

Microscopic anatomy and ultrastructure of the *Camelus dromedarius* ureter

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SUMMARY

The histology and ultrastructure of the ureter of *Camelus dromedarius* were investigated. The ureteric wall included a tunica mucosa of transitional epithelium (urothelium); an underlying loose connective tissue layer (propria-submucosa); a tunica muscularis of smooth muscle forming inner longitudinal, middle circular, and outer longitudinal layers; and a tunica adventitia or a tunica serosa. The smooth muscle layer of the renal pelvis was scarce or lacking. The urothelium consisted of basal cuboidal to columnar cells, intermediate cells, and larger, pale superficial cells with small nuclei. The epithelium of the renal pelvis was only a few cells deep. The transitional epithelium appeared to be adapted for extensibility and water impermeability. The cytoplasm of the superficial and intermediate cells contained many membrane-bound vesicles. Junctional complexes were seen between the lateral membranes of the superficial cells providing a barrier to the passage of substances and urine into the ureteric wall. The junctional complexes and the membrane-bound vesicles may play an important role in membrane turnover and in regulating the expansion and contraction of the urothelium. In this study, we report the presence of submucosal glands in the renal pelvis of

Camelus dromedarius. The serous nature of the submucosal glands, seen under the light microscope, was confirmed with the electron microscope. These glands had nearly pyramidal-shaped cells lying on a basement membrane and a lumen. The cells of the epithelial lining were mainly dark with a central spherical nucleus. A connective tissue capsule could be seen around the gland.

Key words: camel – ureter gland – ureter histology – urinary system – transitional epithelium

INTRODUCTION

The popular myth about the miracle of camels' urine has drawn great attention among scientists. There is a belief in some areas of the Middle East that drinking camel's urine cures some diseases such as liver diseases. Some people believe that it is also beneficial in the treatment of skin diseases such as ringworm, tinea and abscesses. They also claim that it benefits hair when applied to the scalp. Several studies have been carried out to investigate the medicinal value, if any, in camels' urine (Al-Harbi et al., 1996; Khorshid, 2009; Al-Yousef et al., 2012).

The morphology of the kidneys of camels has been the subject of many studies (Abdalla and Abdalla, 1979; Moussa, 1982; Safer et al., 1988). The ureters convey the final urine produced in the

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kidney to the bladder. They arise from the renal pelvis and follow an oblique path through the wall of the bladder. Peristaltic contractions of the smooth muscle in the wall of the ureter move the urine from the renal pelvis through the ureter to the bladder. The histology and the ultrastructure of the ureter have been studied in several species (Hicks, 1965; Shehata, 1965; Firth and Hicks, 1973; Woldemeskel, 1998; Verlamder, 2006) including man (Stein and Weinberg, 1962; Hana et al., 1976); and inter-species variations in the fine structure of the transitional epithelium have been documented (Firth and Hicks, 1973). Some unique microscopic structures have been described in the ureter of some species, such as the horse and the donkey (Shehata, 1965; Verlamder, 2006). However, information about the microscopic features of the ureter of the camel is lacking.

The purpose of this study was to investigate the structure of the ureter of the camel as seen under the light and electron microscopes. We believe that histological and electron microscopical examination of the ureter and the renal pelvis in the dromedary camel can provide a useful morphological baseline to the understanding of its physiology, or more generally, to a better understanding of the physiology of the urinary system in camels.

