Histological and Lectin Histochemical Characterization of the Efferent Ductules in the Dromedary (*Camelus dromedarius*)

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Abstract

The ductuli efferentes in the dromedary were lined with low pseudostratified columnar epithelium. Two morphologically distinct types of epithelium were detected; both included ciliated and non-ciliated cells. Close to the rete testis, the epithelium was less vacuolated and the tubules had narrower lumens. The terminal segment epithelium in some areas had regional height variations similar in appearance to the initial portion of the epididymal duct but the nuclei maintained a pattern specific to the efferent ductules, not to the initial part of the epididymal duct. Histochemical characteristics of the epithelial cells were investigated using an array of six lectins: Con-A, DBA, MAA, SBA, SNA-1, and WGA. Specific sugar residues of MAA, SNA-1, and DBA were not detected. The glycocalyx of both ciliated and non-ciliated cells in both type I and type II epithelia showed binding sites for Con-A, SBA, and WGA. The apical and basal cytoplasm showed strong binding for Con-A and WGA in both type I and type II epithelia, but not for SBA in type I epithelium. The Golgi zone appeared to be un-stained for Con-A; this was different for WGA and SBA. Basal vacuoles showed different binding patterns for Con-A, SBA and WGA. These regional variations suggest a high degree of functional compartmentalization of the epithelium of the efferent ductules.

Key Words

Efferent ductules, dromedary, lectin histochemistry, efferent ductules morphology

Introduction

The excurrent duct system of the testis consists of the ductuli efferentes, the ductus epididymidis, and the ductus deferens (Roberts, 2010). This duct system serves not only to convey spermatozoa to the outside, but is essential for their functional maturation. The efferent ductules (the Anglicized form of ductuli efferentes) connect the rete testis to the ductus epididymidis. These small ducts are unique because they are the only region of the male reproductive tract that is lined with a truly ciliated epithelium. The lining epithelium consists of columnar principal cells that are ciliated in places and non-ciliated in others (Setchell et al., 1994). Scattered free mononuclear cells that have invaded the basal epithelial area have been interpreted as a third genuine cell type but this is probably not the case (Wrobel and Bergmann, 2006).

The ultrastructural features of the ciliated cells lining the efferent ducts suggest that they have only a small capacity for protein synthesis and secretion (Setchell et al., 1994).The ciliated cells help to move the spermatozoa toward the epididymal duct, whose lining epithelium has "stereocilia," (i.e., long microvilli lacking an axoneme and therefore non-motile) but no true ciliation. The non-ciliated cells have resorptive and secretory functions (Ilio and Hess, 1994).

These general morphological features of the lining epithelial cells are known to demonstrate some interspecies variation: previous morphological and histochemical investigations described species specific features of these cells (Jones et al., 1979; Aire, 1980; Goyal and Hrudka, 1981; Jones and Holt, 1981; Lewis-Jones et al., 1982; Aureli et al., 1984; Burkett et al., 1987; Goyal and Williams, 1988; Nagy, 1990; Vicentini et al., 1990; Goyal et al., 1992; Arrighi et al., 1994; Stoffel and Friess, 1994; Wakui et al., 1996; Orsi et al., 1998; Parillo et al., 1998 and 2009; Aire et al, 2003). But a detailed description of the morphology of the efferent ductules and/or the distribution of cellular glycoconjugates in the efferent ductules in the dromedary camel has been lacking.

Dromedaries occupy arid regions of the Middle East through northern India and arid regions in Africa. Camels are of particular economic importance in these regions. They are used as beasts of burden by humans and also provide humans with milk, meat, wool, leather, and fuel from dried manure. In Egypt particularly, they are used for recreational purposes for tourists. As is

true of other economically valuable animals (such as horses and cattle) understanding the morphology of the reproductive organs is fundamental to improve and exploit their reproductive performance.

Lectins are sugar-binding proteins that are highly specific for their sugar moieties (Alroy et al., 1984; Brooks and Hall, 2001). This makes lectin histochemistry a powerful method for mapping the presence of glycans in tissues; this allows accurate detection not only of structurally integral cellular components such as glycoconjugates, but also functionally significant molecules because glycoconjugates can act as cell receptors, tumor markers, or immunomodulators. Histochemistry bridges the gap and clarifies the relationship between morphological description and the functional significance of that variation.

The purpose of this report was to study the microscopic anatomy of the efferent ductules of the dromedary and to map the cellular differences in glycoconjugates demonstrated by lectin histochemistry.

Materials and Methods

Testicles from six apparently healthy adult male dromedaries were collected immediately after slaughter at the central abattoir in Cairo, Egypt. Samples of the efferent ductules were obtained, fixed in Carnoy's solution for about 4 hours, and paraffin-embedded. Sections 6.0 µm thick were prepared. For histological evaluation, sections were stained with Hematoxylin and Eosin or with Masson's Trichrome stain using Gurr's (1956) modification as given in Humason (1979). Weigert's iron Hematoxylin was replaced by Verhoeff's stain. This stain combination allows the differentiation of collagen fibers (blue), muscle (red) and elastic fibers (black) and renders cell nuclei deep mauve; cytoplasmic elements stain in varying shades of red and mauve.

