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MICROSTRUCTURAL AND ULTRASTRUCTURAL STUDIES OF THE TRACHEA IN THE BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

Histology of trachea was studied in 9 bactrian camels (*Camelus bactrianus*) by the use of the light, scanning (SEM) and transmission electron microscopy (TEM). The trachea of camel consisted of 69–74 incomplete cartilaginous rings of hyaline. The trachea was lined with pseudostratified columnar ciliated epithelium and goblet cells. Lamina propria and submucosal layer were loose connective tissue with prominent elastic fibres. The mucosal and submucosal layers were 636.4 ± 83.8 µm (n =27) thick. Submucosal glands were tubuloalveolar with mucous (acidic and neutral) secretions. Tracheal muscle was smooth and lied internal to the open end of the hyaline cartilage. Scanning electron microscopy revealed a dense mat of cilia covering the trachea. Submucosal gland orifices were frequently observed on the surface. Ultrastructural results showed that the pseudostratified epithelium was composed of ciliated, goblet and basal cells. The nuclei in the ciliated cells were flat and an abundant number of mitochondria. Goblet cells contained big nuclei and secretory granules that were different in number, size and electron density. Inter-epithelial granulocytes and lymphocytes were regularly found.

Key words: *Camelus bactrianus,* microstructure, trachea, ultrastructure

Bactrian camels (*Camelus bactrianus*) are vital to the production system of the Chinese desert and semi-desert areas where feeding resources are generally scattered and poor. The animals have adapted well to the harsh climatic conditions and poor feeding resources, not only providing hair, wool, meat and hides for local farmers and herdsmen, but also acting as an indispensable means of transport in this arid zone (Liu, 2005).

The trachea provides the air passage way between the larynx and the bronchi, it is a semi-flexible and semi-collapsible tube in the ventral portion of the neck that extends from the larynx into the thoracic cavity (Raji and Naserpour, 2007). An extensive literature has accumulated on histologsical characteristics of mammalian trachea. For instance, this area has been carefully studied in hamster (Becci *et al*, 1978), mouse (Pack *et al*, 1980), rabbit (Plopper *et al*, 1983), rhesus monkey (Plopper *et al*, 1989), bonnet monkey (Wilson *et al*, 1984), reindeer (Saari, 1995), sheep (Mariassy and Plopper, 1983; 1984), cattle (Lovannitti *et al*, 1985), one-humped camel (Raji and Naserpour, 2007) and human (Fawcett, 1994). It is important to know the details of the trachea to understand the physiological specialisation of the bactrian camel in view of its existence in arid and semiarid areas.

The purpose of this investigation was to study the basic histology of trachea of *C. bactrianus*.

Materials and Methods

Nine specimens of the adult bactrian camels of both sexes were obtained from the slaughterhouse of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. The trachea was dissected free and flushed with normal saline. Samples for LM were fixed in 10% formaldehyde for 72 h, dehydrated, cleared and embedded in paraffin. Embedded tissues were cut into 5-µm thick sections and stained with haematoxylin and eosin (H&E), Van Giesson for collagen fibres, Verhof (V) for elastic fibres, Alcian blue and periodic acid schiff (AB/PAS) for histochemistry of muco-substances.

Small pieces of the tissue samples for TEM were pre-fixed in 3% glutaraldehyde buffer (pH=7.2) and fixed for 3 h. The tissues were then washed thrice in 0.1 M phosphate buffer for 30 min before being cut into 1 mm^3 pieces and post-fixed with osmium tetroxide for 1 h. The samples were washed thrice in 0.1 M phosphate buffer and then dehydrated in

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ascending grades of ethanol before being embedded in Epon 812. Semi-thin sections of each tissue were collected and stained with toluidine blue. Ultra-thin sections (50–70nm) were then collected on copper grids. The ultra-thin sections were stained with a saturated solution of uranyl acetate for 30 min, followed by lead citrate for 7 min in a carbon dioxide-free environment. Sections were then washed in CO_2-free water, dried and examined under a transmission electron microscope (JEOL, JEM-1230).

Tissue samples for SEM were fixed as described above. After dehydration in a series of ascending grades of alcohol, samples were freeze-dried at vacuum and coated with platinum. The mounted specimens were observed under a scanning electron microscope (JEOL, JSM-680LA).