MATERIALS AND METHODS

Ureters from five apparently healthy adult dromedaries were collected immediately after slaughter at Kom Hamada abattoir in El-behera, Egypt. These animals were slaughtered for human consumption, and were examined prior to slaughter by the slaughterhouse

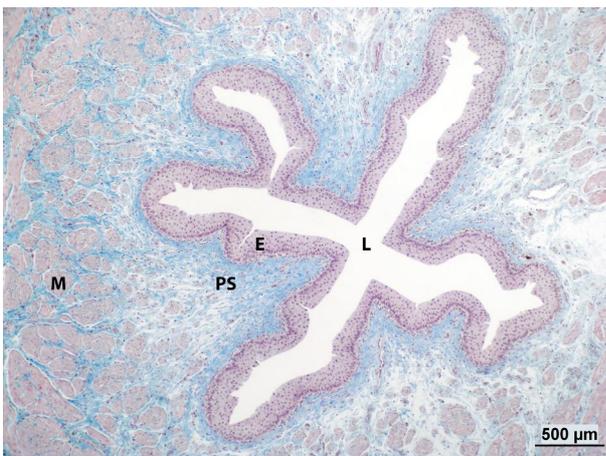


Fig. 1. Ureter of *Camelus dromedarius* stained with Masson's Trichrome and Verhoeff's stains showing the stellate lumen (L) of the un-distended ureter. Wall of the ureter includes tunica mucosa of transitional epithelium (E), propria-submucosa (PS), and a tunica muscularis (M).

veterinarian for approval for human consumption. Samples of the renal pelvis, proximal ureter, and distal ureter were obtained, fixed in 10% buffered formalin solution 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.

For electron microscopy, 1mm samples were obtained from the renal pelvis, and the proximal and distal segments of the ureter. Specimens were immediately fixed in 6% phosphate buffered gluteraldehyde, pH 7.4 at 4°C, serially washed in cold (4°C) 0.1 M phosphate buffer, post fixed in 1% osmium tetroxide, processed and then embedded in Araldite epoxy resin

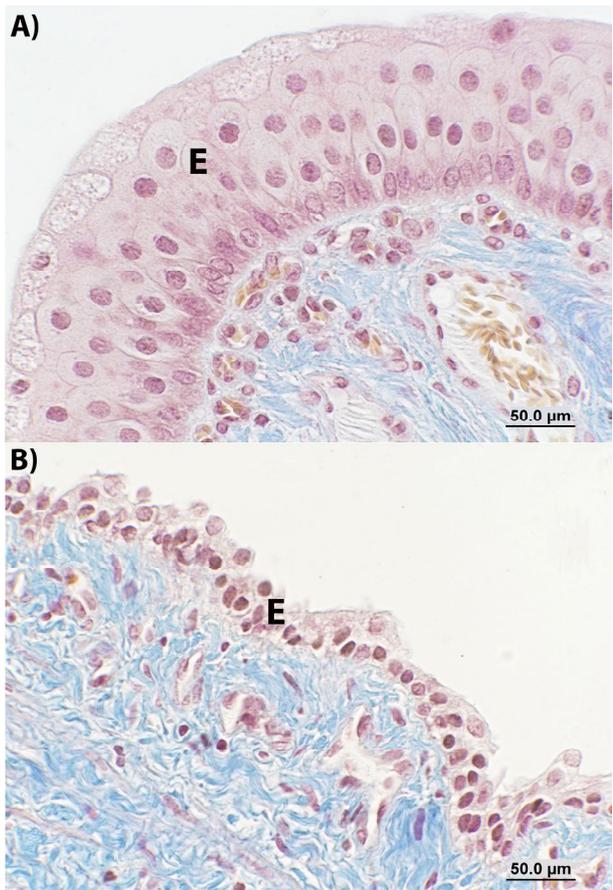


Fig. 2. (A) Distal segment of the ureter of *Camelus dromedarius* stained with Masson's Trichrome and Verhoeff's stains showing the transitional epithelium (E) with basal cuboidal to columnar cells, intermediate cells, and larger, pale, and with small nuclei superficial cells. The epithelium (E) of the renal pelvis in (B) is only a few cells deep.

(Hayat 1986). Semithin sections (1 μ m) were cut and stained with toluidine blue; then ultrathin sections (60-100 nm) were cut and stained with uranyl acetate, followed by lead acetate. The sections were examined and photographed with a JOEL transmission electron microscope at 80 KV.

