JOTRES journal of technical reports in engineering and applied sciences

JOURNAL OF TECHNICAL REPORTS IN ENGINEERING AND APPLIED SCIENCE

Contents available at: www.jotres.com

Journal of Technical Reports on Engineering and Applied Sciences
An International Journal visit us at www.jotres.com

Histomorphological and ultrastructural studies of kidney in mole rats

Huseyin Turker
Ankara University, Science Faculty, Department of Biology, 06500, Ankara, Turkey

ARTICLE INFO | **ABSTRACT** | **ORGIGINAL RESEARCH ARTICLE**

Article History
Received: April 2016
Accepted: April 2016
Keywords:
Mole rats,
kidney,
ultrastructure,
light microscope,
electron microscope

This study was designed to elucidate the histological and ultrastructural variations in the glomerular cells of mole rats (*Spalax leucodon*). The kidney tissues were processed and prepared for light and transmission electron microscopes. The microscopic results revealed that the nephron consisted of renal corpuscles, proximal tubules, distal tubules, intermediate segment and collecting tubules. The renal corpuscles had large capillaries with fenestrated endothelial cells. The proximal tubule was consisted of simple cuboidal cells that showed long microvilli, abundant mitochondria, extensive plasma membrane infoldings, rare endoplasmic reticulum and less cytoplasmic vacuoles. The distal tubule cells lined around the lumen showed few microvilli, less mitochondria, large nuclei situated in basal area and they had plasma membrane infoldings at the basal side. The collecting tubule was consisted of cuboidal epithelial cells that had a few microprojections at the apical surface, large nuclei situated in basal area and less stoplasmic organels. As a result, this study revealed that although the kidney of mole rats had many structural similarities to other animal kidneys, it also had considerable structural differences.

1. Introduction

The kidneys are large bean-shaped organs, located on either side of the lower back, just below the rib cage.Whatever type of kidney may be, all kidneys consist of morphological, structural and functional units called nephrons.The nephrons are composed of renal corpuscle, proximal tubules, distal tubules, intermediate segments and collecting ducts. They regulate the body fluid and electrolyte balance in the urinary sytem (**Ross et al., 1989; Eurell and Frappier, 2007**).

The waste products of metabolism, including nitrogen and inorganic materials were removed actively from the body by kidneys (**Ojeda et al., 2003; Holz and Raidal, 2006**). Also, water availability and conservation of water is arranged by the kidneys. Thats why, there is a strong correlation between the structure of the kidney and urinary concentrating ability. Ultrastructural examinations showed that the kidney cells have marked variations due to the diversity of the factors involved and to considerable differencies between the species and habitats of animals (**Gabri, 1983; Gambarian, 1994; Bozinovic et al., 2003**).

The animals living in arid or semiarid habitats are faced with water preservation problems according to water availability or not. Thats why, the animals show many adaptation features in their kidneys to conserve or reabsorb the water, such as variations in the medullary thickness, increasing in nephron counts, nephron heterogenetiy, thinning of descending epithelium and so on (**Corte´s et al., 2000; Dantzler, 2003; El-Gohary, 2009; Yuan and Pannabecker, 2010).**

Understanding the ultrastructure of kidneys is very important to evaluate the diverse functions of the kidney. Although the kidney structures in different animals have been well investigated (**Eurell and Frappier, 2007; Reece, 2009**); the ultrastructure of mole rats kidney are not completely studied.

The mole rats, *Spalax leucodon* Nordmann, are fossorial animals which live in under ground galleries. They are mainly phytophagous animals and make their habitats by burrowing in agricultural areas, steppes and gardens (**Kuru, 1987**). While the animals can obtain water from drinking or diet, the mole rats can obtain the water only from food or plants. Because of this situation, it is expected to be happened some changes in their kidneys to supply the adaptation. For this reason, the present study was designed to elucidate the histological and ultrastructural variations of glomerular cells in mole rats.