Results

LM results

The trachea of camel consisted of 69–74 incomplete cartilaginous rings of hyaline. The trachea was lined by a pseudostratified ciliated epithelium with numerous goblet and basal cells (Figs 1 and 2). Goblet cells of trachea in camel produce exclusive amounts of acidic and neutral muco-substances (Fig 3). Lamina propria and submucosal layer in trachea of camel were loose connective tissue with prominent and 636.4 ± 83.8 µm (n =27) thick elastic fibres (Fig 2). Numerous submucosal glands (branched, coiled and tubuloalveolar) were observed in the trachea of the camel (Fig 1). Tracheal muscle in the trachea of camel was smooth and lied internal to the open end of hyaline cartilage (Fig 1). The tunica adventitia of trachea in camel consisted of connective tissue with numerous elastic fibres.

SEM results

Most of the luminal surface of the trachea was covered with a dense mat of individually separated, tall and slender cilia orientated in different directions and masking the goblet cells (Fig 4). The primary structure of the viscoelastic layer consisted of glycoprotein and mucin, synthesised by surface goblet cell and mucous cell of the submucosal glands. Submucosal gland orifice was round and surrounded by cilia in copious ciliated cells regions (Fig 5). Trachea was denuded of surface epithelium and submucosal gland orifice was irregular and often easy to see (Fig 6).

TEM results

Three main epithelial cell types were recognised in the trachea of the camel using TEM. These cells were ciliated, goblet and basal cells (Fig 7). Three cells of the pseudostratified mucosa rested on the basal lamina, but only goblet cells and ciliated cells were exposed to the lumen. A small number of granulocytes and lymphocytes were regularly found in the normal tracheal epithelium (Fig 1). Of the 2 cell types the granulocytes occurred more frequently.

Compared to basal cells and goblet cells, the ciliated cells had a relatively electronlucent cytoplasm. Numerous microvilli and cilia, respectively projected from the free surface of the ciliated cells. The nuclei in the ciliated cells were flat and an abundant number of mitochondria existed within the cytoplasm (Figs 7 and 8). The Golgi complex were located in the supranuclear region. Free ribosomes and profiles of rough and smooth endoplasmic reticulum were scattered in the cytoplasm (Fig 8). The ciliated columnar cells showed electron-dense granules with light halo rings (Fig 11), and it seems that these granules are discharged into the lumen of the trachea. Cross sections of the ciliary shaft revealed the classical 9+2 pattern of the motile cilia (Figs 10 and 12).

Goblet cells in trachea of camel contained big nuclei and secretory granules that were different in number, size and electron density (Figs 7 and 8). The ultrastructure of the goblet cells varied considerably, depending on the physiological state. The apical part of goblet cells was relative by wide when filled with secretory granules.

Low and irregularly shaped basal cells were attached to the basal lamina. The centrally located nucleus occupied a large part of the cell. Abundant free ribosomes were dispersed throughout the cytoplasm (Fig 8).

Discussion

The trachea of bactrian camel was lined by a pseudostratified ciliated columnar epithelium with numerous goblet and basal cells, the same as seen in dromedary camel (*C. dromedarius*) (Raji and Naserpour, 2007) or other mammals (Dellman, 1998). Exclusive amounts of acidic and neutral muco-substances are secreted from goblet cells in bactrian camel, which are similar to *C. dromedarius*. However, goat is known to secret acidic mucosubstances (Kahwa and Purton, 1996). Lamina propria and submucosal layer in trachea of the camel, *C. dromedarius* (Raji and Naserpour, 2007), cats and goats (William, 1990) have coincident structures, which were loose connective tissue with prominent elastic fibres.

The present study has shown that numerous submucosal glands (branched, coiled and