RESULTS

Light microscopy

The wall of the ureter included a tunica mucosa of transitional epithelium (urothelium); an underlying loose connective tissue layer (lamina propria-submucosa); a tunica muscularis externa of smooth muscle forming inner longitudinal, middle circular, and outer longitudinal layers; and a tunica adventitia of loose connective tissue or a tunica serosa of mesothelium and connective tissue when peritoneal covering is present.

The mucosal layer was folded, which gave the typical stellate appearance of the lumen in cross section when the ureter was not distended (Fig. 1). The epithelial lining was a typical transitional epithelium with cuboidal to columnar cells in the deep layers and larger, pale cells with small nuclei forming the topmost layer (Fig. 2A). The transitional epithelium showed three different cell types: basal, intermediate and superficial cells. The epithelium of all segments was several cells deep except in the renal pelvis, where it was only a few cells deep (Fig. 2B). The lamina propria was composed of dense irregular connective tissue, which merged with the submucosa, because there was no muscularis mucosa.

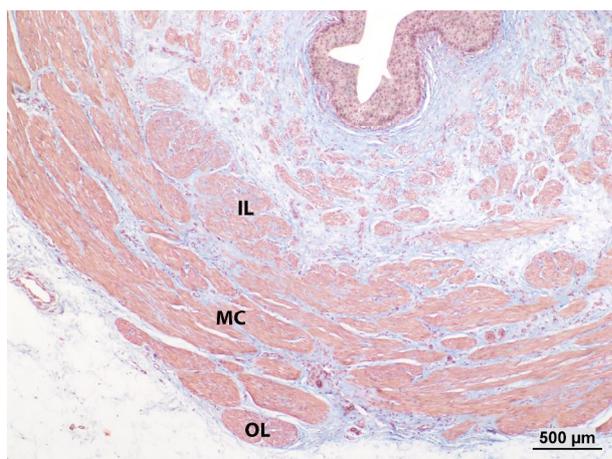


Fig. 3. Ureter of *Camelus dromedarius* stained with Masson's Trichrome and Verhoeff's stains showing the arrangement of the ureteric musculature. IL: inner longitudinal layer; MC: middle circular layer; OL: outer longitudinal layer.

The layered arrangement of the ureteric muscle coat was not clearly distinguishable. The layers generally appeared as loose, anastomosing strands of smooth muscle separated by abundant collagenous connective tissue (Fig. 3). The middle circular layer of the tunica muscularis was the most distinct among the layers of the muscularis. The outer longitudinal layer was least distinct in the distal portion of the ureter. There was a lot of branching amongst the different muscle bundles, so there was no true spiraling of the muscles around the ureter. The smooth muscle layer of the renal pelvis was scarce or lacking. Blood vessels and lymphatics were scattered in all layers except the epithelium.

Glands were observed in the lamina propria-submucosa of the renal pelvis mostly deeper in the submucosa. The glands were arranged in groups and were lined with cuboidal epithelium, usually a single cell in thickness. The acini and the secretion of the glands showed eosinophilia (with basophilic nuclei) when stained with hematoxylin and eosin. With Masson's trichrome stain (Fig. 4A), the acini and/or their