Lectin histochemistry was performed using biotinylated lectins following the protocol of Brooks and Hall (2001). Embedded sections were deparaffinized in xylene and rehydrated in increasingly dilute ethanol/distilled water baths. For antigen retrieval, slides were trypsinized for 10 minutes at 37°C (Trypsin-EDTA, Media Tech Inc., Manassas, VA, USA). Slides were incubated in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase, then washed and incubated with Carbo-Free Blocking solution (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature followed by incubation with the biotiny-

lated lectin for 60 min in a humid chamber at room temperature.

Table (1) lists the lectins (E.Y. Laboratories Inc., San Mateo, CA, USA) used in this study, their source, their abbreviations, the lectin concentration used, their major sugar specificities, and their inhibitory sugars. Concentrations were selected that gave minimal background staining yet were sufficient to detect relatively low levels of specific sugar residues. A final incubation, after washing, with ABC (avidin: biotinylated horseradish peroxidase complex) reagent (Vector Laboratories) lasted 30 minutes under the same conditions. Immunoreactivity was visualized with 3, 3-diaminobenzidine (DAB substrate kit, Vector Laboratories) in a dark place as outlined in the manufacturer's protocol. Slides were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted.

Some sections were treated with neuroaminidase (0.1 units/ml of type VI from *Clostridium perfringens*, Sigma, USA) (Jones et al., 1995). Controls for non-specific staining included incubation in which the biotinylated lectins were replaced with unconjugated lectins, biotinylated bovine serum albumin (E.Y. Laboratories) or buffer, preincubation of a lectin, except MAA and SNA-1 with its competing sugar (Vector Laboratories), or sections to be stained with MAA or SNA-1-were preincubated with neuroaminidase (Sigma, USA).

Results

The efferent ductules were lined with low pseudostratified columnar epithelium. There were two types of this epithelium; each type consists of both ciliated and non ciliated principal cells (Fig. 1). The initial segment of each duct was lined with type I epithelium. The segment close to the epididymal duct was lined with type II epithelium. The epithelium of the terminal segment showed in some areas regional height variations from place to place, similar to what is seen in the initial segment of the epididymal duct; but in the efferent ductules this variable appearance was much less pronounced compared to the initial part of the epididymal duct. The initial segment of the epididymal duct and the terminal segments of the efferent ductules could generally be distinguished by the position of the nuclei. The nuclei in the type II epithelium were located more or less in the mid portion of the epithelial cells with only a few of them showing basally located nuclei. The terminal segments of the tubules had wider lumens than the other regions; and the epithelium of the terminal seg-

ments was more vacuolated; in addition there was decreased distance between the nuclei and the luminal surface. Delicate peritubular smooth muscle cell layers lined the tubules and a loose connective tissue was dispersed in the space between the adjacent tubules (Fig. 2).

There were six lectins studied, but only three gave positive labeling: binding sites for DBA, MAA, and SNA-1 were not detected in the epithelium. The lectin binding profile of ciliated and non-ciliated cells of type I and type II epithelia are reported in table (2). Binding sites for Con-A appeared in the glycocalyx, the apical cytoplasm, and the basal cytoplasm of both ciliated and non-ciliated cells in both type I and type II epithelia (Fig. 3). The intensely stained glycocalyx was interrupted by the less-strongly stained cilia of the ciliated cells. The apical cytoplasm of the epithelial cells of type II epithelium showed Con-A positively stained granules and some unstained areas. No binding sites for Con-A were detected in the Golgi zone of either cells of type I or type II epithelia; but in the lateral cytoplasm (on either side of the Golgi zone) there appeared to be positively stained granules in type I cells. This was not seen in the type II epithelium. Con-A staining was positive in the cilia of both type I and type II epithelia. Basal vacuoles of type II epithelium did not show binding sites for Con-A.

Figure (4) shows that SBA strongly bound to the glycocalyx of ciliated cells in type I epithelium, but the non-ciliated cells were weakly stained. Neither the apical and the basal cytoplasm nor the Golgi zone of the type I epithelial cells had binding sites for SBA. The glycocalyx and the apical and basal cytoplasm of the epithelial cells of type II epithelium showed binding sites for SBA. In the type II cells, the Golgi zone and the basal vacuoles appeared only weakly stained. SBA bound with cilia in both types of epithelia.

WGA binding was detected in the glycocalyx, the apical cytoplasm, and the basal cytoplasm in both types of epithelia (Fig. 5). WGA showed more intense binding with glycocalyx in some areas than in other areas in type I epithelium. The apical cytoplasm of both types contained both unstained areas and positively stained areas. In general the Golgi zone of neither type showed binding sites for WGA except in a subgroup of type I cells and a few positively stained granules were detected in the basal vacuoles of type II epithelium. WGA bound with cilia in both type I and type II epithelia.