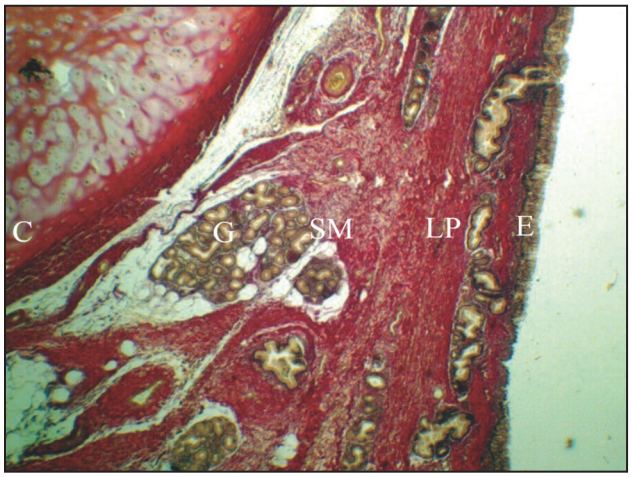

Fig 1. Histological structure of the trachea of camel, epithelium (E), lamina propria (LP), tela submucosa (SM), tracheal muscle (T), cartilage (C), gland (G), adventitia (AD) and Van Giesson×100.

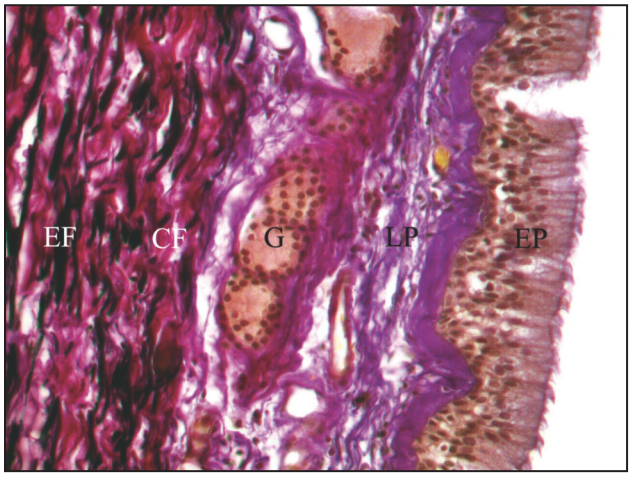

Fig 2. Elastic fibre in the tunica submucosa. Epithelium (EP), lamina propria LP, elastic fibres (EF), collagen fibres (CF), Verhoeff×400.

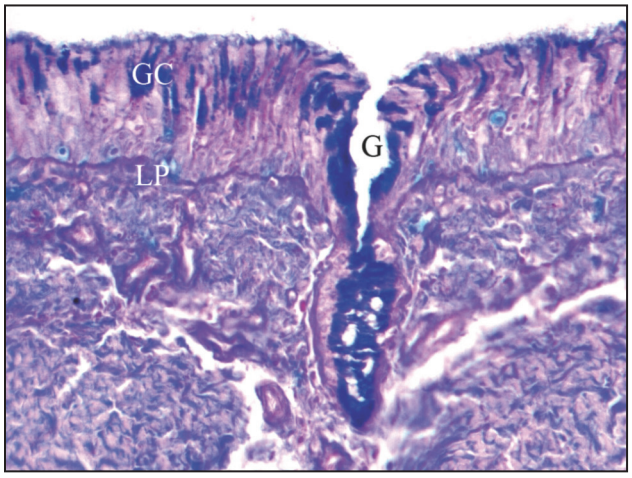

Fig 3. Tunica mucosae of the trachea of camel, goblet cell (GC), lamina propria LP, gland G, Alcian blue-PAS×400.

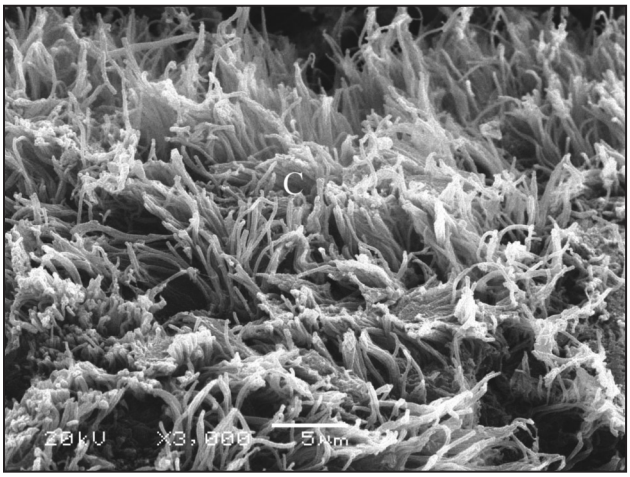

Fig 4. Epithelial surface of trachea, cilia (C), SEM.