2. Materials and methods

Five adult mole rats (*Spalax leucodon* Nordmann) of both sexes weighing 180-200 g were used in this study. All mole rats were caught within the rural area of Ankara, Turkey. The mole rats were housed individually in special cages until the euthanization and fed with carrot, potato and plant roots. During the euthanasia the animals were anesthesied and the kidneys were removed immediately and cut into small pieces.

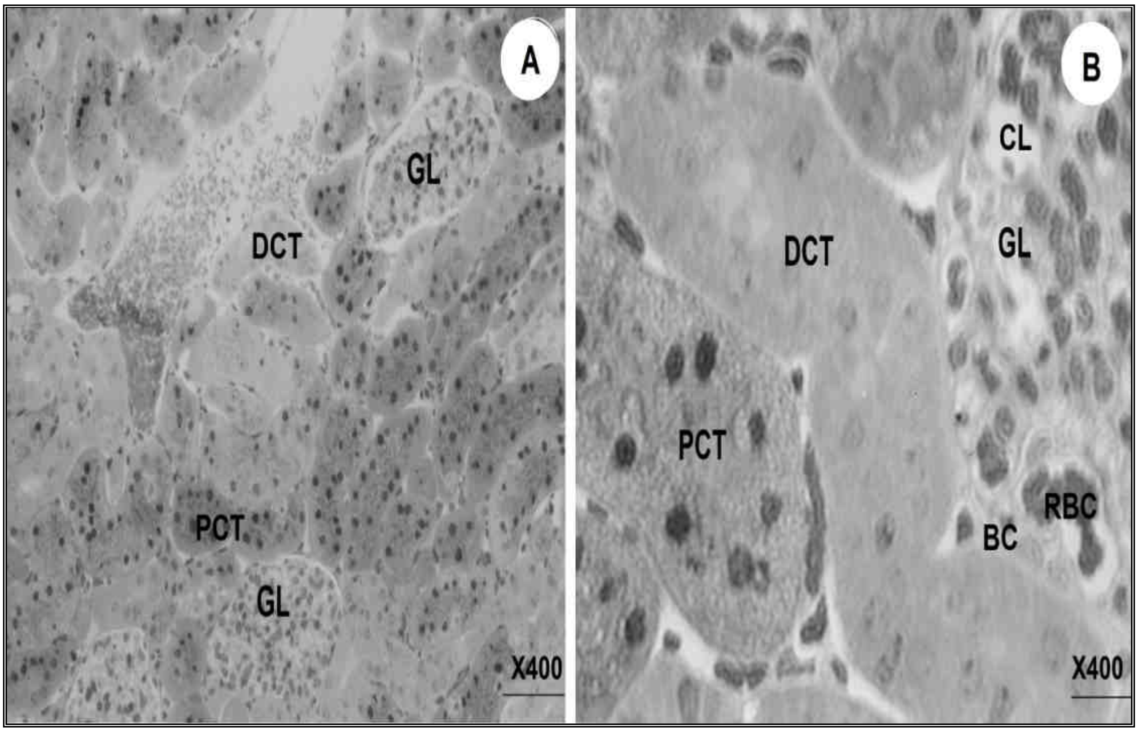
The kidney samples of 1 mm^3 size were fixed in glutaraldehyde (3%), washed in phosphate buffered saline (pH 7.2) at 4 ^{0}C for three hours and post fixed with osmium tetroxide (1%) for one hour. Then osmium tetroxide was washed away with buffered saline. Ethyl alcohol was used for dehydration and specimens were embedded in Araldite CY- 212.

Ultrathin sections were obtained by an ultramicrotome and picked up onto lams and the finder grids. The thin sections were stained by hamotoxylin and eosin, ultrathin samples were stained with saturated uranyl acetate (for 20 min.) and lead citrate (for 10 min.)(**Sato, 1967**). While thin specimens were examined under the light microscope, the ultrathin specimens were examined on Jeol JEM 100 CX-II electron microscope in Gulhane Academic and Medical University, Ankara.