Peritubular smooth muscle cells of type I epithelium showed binding sites for WGA. The peritubular smooth muscle of both types showed binding sites for Con-A. SBA did not bind with peritubular smooth muscle cells of either type. Con-A, WGA, and SBA bound with the luminal contents in both types of epithelia (Figs. 3, 4, and 5). The intertubular connective tissue showed binding sites for Con-A and WGA only in regions of type I epithelium.

Discussion

Con-A is specific for trimannoside core, which is common for N glycans. Binding sites for Con-A were detected readily in various compartments in both type I and type II epithelia. The presence of Con-A binding sites in the basal cytoplasm may correspond to the binding at the cisternae of the rough endoplasmic reticulum (RER). This finding agreed with both ultrastructural investigation of this cytoplasmic site by Con-A (Chan and Wong, 1992) and at the light microscope level by Con-A (Parillo et al., 2009). It has been reported that mannose moieties present in the RER may be associated with the initial assembly of the N-linked oligosaccharides of glycoproteins, which is completed in the Golgi stacks (Parillo et al., 2009).

WGA has been used in this study to detect sialoglycoconjugates, and the detailed sialic acid binding pattern to internal galactose can be recognized using SNA and MAA; SNA detects N-acetylneuraminic acid and α-N-acetylgalactosamine, and MAA detects N-acetylneuraminic acid. Sialic acid residues were detected in some areas in both type I and type II epithelia as demonstrated by binding to WGA. Detailed sialic acid binding was not detected due to lack of binding sites for SNA and MAA. Differences in staining of the same population of cells as in some cases of WGA binding indicate various stages of secretion as well as cellular compartmentalization.

No binding sites for DBA and SNA were detected in our study. Both DBA and SNA react to α N-acetylgalactosamine. SBA reacts to both α and β N-acetylgalactosamine. Binding sites for SBA were detected in some compartments in both type I and type II epithelia, these same areas lacked binding sites for DBA and SNA. This indicates that these areas showing binding sites for SBA contain β N-acetylgalactosamine and not α N-acetylgalactosamine.

The Golgi zone did not appear to be stained for most of the lectins used in this study with the exception of some weak staining in the case of

SBA and WGA. If the cells had been actively involved in the synthesis and secretion of glycoconjugates, the Golgi zone should reveal strong binding sites. Camels are seasonal breeders with a relatively short breeding season during the cooler months from December to April (Hafez and Hafez, 2001). The specimens were collected outside the rutting season; this might explain the lack of strong staining in the Golgi zone.

The glycocalyx of both ciliated and non-ciliated cells in both type I and type II epithelia showed binding sites for Con-A, SBA, and WGA; this indicates the functional complexity of the glycocalyx in the two types of epithelium.

Lectin staining at the apical cytoplasm might indicate the presence of glycoconjugates preceding their extrusion. However, cellular blebs were not detected on the luminal surface, which either indicates that apocrine mode of secretion is not detected in the efferent ductules of the dromedary, or high degree of secretion was not detected during the time when the specimens were collected.

While the results of this study may not be of immediate application to dromedary production, it is worth noting that baseline data on variations of reproductive system histology have in the past been shown to be significant in other species. The regional differences in the epithelium we have demonstrated may be related to the male dromedary's ability to fertilize a receptive female in the proper season of the year. Had these specimens been collected at a different time of the year, the pattern might have been different. While further work is needed to determine whether this is the case, it seems unlikely that distinct regional differences at the molecular level are present without some functional significance, and it would be worth knowing whether there may be temporal ones as well.

Conclusion

This study demonstrates that morphological and histochemical features of the efferent ductules of the dromedary provide evidence for the existence of two different types of epithelium (Type I, II), and suggest a high degree of functional compartmentalization of the epithelium of the efferent ductules.

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References

Aire, T. A. (1980): The ductuli efferentes of the epididymal region of birds. J Anat 130 (Pt 4), 707-723.

Aire, T. A., and M. van der Merwe (2003): The ductuli efferentes testis of the greater cane rat (*Thryonomys swinderianus*). Anat Embryol (Berl) 206 (5), 409-417.

Alroy J, Ucci AA, and M. E. A. Pereira (1984): Lectins: histochemical probes for specific carbohydrate residues. In Diagnostic immunochemistry, edited by DeLellis, R. A. New York, 67-88.

Arrighi, S., M. G. Romanello, and C. Domeneghini (1994): Ultrastructure of the epithelium that lines the ductuli efferentes in domestic equidae, with particular reference to spermatophagy. Acta Anat (Basel) 149 (3), 174-184.