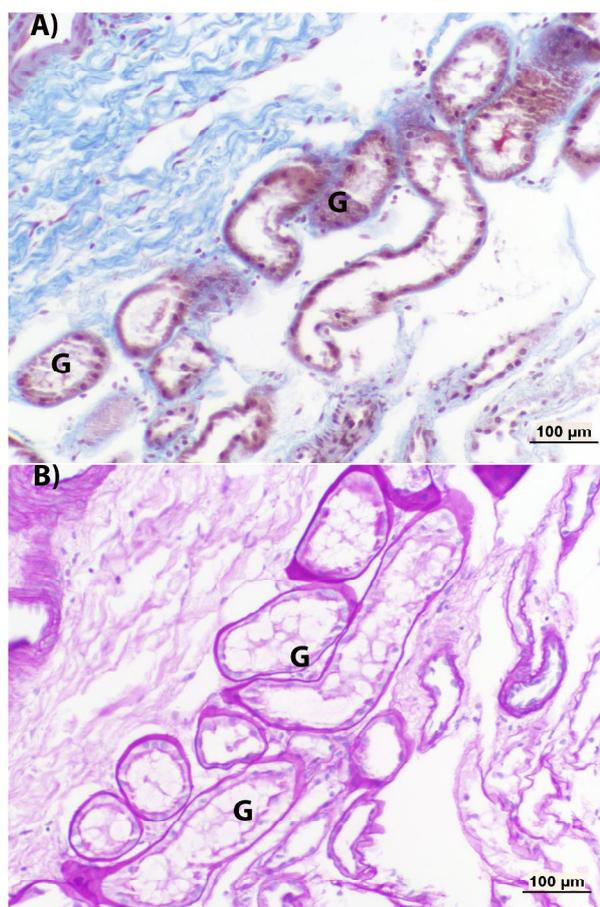


Fig. 4. (A) Masson's Trichrome staining of the renal pelvis of *Camelus dromedarius* showing serous submucosal glands (G). (B) PAS staining of these glands (G).

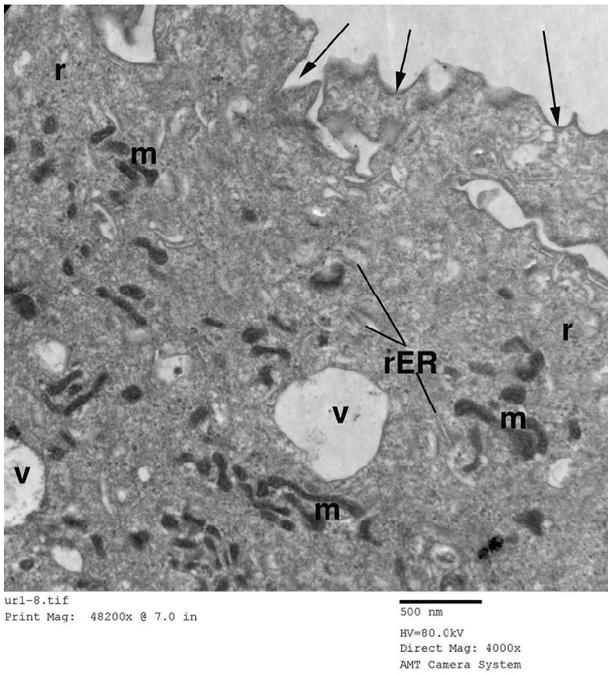


Fig. 5. Transmission electron micrograph of the proximal part of the ureter of *Camelus dromedarius* showing the cytoplasm of the cell layer closest to the lumen. These cells exhibit vacuoles (v), mitochondria (m), rough endoplasmic reticulum (rER), and free ribosomes (r). Note the irregular free luminal surface of the cells (arrows).

secretion colored mauve. These glands exhibited a strong PAS-positive basement membrane (Fig. 4B). The displacement of the nuclei toward the lumen, lack of PAS-positive staining of the secretion, and the rounded nuclei suggested the serous nature of the secretion of these glands.

Transmission electron microscopy

The epithelium showed three different cell types. The cells in the layer facing the lumen were large, umbrella-shaped, and somewhat electron-lucent with an irregular dense cell membrane facing the lumen (Fig. 5). Their cytoplasm contained many membrane-bound vesicles, mainly at the apical part of the cell. Clusters of mitochondria and free ribosomes were also scattered in the cytoplasm with a few profiles of rough endoplasmic reticulum, some lysosomes, and a Golgi apparatus. Nuclei of these cells were spherical to ovoid with prominent nucleoli and were located mainly at the base of the cell.