All experiments were carried out in accordance with the university guidelines for the care of experimental animals. Also, guiding principles for experimental procedures found in Declaration of Helsinki of the World Medical Association regarding animal experimentation were followed in the study**.**

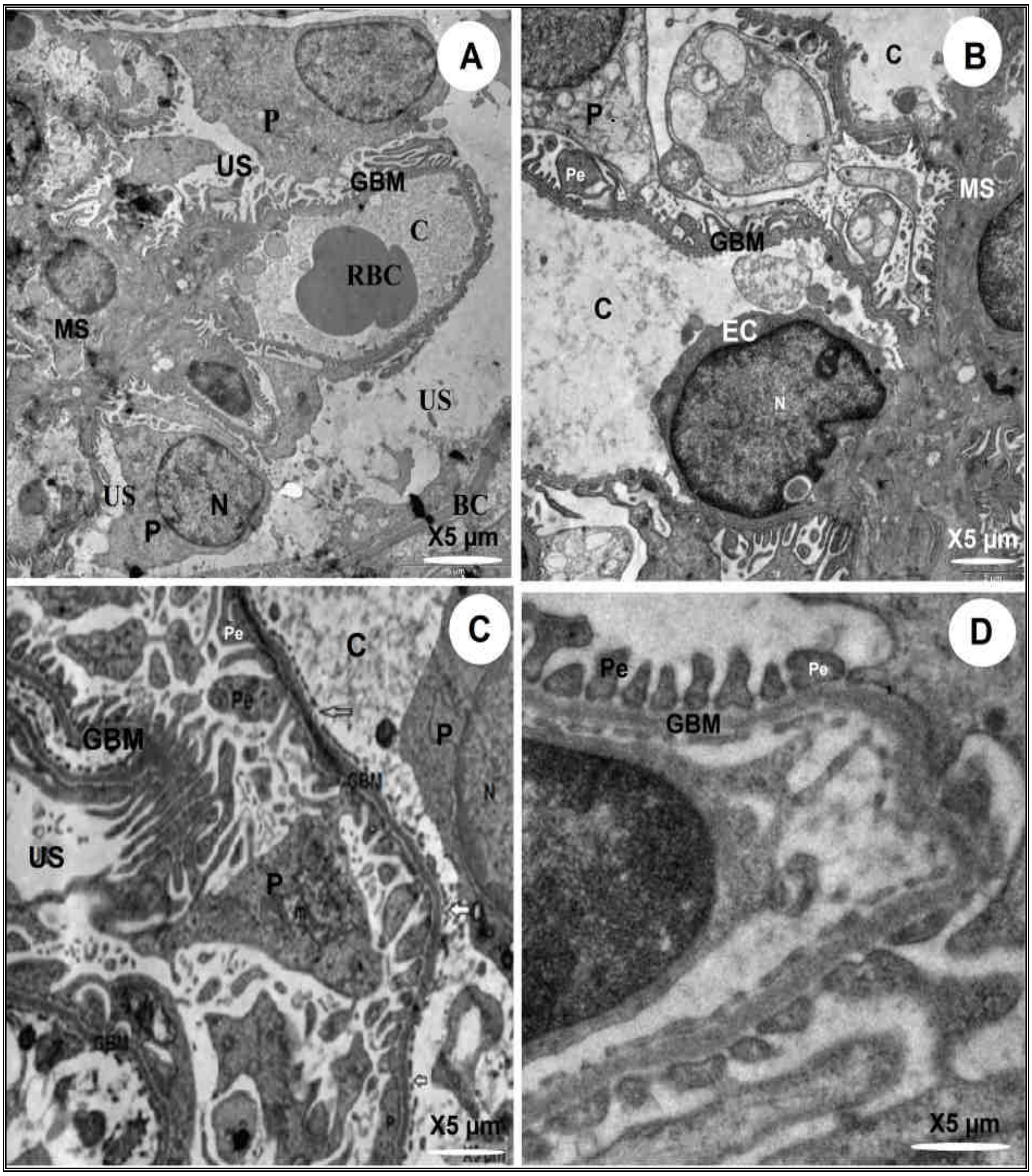
Results

Histological results: Light microscopic examination of kidney section of mole rats showed normal structure of the renal cortex, proximal and distal convoluted tubules. Renal cortex indicated multiple glomeruli, each consisted of tuft capillaries surrounded by Bowman's capsula. While the proximal convoluted tubules were lined with somewhat dark cuboidal cells, the distal convoluted tubules were lined with pale cuboidal cells. These cells had spherical nuclei and red blood cells (**Fig. 1 A-B**).



Figs. 1 A-B. Light micrographs of glomerular cells in mole rats. Glomerulus (GL), proximal convoluted tubule (PCT), distal convoluted tubule (DCT), capillary lumen (CL), Bowman's capsula (BC) and red blood cells (RBC) (H & E).

Ultrastructural results: Electron microscopic examination of kidney section of mole rats showed that the renal glomerulus, surrounded by a double-walled epithelial capsule namely Bowman's capsule. The internal leaflets of the capsule enveloping the glomerular capillaries was the visceral layer, whereas the outer limit of the renal glomerulus was the parietal layer. The space between the two layers was the urinary space. The wall of each glomerular capillary was formed by the glomerular basement membrane (GBM) and an inner endothelial lining. The GBM was surrounded by a discontinuous layer (visceral layer) composed of the podocytes; each had a cell body from which extend several primary foot processes that gave rise to numerous secondary processes embracing the glomerular capillaries. The secondary processes of visceral epithelial cells or podocytes were interdigitated, delimiting elongated spaces called the filtration slits between the adjecent processes**.