Aureli, G., S. Arrighi, and M. G. Romanello (1984): Ultrastructural and cytochemical study on the epithelium lining ductuli efferentes in Equus asinus. Basic Appl Histochem 28 (2), 101-115.

Brooks, S. A., and D. M. S. Hall (2001): Lectin Histochemistry to Detect Altered Glycosylation in Cells and Tissues. In Metastasis Research Protocols: Volume I: Analysis of Cells and Tissues, edited by Brook, S. and Schumacher, U., 49-65.

Burkett, B. N., B. A. Schulte, and S. S. Spicer (1987): Histochemical evaluation of glycoconjugates in the male reproductive tract with lectin-horseradish peroxidase conjugates: I. Staining of principal cells and spermatozoa in the mouse. Am J Anat 178 (1), 11-22.

Chan, L., and Y. C. Wong (1992): Localization of prostatic glycoconjugates by the lectin-gold method. Acta Anat (Basel) 143 (1), 27-40.

Goyal, H. O., and F. Hrudka (1981): Ductuli efferentes of the bull-a morphological, experimental and developmental study. Andrologia 13 (4), 292-306.

Goyal, H. O., V. Hutto, and D. D. Robinson (1992): Reexamination of the morphology of the extratesticular rete and ductuli efferentes in the goat. Anat Rec 233 (1), 53-60.

Goyal, H. O., and C. S. Williams (1988): The ductuli efferentes of the goat: a morphological study. Anat Rec 220 (1), 58-67.

Hafez, E. S., and B. Hafez (2001): Reproductive parameters of male dromedary and bactrian camels. Arch Androl 46 (2), 85-98.

Humason, G. L. (1979): Animal Tissue Techniques: W.H. Freeman and company.

Ilio, K. Y., and R. A. Hess (1994): Structure and function of the ductuli efferentes: a review. Microsc Res Tech 29 (6), 432-467.

Jones, C. J., V. Dantzer, and R. W. Stoddart (1995): Changes in glycan distribution within the porcine interhaemal barrier during gestation. Cell Tissue Res 279 (3), 551-564.

Jones, R., D. W. Hamilton, and D. W. Fawcett (1979): Morphology of the epithelium of the extratesticular rete testis, ductuli efferentes and ductus epididymidis of the adult male rabbit. Am J Anat 156 (3), 373-400.

Jones, R. C., and W. V. Holt (1981): Studies of the deferent ducts from the testis of the African elephant, Loxodonta africana. III. Ultrastructure and cytochemistry of the ductuli efferentes. J Anat 133 (Pt 2), 247-255.

Lewis-Jones, D. I., R. G. Harrison, and E. W. Macmillan (1982): A reexamination of rat ductuli efferentes. Anat Rec 203 (4), 461-462.

Nagy, F. (1990): On the ultrastructure of the male reproductive tract in the Siberian hamster (Phodopus sungorus campbelli). I. The ductuli efferentes. J Submicrosc Cytol Pathol 22 (4), 615-625.

Orsi, A. M., S. M. Matheus, E. A. Gregorio, and C. C. Beu (1998): Morphological investigations of the surface epithelium of ductuli efferentes of black isogenic mice (Mus musculus). Anat Histol Embryol 27 (4), 215-218.

Parillo, F., G. E. Magi, S. Diverio, and G. Catone (2009): Immunohistochemical and lectin histochemical analysis of the alpaca efferent ducts. Histol Histopathol 24 (1), 1-12.

Parillo, F., G. Stradaioli, A. V. Supplizi, and M. Monaci (1998): Lectin-staining pattern in extratesticular rete testis and ductuli efferentes of prepubertal and adult horses. Histol Histopathol 13 (2), 307-314.

Roberts, K. P. (2010). What are the components of the male reproductive system? In Handbook of Andrology, edited by Robaire B and P. Chan.

Lawrence, KS: Allen Press, Inc., 1-5.

Setchell, B. P., S. Maddocks, and D. E. Brooks (1994): Anatomy, Vasculature,Innervation, and Fluids of the Male Reproductive Tract. In The Physiology of Reproduction, edited by E. Knobil and J. D. Neill. New York: Raven Press, 1063-1176.

Stoffel, M. H., and A. E. Friess (1994): Morphological characteristics of boar efferent ductules and epididymal duct. Microsc Res Tech 29 (6), 411-431.

Vicentini, C. A., A. M. Orsi, and E. A. Gregorio (1990): Fine structure of the ductuli efferentes of the hamster (*Mesocricetus auratus*). Gegenbaurs Morphol Jahrb 136 (1), 111-118.

Wakui, S., M. Furusato, H. Takahashi, M. Motoya, and S. Ushigome (1996): Lectin histochemical evaluation of glycoconjugates in dog efferent ductules. J Anat 188 (Pt 3), 541-546.

Wrobel, K., and M. Bergmann (2006): Male Reproductive System. In Dellmann's Textbook of Veterinary Histology, edited by Eurell J and F. B. Iowa: Blackwell Publishing.