The cells of this superficial layer were connected to each other on the luminal portion of the lateral membrane through junctional complexes composed of a Zonula occludens, at the luminal end, with a Zonula adherens deep to that, and deep to those a macula adherens or desmosomes. On the distal portion of the lateral border and the distal border, the superficial cells were

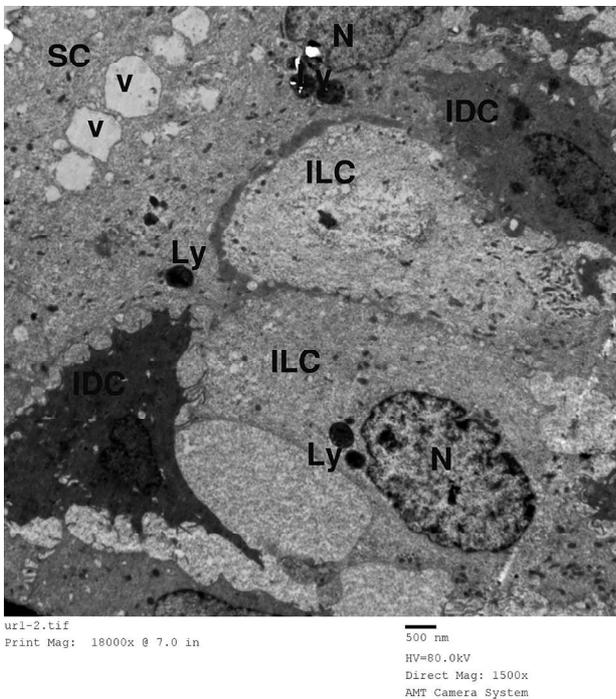


Fig. 6. Transmission electron micrograph of the proximal part of the ureter of *Camelus dromedarius* showing the intermediate light cells (ILC) and intermediate dark cells (IDC). Part of the superficial cells (SC) is in view. V: cytoplasmic vesicles; Ly: lysosomes; N: nucleus.

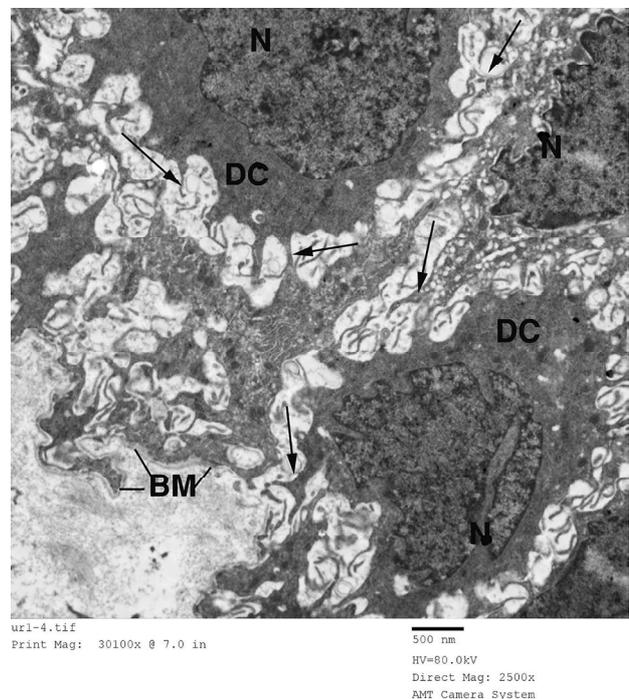
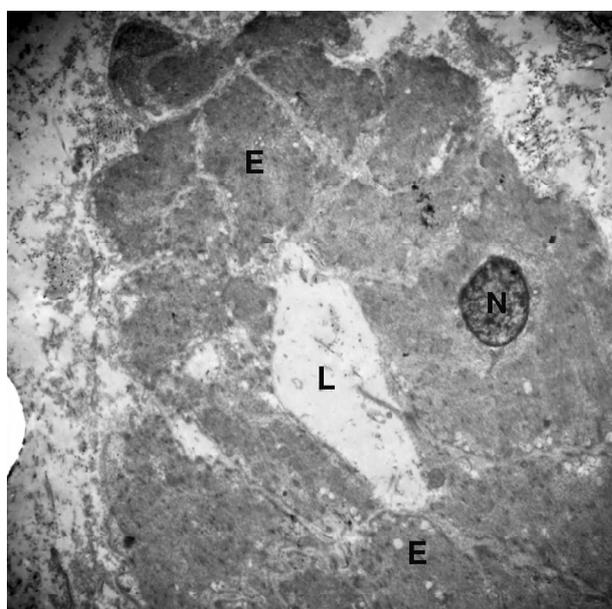