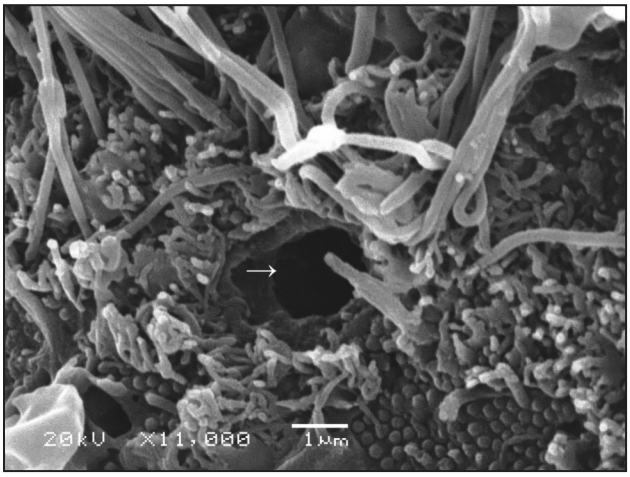

Fig 5. Epithelial surface of trachea, showing a submucosal gland openings (arrow), SEM.

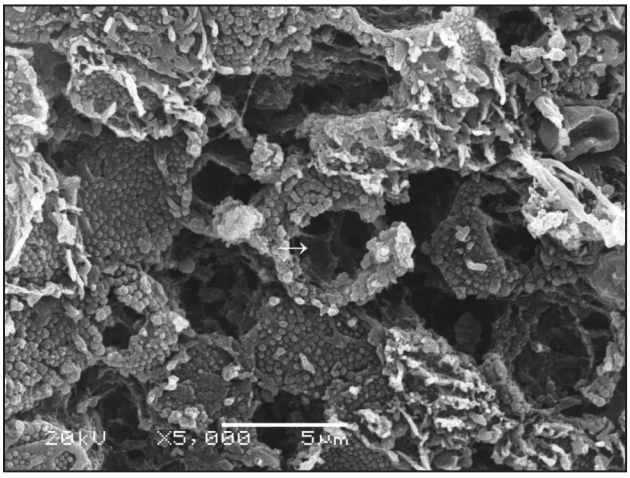

Fig 6. Denudation of surface epithelium, submucosal gland orifice (arrow), SEM.

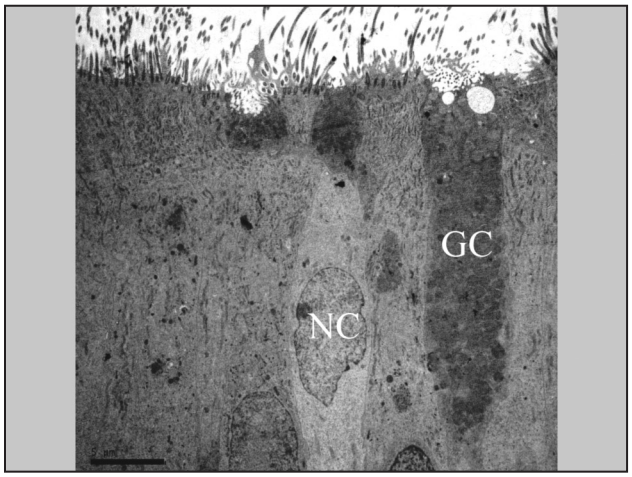

Fig 7. A transmission electron micrograph showing nucleus of ciliated cell (NC) and goblet Cell (GC)×4000.

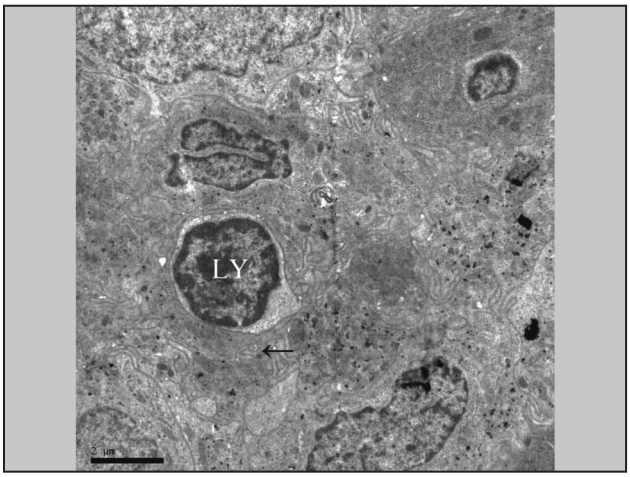

Fig 10. A transmission electron micrograph showing lymphocyte (LY) and smooth endoplasmic reticulum (arrow)×10000.