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Table 1: lectins used for identifying carbohydrate residues in the efferent dutcules of the dromedary

Source of lectin	Common name	Acronym	Concentration (ug/ml)	Carbohydrate specificity	Inhibitory sugar
Canavalia ensiformis	Jack bean	CON A	10	α-D-mannose/α-D-glucose	α-D-methylmannose
Dolichos biflorus	Horse gram	DBA	50	α-D-*N*-acetylgalactosamine.	α-D-*N*-acetylgalactosamine
Maackia amurensis	Maackia seed	MAA	50	*N*-acetylneuraminic acid	NA
Glycine max	Soybean	SBA	50	α- and β- D-*N*-acetylgalactosamine.	α- and β- D- *N*-acetylgalactosamine
Sambucus nigra	Elderberry	SNA-1	50	*N*-acetylneuraminic acid/ α-*N*-acetylgalactosamine	NA
Triticum vulgaris	Wheat germ	WGA	50	*N*-acetylglucosamine/ sialic acid	*N*-acetylglucosamine

NA: not applicable

Table 2: lectin binding profiles in the efferent ductules of the dromedary

	Type I epithelium		Type II epithelium	
	Ciliated cells	Non-ciliated cells	Ciliated cells	Non-ciliated cells
CON-A				
Cilia	+++	NA	+++	NA
Glycocalyx	+++	+++	+++	+++
Apical cytoplasm	+++	+++	+++ (-)	+++ (-)
Golgi zone	-	-	-	-
Basal cytoplasm	+++	+++	+++	+++
Basal vacuoles	NA	NA	-	-
Peritubular sm	++		++	
Intertubular CT	+		-	
Luminal contents	++		++	
SBA				
Cilia	+++	NA	+++	NA
Glycocalyx	+++	+	+++	+++
Apical cytoplasm	-	-	+++	+++
Golgi zone	-	-	+	+
Basal cytoplasm	-	-	++	++
Vacuoles	NA	NA	+	+
Peritubular sm	-		-	
Intertubular CT	-		-	
Luminal contents	++		++	
WGA				
Cilia	+++	NA	+++	NA
Glycocalyx	+++	+++	+++	+++
Apical cytoplasm	+++ (-)	+++ (-)	++ (-)	++ (-)
Golgi zone	- (+)	- (+)	-	-
Basal cytoplasm	++	++	++	++
Vacuoles	NA	NA	+ (-)	+ (-)
Peritubular sm	+		-	
Intertubular CT	+		-	
Luminal contents	++		++	

All control sections failed to disclose appreciable reactivity at any of the above sites