** The glomerular capillaries of lumen had certain cells, known as the mesangial cells adhering to their walls in places where the basal membrane forms a sheath that was shared by two or more capillaries (**Fig. 2A-D**).



Figs. 2 A-D. Electron micrographs of a renal cortex of mole rats. Bowman's capsule (BC), urinary space (US), capillary lumen (C), glomerular basement membrane (GBM), red blood cell (RBC), mesangial cells (MS), endotelial cell (EC), podocytes (P), pedicel (Pe), filtration slits (Arrow) and mitochondria (m).

The proximal convoluted tubules (PCTs) had their lumen mostly occupied with the microvilli or brush border extending from the apical portion of the lining cells. The cytoplasm of these cells contained many spherical or elongated mitochondria, Golgi apparatus and scarce glomerular endoplasmic reticulum (GER). The basal membrane of cells displayed multiple infoldings to increase the cell surface at the basal regions (**Fig. 3A**).

The distal convoluted tubules (DCT) had larger diameter than that of the proximal tubules and the luminal borders of their lining cells lack the brush borders and had small microvilli. The basal regions of these cells showed basal infoldings that were almost similar to the proximal tubule cells. The apical regions of cells had small vesicles and a few vacuoles. The cytoplasm of these cells had less cytoplasmic organels (**Fig. 3B,C**).

The medullary collecting duct cells had a few short apical microprojections and randomly oriented mitochondria. The nuclei were oval and located at the basal region (**Fig. 3D**).

