrian camel is 15-55 μm and 13-50 μm in cattle (Gao Huiying *et al,* 1997). Histological characteristics of harderian gland in bactrian camel is similar to that in cattle. The anterior part consisted of compound tubuloacinar unit and the mid-to-distal part of the gland was composed of large lumen acini lined with cuboidal epithelial cells.

The main 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 Evcrson Pcarso (1980), glycoprotein secretory products can be identified with PAS, AB Neutral glycoproteins are PAS-positive and AB-negative and acid glycoproteins are PAS-negative and AB-positive. In this study, the main lacrimal glands of bactrian camels revealed acini and/or cells within an acinus that contained both neutral and acidic glycoproteins, which included sialylated and sulphated acidic glycoproteins. The superior glands of the third eyelid, as well as the proximal part of the harderian glands of bactrian camels 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 (Evcrson Pcarso, 1980). Therefore, our results suggest that the acinar cells of the main lacrimal gland in bactrian camel are a mixture of serous and mucous secretory cells and that of the superior glands of the third eyelid and the proximal part of the harderian gland are serous. This is similar to the canine and cattle lacrimal gland (Martin *et al,* 1988; Vânia Pais Cabral *et al,* 2005; Pinard *et al,* 2003). The histochemical analysis of the large lumen acini seen in the bactrian camels harderian gland did not react with PAS, AB. Pinard (2003) reported similar findings with PAS staining in cattle glands.

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 difference in the appearance of granules in lacrimal cells between samples could be attributed to the various secretory phases of the same cells (Evcrson Pcarso, 1980).

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