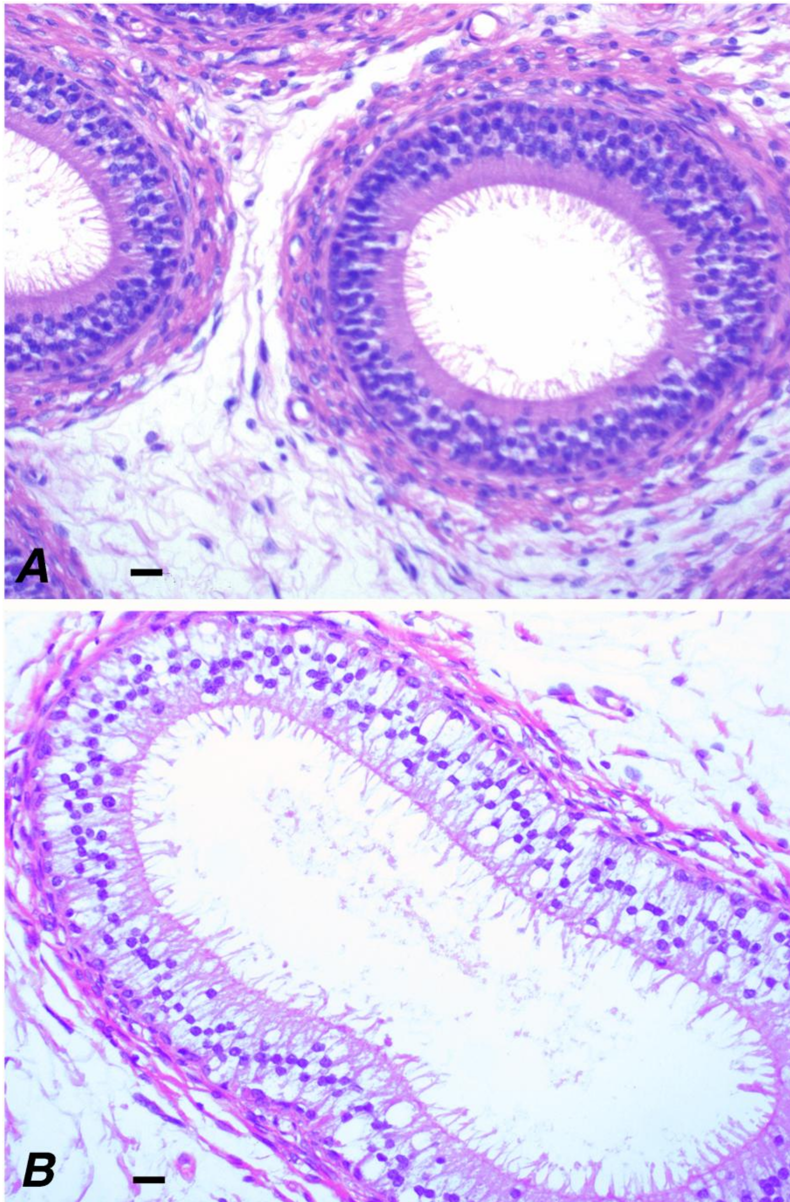

Fig (1): Ductuli efferentes of *Camelus dromedarius* stained with hematoxylin and eosin. The epithelial lining is a low pseudostratified columnar type containing two populations of cells, in which both ciliated and non-ciliated varieties occur. A. Type I epithelium showing apical cytoplasmic granules and a greater distance between the nuclei and the luminal surface; B. Type II epithelium showing basal vacuoles. Bar = 200 µm.

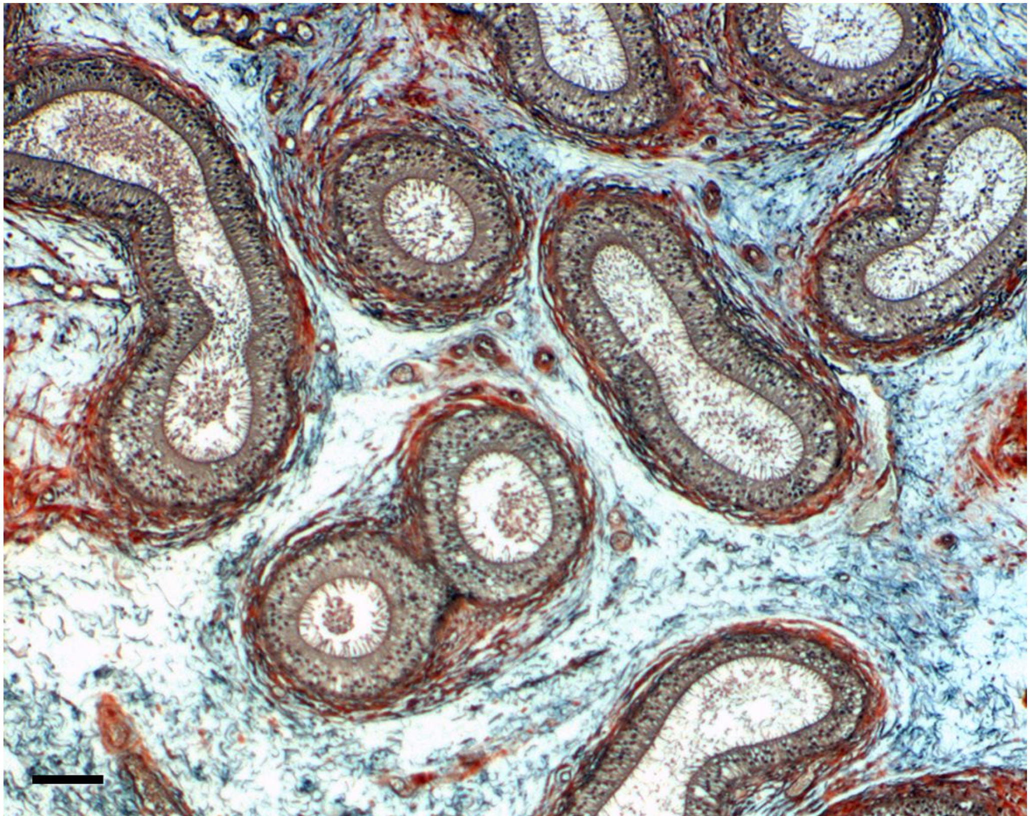

Fig (2): Ductuli efferentes of *Camelus dromedarius* stained with Masson's Trichrome and Verhoeff's stains showing that the tubules were surrounded by delicate peritubular smooth muscle cell layers (red color) with a loose collagenous connective tissue (bluish color) dispersed in the space between adjacent tubules. Bar = 100 µm