Fig. 7. Transmission electron micrograph of the proximal part of the ureter of *Camelus dromedarius* showing the basal dark cells (DC) resting on a corrugated basement membrane (BM) and the indented nuclei (N) of the cells. Note the cytoplasmic interdigitation between cells (arrows) leaving wide intercellular spaces.

connected to each other and to the intermediate cells through interlocking cytoplasmic processes.

The multi-layered intermediate zone consisted of both electron-dense and electron-lucent cells (Fig. 6). Electron-dense cells were generally smaller in size than the electron-lucent ones. Long Intercellular cytoplasmic processes were evident among cells of this zone and between these cells and the underlying basal cells. Intercellular spaces varied in size and they were more or less larger between dark cells than between light cells. Desmosomes were evident between cells of the intermediate zone. Membrane-bound cytoplasmic vesicles were found in the cells of this zone, but they were less numerous than those of the superficial layer. The nuclei were oval with some indentations in the nuclear envelope.

The basal cell layer showed a corrugated basement membrane and consisted of electron-dense and electron-lucent cells, of which the former were predominant (Fig. 7). Cytoplasmic vesicles and lysosomes were very rare. Abundant rough endoplasmic reticulum was seen around the nuclear envelope. Lateral borders of the basal cells exhibited extensive cytoplasmic interdigitation and wide intercellular spaces. This interdigitation was also seen between the basal portion of the cells and the basal lamina leading to an extensive infolding of the basal lamina. The nuclei were large oval with indentations.



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Fig. 8. Transmission electron micrograph of the renal pelvis of *Camelus dromedarius* showing the submucosal glands with a lumen (L) and epithelial cell lining (E). N: nucleus.

The collagenous lamina propria-submucosa contained numerous blood vessels. The serous nature of the submucosal glands seen under the light microscope was confirmed with the electron microscope (Fig. 8). These glands had nearly pyramidal-shaped cells resting on a basement membrane and a lumen. The cells of the epithelial lining were mainly dark with a central spherical nucleus. A connective tissue capsule could be seen around the gland.

DISCUSSION

The histological organization of the ureter of the camel is similar to that of other species, but has unique species-specific features (Stein and Weinberg, 1962; Shehata, 1965; Murnaghan, 1967; Hanna et al., 1976). This is the first study to report the presence of submucosal glands in the renal pelvis in *Camelus dromedarius*.

The displacement of the nuclei toward the lumen, lack of PAS-positive staining of the secretion, and the rounded nuclei suggest the serous nature of the secretion of these glands. The serous nature of the submucosal glands seen under the light microscope was confirmed with the electron microscope. In the horse, mucosal mucous glands present in the renal pelvis and the proximal ureter contribute to the viscous, stringy nature of horse urine. These glands are found beneath the epithelium in the horse (Verlander, 2006). In the dromedary camel, glands are found farther away from the epithelium. Also the nature of the glands found in the camel was different from the ones found in the horse. The glands in the horse are mucous in nature, while histological features of the glands in the dromedary camel suggest a serous-type gland. This might explain the lack of foamy urine in the dromedary camel and the foamy nature of the urine in the horse. The presence of mucous gland at the distal end of ureter was reported in the donkey (Shehata, 1965). These glands were found in the submucosa of the donkey ureter and were limited to the dorsal wall of the ureter.