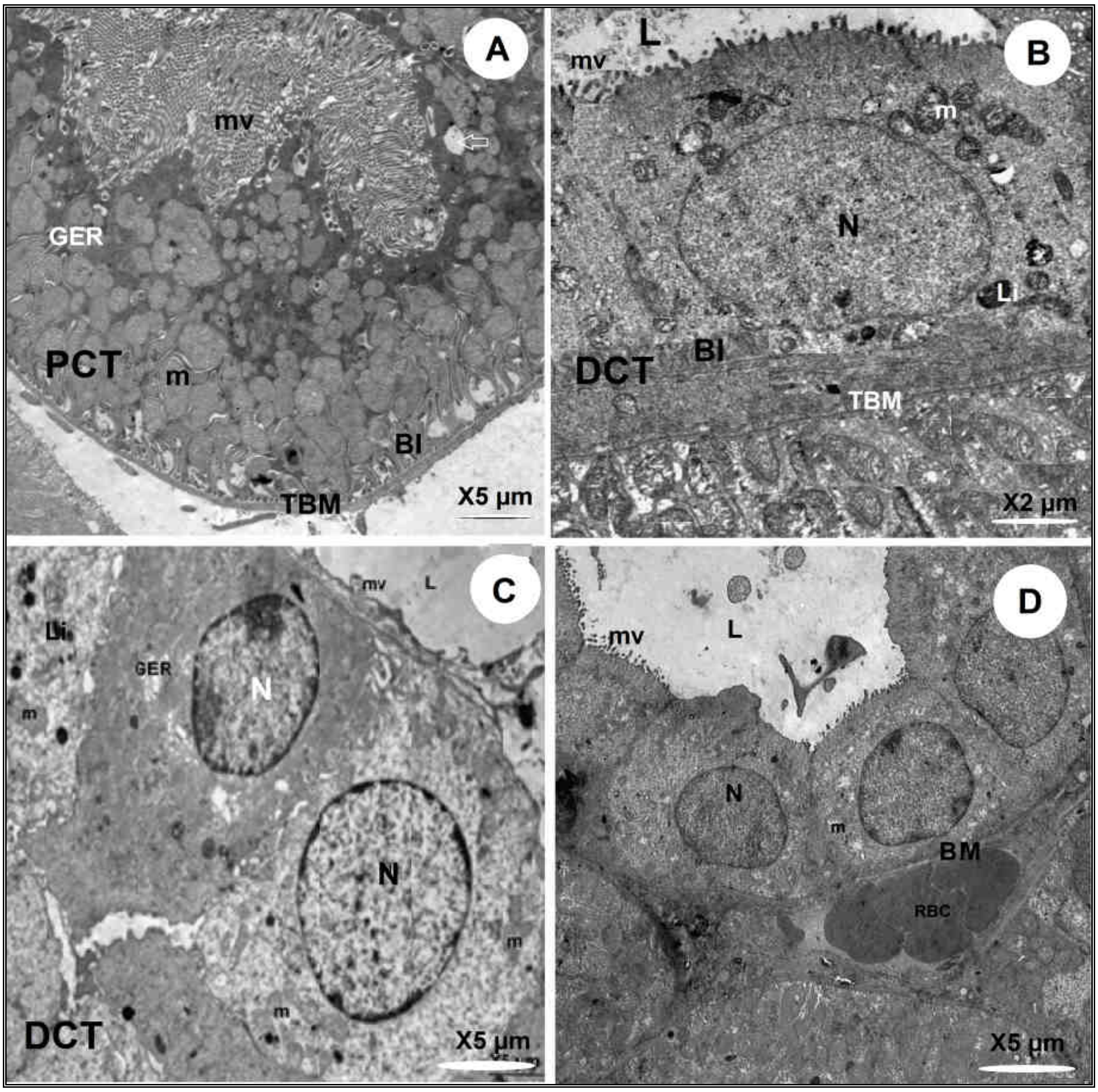


Fig. 3 A-D. Electron micrographs of a proximal convoluted tubule cells (A), distal convoluted tubule cells (B, C) and medullary collected cells (D). Microvilli (mv), mitochondria (m), vacuole (arrow), basal infoldings (BI), tubular basal membrane (TBM), lumen (L), nucleus (N), granular endoplasmic reticulum (GER), basal membrane (BM), red blood cell (RBC).

4. Discussion

This study showed that the kidney of mole rats have some similarities and differences with the other animals. These ultrastructural similarities included the renal Bowman's capsule, proximal tubules, distal tubules, intermediate segments and collecting ducts. Although a big mamalian kidney had more than million nephrons, a mole rat kidney had a few thousand of nephrons. These structural features were consistent with the other species structures (**Ross et al., 1989; Tisher and Madsen, 1993; Eurell and Frappier, 2007**). Ultrastructural modifications of nephrons enabled vertebrates to solve the problems of their environmental conditions whether they had water or not (**Gambarian, 1994**).