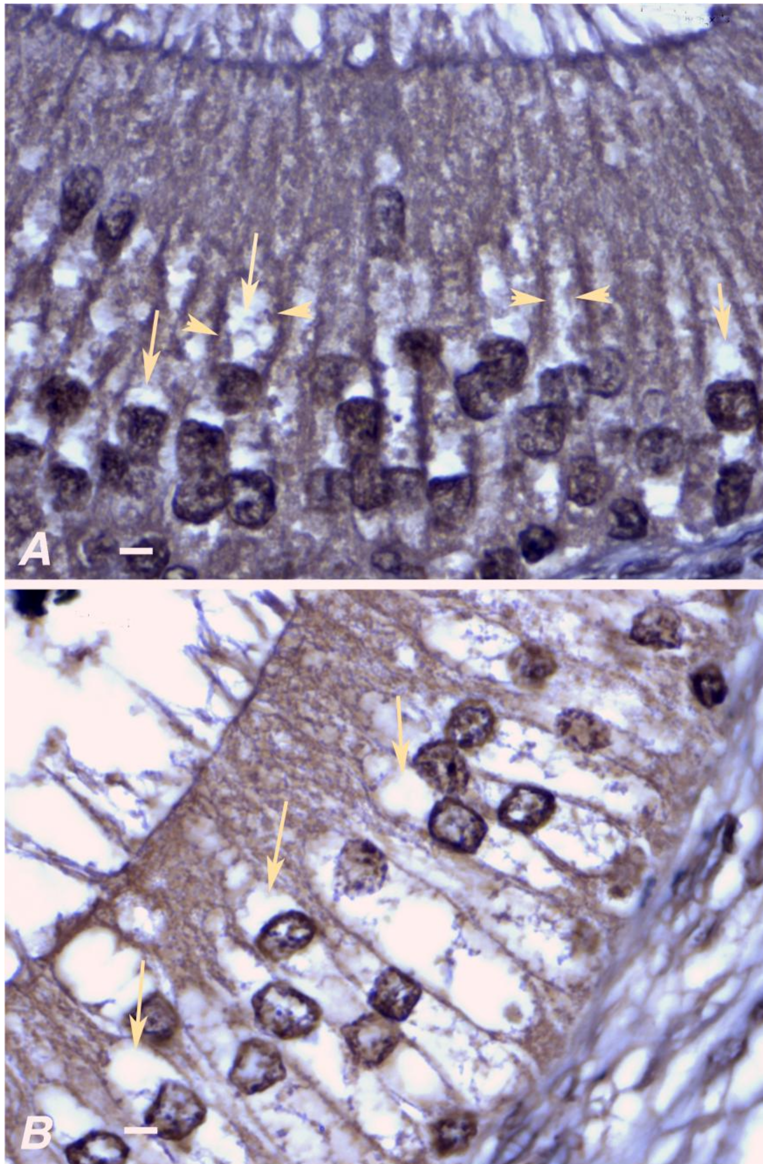

Fig (3): Con-A binding in the glycocalyx and the apical and basal cytoplasm in type I epithelium (A) and type II epithelium (B). No binding sites for Con-A were detected in the Golgi zone of epithelial cells of either type I or type II epithelium (arrows); however, the lateral cytoplasm on either side of Golgi zone appeared to have positively stained granules in type I (arrow heads in A), but not in type II epithelium. Con-A bound with Cilia in A and B, and did not bind to basal vacuoles in B. Bar = 40 μm.