The differences in the glandular profiles in two very similar species, horse and donkey, lead to the speculation that other camelids, such as the Bactrian camel and the llama, might similarly display such variations. As those species have yet to be investigated, further work would be needed to establish whether the pattern of variations-within-a-group exists and what (if any) those variations might be. Since anatomical variations are usually the result of very subtle differences in selection pressures from the external environment, if such differences exist and could be demonstrated, they might allow for a better understanding of the unique physiology of the dromedary.

The transitional epithelium appeared to be adapted for extensibility and water impermeability. The cells of the superficial layer are connected to each other on the luminal portion of the lateral membrane through junctional complexes. Walton et al. (1982) suggested that the tight junctions between the lateral membranes of the superficial cells provide a barrier to the passage of substances and urine into the bladder wall. This would be appropriate as well in the ureter, since the epithelium functions in both the urinary bladder and the ureter to provide a barrier against fluid flow between the epithelial cell and the hypertonic urine.

The presence of membrane-bound vesicles has been reported in the urothelium of other species; in rats, rabbits, bats, mice, lemmings, cows, cats, dogs, common seals, grey seals, and rhesus monkeys (Firth and Hicks, 1973); in pigs (Firth and Hicks, 1973; Woldemeskel et al., 1998); in gerbils, and guinea-pigs (Firth and Hicks, 1973; Minski and Chlapowski, 1978); in hamsters (Minski and Chlapowski, 1978); as well as in man (Firth and Hicks, 1973; Tannenbaum, 1979; Jost et al., 1989). All the afore-mentioned studies reported the presence of the vesicles in the superficial cell layer, but only some of these studies also reported the presence of these vesicles in the intermediate cell layer. Disagreement even exists in between studies performed on the same species; *i.e.* Firth and Hicks (1973) reported the presence of vesicles only in the superficial cells of the urothelium in pigs. In contrast to their findings, Woldemeskel (1998) reported the presence of vesicles in both the superficial and intermediate cells of urothelium in the same species. These authors supported the idea that the intermediate cells may be precursors of the superficial cells suggested initially by Hicks (1965).

Vesicular profiles at the surface of an epithelial sheet imply that something is being moved into or out of the cells. Given the "tight" nature of the urothelium, such a mechanism of bulk transport would be of obvious utility in adjusting the contents of the urine or maintaining the integrity of the cells of the urothelium. Tracer studies might establish the nature of the traffic between the two compartments, and provide valuable clues to the function of the vesicles. That they are so widely distributed among very dissimilar species argues that the vesicles are of some physiological importance.

The junctional complexes and the membrane-bound vesicles may play an important role in membrane turnover and in regulating the expansion and contraction of the urothelium (Woldemeskel et al., 1998).

The variations in the number of cell layers and

the size of the intercellular spaces observed in the present study and as reported by others (Stein and Weinberg, 1962; Hicks, 1965; Hanna et al., 1976; Woldemeskel et al., 1998) may reflect more the variable physiological status of the epithelium at the time of sample collection, and/or at the time of sacrifice of the animal than interspecies variations.

The ill-defined arrangement of the muscle bundles in the tunica muscularis of the ureter was previously noted in a study of the human ureter (Murnaghan, 1967). Due to such arrangements the author of that study described the ureter as "a non-layered muscular tube". It is not obvious why the tunica muscularis externa should be "non-layered" and/or how this situation would contribute to efficient movement of urine from the renal pelvis to the bladder.

We conclude that the distinctive architecture of the ureter and the renal pelvis in the dromedary, while it bears similarities to other species', is sufficiently distinct to warrant further investigation, as it must clearly be related to this animal's extraordinary tolerance for heat and desiccation. While the present study provides baseline morphological information, it is clearly desirable to relate these observations to the physiology of the dromedary's renal function. We suggest as an initial step that histochemical investigations be conducted to complement the morphology; and that comparative studies of other camelids would have significant value as well. From there, controlled experiments could be done to discern what – if any – changes in the structure and/or renal function may be observed in camels that are subjected to non-desert environmental conditions. These sorts of studies would provide greater insight into the evolutionary development of an economically important species, and perhaps advance the husbandry of these fascinating animals.

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