The fine structure of the renal corpuscle of the mole rats were resembled to the other animal structures. The renal corpuscles were consisted of Bowman's capsule, tubule cells, urinary space, mesangium cells and epithelial cells or podocytes. Some places the proceseses of podocytes were connected to pedicles. The glomerular capillaries were enclosed glomerular filtration bariers (GFB) which comprised the endothelium, the glomerular basement membrane and the podocyte foot processes. Similar observations had been described in different animal kidneys (**Gabri, 1983; Mundel and Kriz 1995; Casotti et al., 2005; Eurell and Frappier, 2007**).

The glomerular filtration of barier consisted of three layers. These were the lamina rara interna (the electron-lucent layer adjacent to the endothelial cells), the lamina densa (the electron-dense layer), and the lamina rara externa (the electron-lucent layer adjacent to the visceral epithelial cells). The podocytes were large and had irregular pedicels. They were located on the filtrate side of the capillary loops. In addition, the basament membrane had relatively low density of narrow filtration slits. It was thougt that these cells had endocytic and phagocytic properties and contributed to the structural stability of the capillary tuft and selective permeability of the filtration barrier, synthesized the basal membrane (**Eurell and Frappier, 2007; Galaly, 2008).**

The mesangial cells were numerous and their irregular shapes with long cytoplasmic processes extended to the capillary loops and some of them attached to the capillary basal membrane. It was thougt that these cells regulate the glomerular filtration rate by altering the capillaries. Similar findings were observed in some animal glomerular cells (**Schlondorff, 1996; Galaly, 2008**).

The ultrastructure of the proximal convoluted tubule cells (PTC) showed well developed brush border of numerous long microvilli and fewer small vesicules, huge numbers of spherical and elongated mitochondria with densely packed cristea. The mitochondria were located into the deep infoldings of the basolateral plasma membrane**.** Because of these features, it was thougth that the proximal convoluted tubular cells might reabsorb the water and suply the ion balance in the kidneys. Similar findings were observed in some animals glomerular cells (**Welling and Welling, 1975; Casotti et al., 2005; Nabipour et al., 2009**).

The basolateral plasma membrane infoldings significantly increased the surface of proximal convoluted tubule and these were permited the high rates of epithelial transport. The microvilli played a mechanosensory function in transmitting and reabsorbing the ions in proximal tubules (**Dantzler, 2003; Du et al., 2004; Eurell and Frappier, 2007**).Although marked plasma membrane infoldings were observed in some reptiles (**Solomon, 1985**), these were not encountered in the rabbit kidneys (**Ross et al., 1989).**

Distal convoluted tubularcells (DTC) were a little taller than proximal tubular cells and they had less basolateral plasma membrane infoldings and lessmitochondria. The distal convuloted tubules were mainly involved in ion reabsorption from the tubule fluid as mentioned by **Burkitt et al**. **(1993).** These observations were also seen in the other studies (**Bohrer et al., 1978; Kriz and Kaissling, 1992**; **Plotkin et al., 1996**).

The small microvilli and vesicles observed in the apical surface of distal convoluted tubular cells indicated that the absorptive function of this segment is very low. The cytoplasmic features and the apical surfice of distal tubular cells were resembled those seen in some reptile kidneys. Also, the apical vesicles were thought to be involved in the reabsorption processes of water. These observations were cosistent with the studies done on some animals (**Danzler and Holmes, 1974; Ross et al., 1989; Casotti et al., 2005**; **Reece, 2009**).

A lot of protrusions were observed at the apical cytoplasm of distal tubular cells as observed in some animal kidney cells. It was assumed that these protrusions were related with secretion of uric acid and exchange of fluids (**Gabri and Butler, 1984; Ross et al., 1989**).