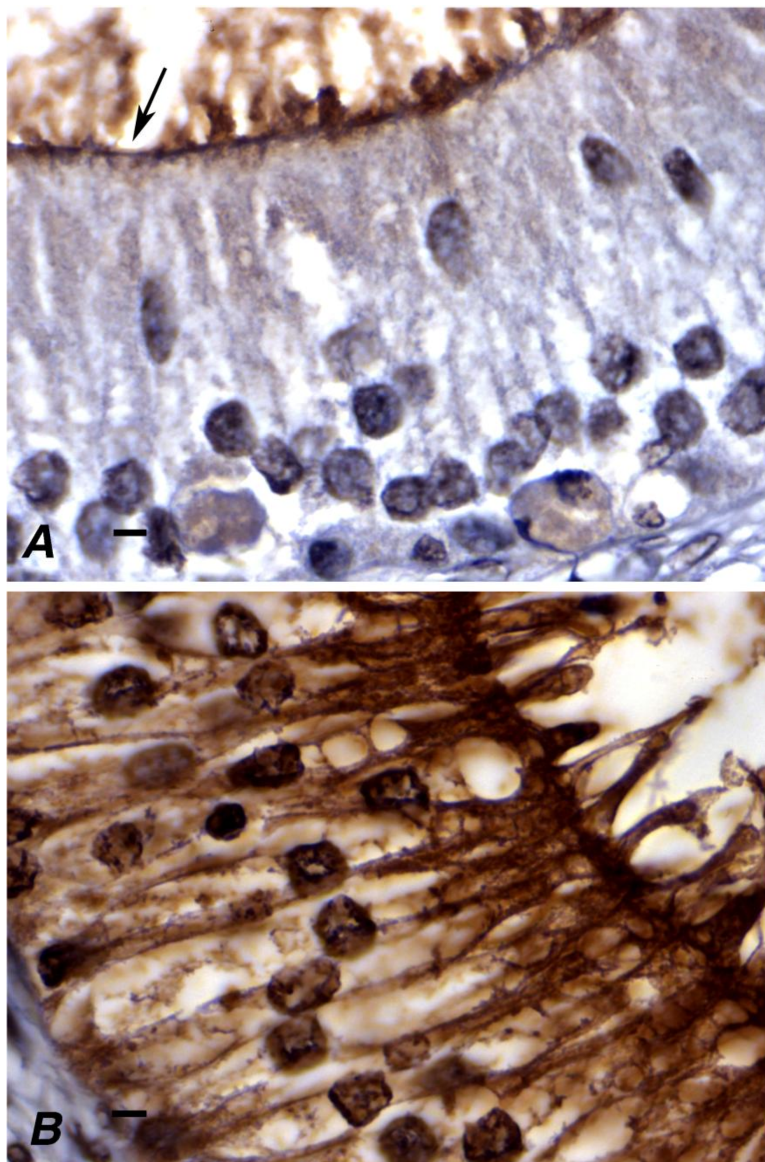

Fig (4): SBA binding in the glycocalyx of ciliated cells and non-ciliated cells (with less intensity, arrow) in type I epithelium (A). The glycocalyx and the apical and basal cytoplasm of the epithelial cells of type II epithelium (B) showed binding sites for SBA. Golgi zone and the basal vacuoles of type II epithelium appeared weekly stained. SBA bound with cilia in both types of epithelia. Bar = 40 µm.

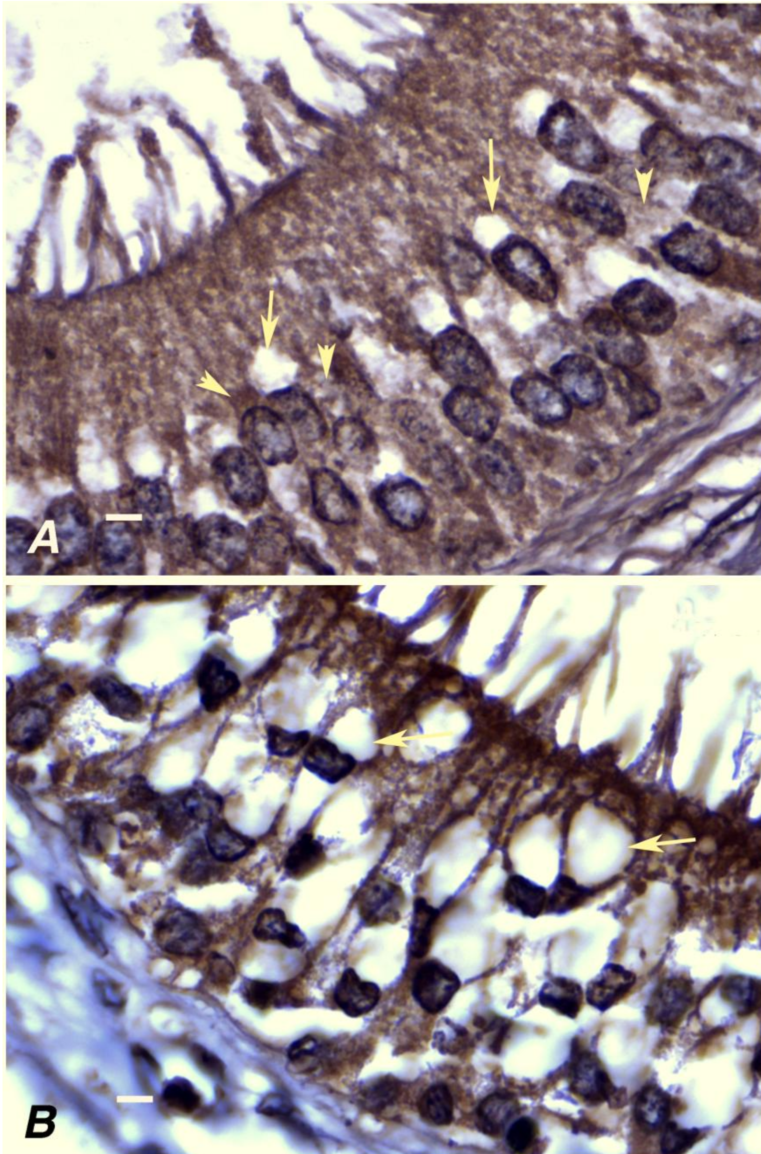

Fig (5): WGA binding in the glycocalyx, the apical cytoplasm, and the basal cytoplasm in type I epithelium (A) and type II epithelium (B). The apical cytoplasm showed unstained areas in addition to positively stained areas. The Golgi zone (arrows) in neither type of epithelia showed binding sites with WGA except in a subgroup of cells (arrow heads) in type I epithelium. Some positively stained granules were detected in the basal vacuoles of type II epithelium. WGA bound with cilia in type I and type II epithelia. Bar = 40 µm.