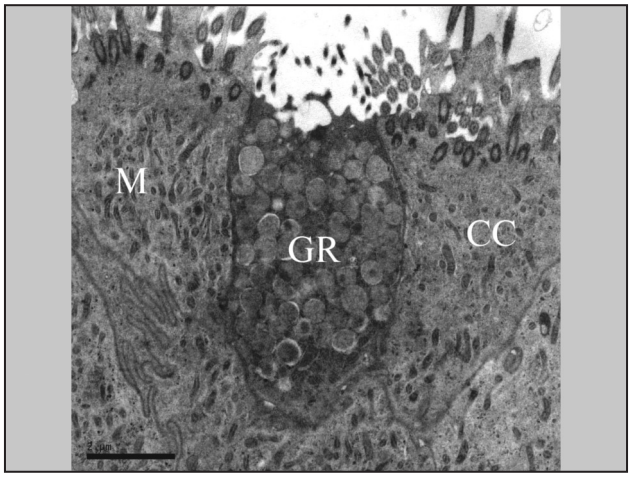

Fig 8. A transmission electron micrograph showing mitochondria (M) in a ciliated cell (CC) and secretory granules (GR) in GC×12000.

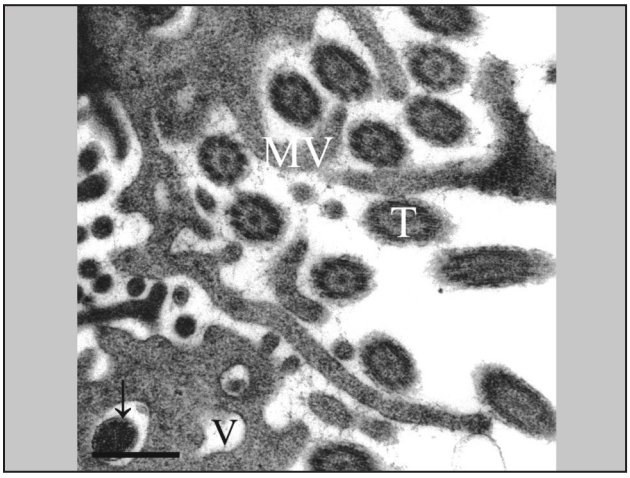

Fig 11. A transmission electron micrograph showing transverse section of cilia (T), vacuole (V), microvilli (MV) and granules (arrow)×50000.

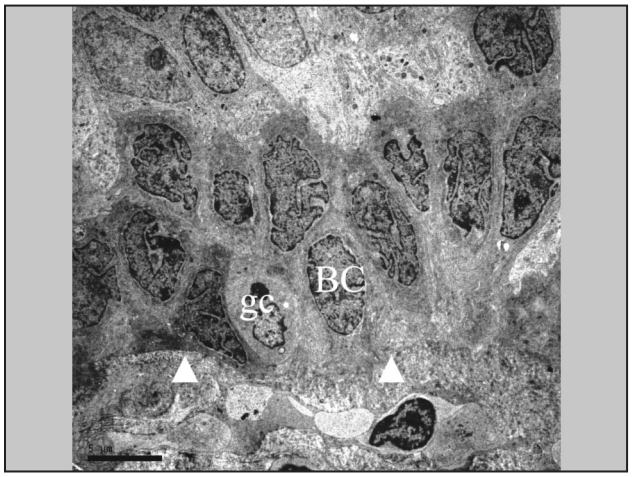

Fig 9. A transmission electron micrograph showing basal cell (BC), granulocyte (gc) and the basal lamina (arrowheads)×4000.