The collecting tubules were consisted of cuboidal epithelial cells. These cells had basal nuclei and less cytoplasmic organels. Like distal tubular cells, these cells played a significant role in producing urine and reabsorbing the water from the tubular lumen. It was assumed that these cells were secreted mucine, which might aid in eliminating the uric acid from the cells **(Casotti et al., 2000, 2005).** Although mucous cells were abundant in some reptiles and snakes (**Gabri, 1983; Soares and Moares, 1984**), there was not encontered any mucous cells in mole rats.

Conclusion: The kidneys are very important organs that regulate the composition and volume of body fluids. The functional nephron is comprises of a glomerulus, a proximal and a distal convoluted tubule and a collecting duct. Both the proximal and distal tubules exhibite the membrane infoldings on their basal and lateral cell walls as observed in a lot of mamalian tubules. It is hoped that the results of this study will encourage further research in this field.

Financial support: There was no financial support taken for this manuscript.

Conflict of interest: The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments: The authors thank Prof. Dr. Turan GUVEN and Prof. Dr. Mustafa YEL (Department of Biology, Gazi Education Faculty, Gazi University, Ankara) for assisting with the experiment and proofreading the manuscript.

References

- Bohrer MP, Baylis C, Humes HD, GIassock RJ, Robertson CR and Brenner BM. Permselectivity of the glomerular capillary wall. Facilitated filtration of circulating polycations. J. Clin. Invest., 1978, 61: 72-78. **doi:10.1172/JCI108927**
- Bozinovic F, Gallardo P A, Visser RH and Corte's A. Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological process and ecological implications. J. Exp. Biol., 2003, 206: 2959-2966. **doi: 10.1242/jeb.00509**
- Burkitt HG, Young B. and Heath JW. Wheater's Functional Histology, 3rd ed. ELPS Churchill Livingstone, 1993.
- Casotti G, Herrera LG, Flores JJ, Mancina CA and Braun EJ. Relationships between renal morphology and diet in 26 species of new world bats (suborder microchiroptera). J. Zool., 2005, 109: 196-207. **doi:10.1016/j.zool.2006.03.003**
- Casotti G, Lindberg KK and Braun EJ. Functional morphology of the avian

medullary cone. Am. J. Physiol. Regul. Integr. Comp. Physiol., 2000, 279: 1722-1730. **PMID:11049855 [PubMed - indexed for MEDLINE]**

- Corte´s A, Rosenmann M and Bozinovic F. Water economy in rodents: evaporative water loss and metabolic water production. Rev. Chil. Hist. Nat., 2000, 73, 311–321. **www.scielo.cl/pdf/rchnat/v73n2/art06.pdf**
- Dantzler WH. Regulation of renal proximal and distal tubule transport. Sodium, chloride and organic anions. Comp Biochem Physiol. A Molec. Integr. Physiol., 2003, 136(3): 453–478. **doi:10.1016/S1095-6433(03)00135-1**
- Dantzler WH and Holmes WN. Water and mineral metabolism in reptilian, pp. 277–377. In: Florkin M. and Scheer B.T. (eds), Chemical Zoology Vol. IX, Amphibia and Reptilia, Academic Press, New York London, 1974, 537. **ISBN: 0122610393 ISBN-13: 9780122610394**
- Du Z, Duan Y, Yan O, Weinstein AM, Weinbaum S and Wang T. Mechanosensory function of microvilli of the kidney proximal tubule. PNAS, 2004, 101: 13068-13073. **doi:10.1073/pnas.0405179101**
- El-Gohary ZMA. Structural- functional adaptations in the kidneys of two rodent species inhabiting different habitats. Egypt. J. Zool., 2009, 52: 337-362.
- ***Eurell JA and Frappier BL. Dellmann's Textbook of Veterinary Histology (6th Edition). Australian Vet. Journal., 2009, 85(8): 310, First published online in 2007, revised in 2013,*** *doi: 10.1111/j.1751-0813.2007.00177.x*
- Gabri MS. Ultrastructure of the tubular nephron of the lizard *Podarcis taurica*. J. Morphol., 1983, 175(2): 131–142. **doi: 10.1002/jmor.1051750204**
- Gabri MS and Butler RD. The ultrastructure of the renal corpuscle of the lizard. Tissue Cell., 1984, 16(4): 627–634. **doi:10.1016/0040-8166(84)90036-3**
- Galaly SR. Histology, histochemistry and ultrastructure of the nephron of the (freshwater) *Varanus niloticus* and (desert) *Uromastyx aegyptius*. J. Egypt. Ger. Soc. Zool., 2008, 57: 79–106. **doi: 10.2478/s11756-013-0181-7**
- Gambarian SP. Micro-dissectional investigation of the nephron in some fishes, amphibians and reptiles inhabiting different environment. J. Morph., 1994, 219(3): 319–339. **doi:10.1002/jmor.1052190311**
- Holz PH and Raidal SR. Comparative renal anatomy of exotic species. Vet. Clin. North. Am. Exot. Anim. Pract., 2006, 9: 1-11.
- Kriz W and Kaissling B. Structural organization of the mammalian kidney. In: The Kidney: Physiology and Parhopliysiology, DW Seldin and *G* Giebisch (eds). Raven Press, New York, 1992.
- Kuru M. Omurgalı Hayvanlar. Atatürk Üniv. Basımevi, Erzurum (In Turkish), 1987.
- Mundel P and Kriz W. Structure and function of podocytes: An update. Anat. Embriol. (Berl.), 1995, 192: 385-397. **PMID:8546330 [PubMed - indexed for MEDLINE]**
- Nabipour A, Alishahi E and Asadian M. Some histological and physiological features of avian kidney. J. Appl. Anim. Res., 2009, 36: 195-198. **doi: 10.1080/09712119.2009.9707058**
- Ojeda JL, Icardo JM and Domezain A. Renal corpuscle of the sturgeon kidney: An ultrastructural, chemical dissection and lectin binding study. Anatomical

Record, 2003, 272A: 563-573. **doi:10.1002/ar.a.10068**

- Plotkin MD, Kaplan MR, Verlander JW, Lee WS, Brown D, Poch E, Gullans SR and Herbert SC. Localization of the thiazide sensitive Na-CI cotransporter, rTSCl in the rat kidney. Kidney Int., 1996, 50: 174-183.
- Reece WO. Functional anatomy and physiology of domestic animals. Wiley-Blackwell, Fourth Edition, Ames, USA, 2009.
- Ross MH, Reith EJ and Romrell LJ. Histology. A Text and Atlas. 2nd Ed. 1989, 527-561.
- Sato T. A modified method for lead staining of thin sections. J. Electron. Microsc., 1967, 16,133.
- Schlondorff D. Roles of the mesangium in glomerular function. Kidney Int., 1996, 49: 1583-1585. **PMID: 8743459 [PubMed - indexed for MEDLINE]**
- Soares AV and Moraes F. Morphological and morphometrical study of the kidney of the male tropical lizard *Tropichurus torquatus*. Anat. Anz. Jena., 1984, 157: 365–373.
- Solomon SE. The morphology of the kidney of the green turtle (*Chelonia mydas* L). J. Anat., 1985, 140: 355–369. **PMCID: PMC1165102**
- Tisher CC and Brenner BM. Structure and function of the glomerulus. In: Renal Pathology: With Clinical and Functional Correlations, CC Tisher and BM Brenner (eds). J B Lippincott, Philadelphia, 1994, 143-161.
- Welling LW and Welling DJ. Surface areas of brush border and lateral cell walls in the rabbit proximal nephron. Kidney Int., 1975, 8: 343-348**. PMID:1206850 [PubMed - indexed for MEDLINE]**
- Yuan J and Pannabecker TL. Architecture of inner medullary descending and ascending vasa recta: Pathways for countercurrent exchange. Am. J. Physiol., 2010, 10: 1152. **doi:10.1152/ajprenal.00071.2010**