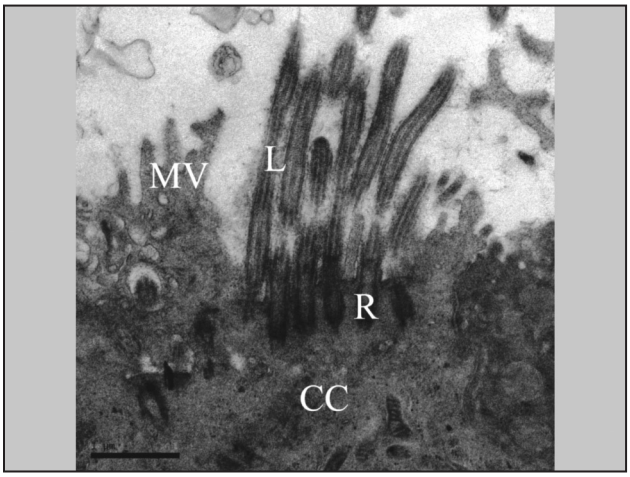

Fig 12. A transmission electron micrograph showing a ciliated cell (CC), microvilli (MV), longitudinal section of cilia (L) and root of cilia (R)×25000.

tubuloalveolar) were observed in the trachea of the camel as previously reported in *C. dromedarius* (Raji and Naserpour, 2007), sheep (Goco *et al*, 1963; Mariassy and Plopper, 1983; 1984) and cat (Gallagher *et al*, 1975). The trachea of mouse and rabbit were gland-free. In contrast, glands were present along the full lengths of rat and guinea pig trachea, almost exclusively between the cartilaginous rings in the ventral wall (Widdicombe *et al*, 2001). In ox, goat, dog and sheep, the volume of glands per unit tracheal surface area was markedly greater in the ventral than the dorsal aspect of the trachea. As mucous serves to trap and remove inhaled particles, the increase in gland density with increasing airway size is presumably correlated with greater rates of particle deposition in larger airways (Choi *et al*, 2000). As seen in other ruminants, including *C. dromedarius*, tracheal muscle in the trachea of camel was smooth and lied internal to the open end of the horseshoe-shaped hyaline cartilage. However, tracheal muscle lies external to the cartilages in the carnivores (Nickel and Schumer, 1979). The tunica adventitia of trachea in camel consisted of connective tissue with numerous elastic fibres that are similar to cat (William, 1990) and *C. dromedarius* (Raji and Naserpour, 2007).

The pseudostratified epithelium in the trachea of the camel was composed of ciliated, goblet and basal cells. Ciliated cells were numerous, structurally similar to those of *C. dromedarius* and other mammals. Interepithelial granulocytes and lymphocytes were occasionally seen, which was observed in the present study, has also been reported in the tracheal epithelium of guinea-pig (Dalen, 1983). Cross sections of the ciliary shaft revealed that the motile cilia has the same classical 9+2 pattern in trachea of *C. bactrianus* and *C. dromedarius*. The microvilli extend into the lumen from the ciliated cell in the camel trachea. The corresponding structures on the tracheal microvilli, in collaboration with the negatively charged mucopolysaccharides which have a strong water-binding capacity, may play an essential role in fluid reabsorption (Dalen, 1983).

It was observed in the present study that the epithelium lining the trachea was heavily ciliated, which essentially resembles to that found in cattle (Lovannitti *et al*, 1985), goat (Kahwa and Purton, 1997), neonatal kids (Kahwa *et al*, 2000) and *C. dromedarius* (Raji and Naserpour, 2007). Submucosal gland orifice was observed in copious ciliated cells regions and denuded of surface epithelium. The primary structure of the viscoelastic layer consists of glycoprotein and mucin, synthesised by surface goblet cell and mucous cell of the submucosal glands. In the normal tracheal epithelium, the mucociliary apparatus constitutes the major defence mechanism against inhaled foreign material (Kilburn, 1968). The mucous blanket originates from 2 sources, from the surface mucous-producing cells and from the submucosal glands. Whereas, the latter contributes to the sol layer of the mucous blanket, the former contributes significantly to the gel layer (Dulfano, 1973). A visco-elastic mucous blanket or gel floats on top of a sol layer which, under normal conditions, creates an ideal micro-environment for the ciliary motion. During the effective stroke, the ciliary tips penetrate into the superficial mucous layer, and during the recovery stroke, they withdraw from this layer (Dalen, 1983). Goblet cells in trachea of camel contained big nuclei and secretory granules that were different in number, size and electron density. The ultrastructure of the goblet cells varied considerably, depending on the physiological state. The apical part of goblet cells was relatively by wide when it was filled with secretory granules.

Conflict of interest statement

None of the authors of this paper has a financial of personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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