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RESEARCH ARTICLE

Histo- and Ultrastructural study of the Kidney in three species of Family Rallidae

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Abstract

In birds, the renal system is unique among vertebrate kidneys. Due to vital function of kidneys so the major aim of this examination is to study the histological and ultrastructural structures of kidneys in three water birds; Allen's Gallinule, Purple Swamphen and Water Rail. These species belong one order, Gruiformes and to one family, Rallidae. Kidneys were removed from the synsacrum and examined histologically. Investigations for light microscopy represented relatively large medulla found in Allen's Gallinule and showed also that the renal corpuscle was smaller in size and number in Allen's Gallinule than in Water Rail and Purple Swamphen. In Water Rail and Purple Swamphen collecting tubules were much larger in size and number than in Allen's Gallinule, they lined by pale cells and cuboidal shape. These differences seem to be the adaptive advantage of species which have to mitigate scarcity of water or excessive evaporative water loss by maximum renal water conservation during migration. On the other hand the electron microscopy study demonstrated that podocytes process was present and large in Purple Swamphen while in Allen's Gallinule and Water Rail wasn't visible.

INTRODUCTION

Many detailed studies have been published on the histology of the mammalian kidney but comparatively few have been of the avian kidney. Birds have paired kidneys located within a cavity formed by the ventral surface of the synsacrum. The kidneys extend from the caudal edge of the lungs to the caudal synsacrum (Canny 1998).

One of the most unique features of avian kidneys is the presence of two types of nephrons, with and without a loop of Henle (Casotti and Braun 2000). The loop of Henle allows for urine concentration and is the primary reason that birds and mammals are the only classes of vertebrates that can consistently produce hyperosmotic urine (Casotti 2001). In birds, only about 10 to 30% of the nephrons are of the mammalian type (Frazier et al 1995; Casotti et al 2000; Lumeij 2000). Most avian nephrons are loopless ("reptilian" type) and stay within the cortex. The looped nephrons ("mammalian" type) extend from the cortex into the discrete medullary areas known as medullary cones. Since birds have primarily "reptilian"type nephrons, which produce iso osmotic urine, urine concentration is limited.

Instead, kidneys conserve fluid components necessary to maintain homeostasis while ridding the body of metabolic waste products, as well as excess water and electrolytes, in the form of urine (Holz and Raidal 2006). An understanding of the structure the kidney and especially the interrelation ship of the renal tubules and renal vasculature is essential to appreciate the diverse function of kidney (Dellman and Eurell 1998; Nabipour and

Dehghani 2012). Due to vital function of kidneys so the major aim of this examine to study the histological structure of kidneys in Water rail, Purple Swamphen and Allen's Gallinule.

MATERIAL AND METHODS

Experimental birds

Allen's Gallinule (*Porphyrio alleni*), Purple Swamphen (*Porphyrio porphyrio*) and Water Rail (*Rallus aquaticus*) Fig. 1a, b & c are water birds which belong to one order, Gruiformes and to one family, Rallidae. Both Water Rail and Purple Swamphen are resident birds while Allen's Gallinule is a migratory one.

Three bird of every species were sacrificed, abdomen of each bird was opened by a single medial ventral incision, and the viscera were removed to expose the kidneys.

Light Microscopy

After making transverse sections, samples from kidney were fixed in 10% aqueous formaldehyde solutions for 48 hrs. Next, the samples were dehydrated in an ascending grade of ethanol series, cleared in xylene. They were embedded in paraffin blocks and were cut serially (5 µm). All sections were stained using Harris hematoxylin-eosin (Bancroft and Stevens 1997).

Electron Microscopy

Another tissue sample of kidneys was processed for transmission electron microscopy. Tissue samples immediately transferred into the solution of 2.5% glutaraldehyde in 0.1M phosphate buffer for 2 hours at room temperature. The fixation solution was refreshed after 2 hours and samples were kept in the fresh solution at 4°C overnight and washed in buffer, pH 7.2. Then, tissue samples were subjected to a post-fixation step with osmium tetroxide for 1½ hours at 4° and washed in buffer, pH 7.2. After that, tissue samples were placed in a series of graded ethanol steps of 70, 90, 95, and two changes in 100% ethanol, with incubations for 5 minutes at each step, and 10 minutes in 100% ethanol. Dehydrated tissues were infiltrated with propylene oxide and embedded in Araldite 502 epon resin. Sections of 90 nm were obtained by ultramicrotome, were picked up onto the finder grids, and stained with uranyl acetate and lead citrate (Makanya et al 2011). Then the specimens were viewed in a JEOL transmission electron microscope at an accelerating voltage of 80 kV at Electron Microscopy Unit in Faculty of Science, Alexandria University.

RESULTS

In the present study the kidneys of the studied species consisted of two zones, the cortex and medulla. The cortex made up the majority of the kidney with only a small portion being medulla. The cortex and the medulla were arranged in cones of different lengths, which were distributed randomly within the kidney (Fig. 2 a, b & c).

Investigation represented also that the cortex contain the reptilian type of nephrons without loop of henle while the medulla contain the mammalian type of nephrons with loop of henle (Fig. 2 d, e & f). The cortical nephrons have smaller renal corpuscles than the medullary nephrons, the large renal corpuscles of medullary nephrons lie close to the medulla.

In this study, both Water Rail and Purple Swamphen have larger renal corpuscle than Allen's Gallinule. The renal corpuscle consisted of an outer Bowman's capsule separated by Bowman's space from a centrally located glomerulus. The glomeruli consisted of tightly packed central core of mesangial cells, surrounded by capillary loops (Fig. 2 g, h & i).

The resulted showed also that the proximal convoluted tubules were lined by simple low cuboidal epithelium in the three birds. The distal convoluted tubules were also lined by simple cuboidal epithelium, the lumen of the distal convoluted tubules were more clearly defined (Fig. 2 d, e & f).

Collecting tubules were occurred in the peripheral part of the cortex, in Water Rail and Purple Swamphen collecting tubules were much larger in size and number than in Allen's Gallinule, they lined by pale cells and cuboidal shape and were intermediate in size between the proximal and distal convoluted tubules (Fig. 2 d, e & f).

Thick and thin segments of henles loop were separated by collecting ducts, thick segments were restricted to the peripheral of the medullary cones and surrounded by a ring of collecting ducts, the thick and thin segment consisted of simple cuboidal epithelium, the medullary collecting ducts continued into a distal papillary duct which consisted of a columnar epithelium (Fig. 2 d, e & f).

Electron microscopy study showed that, the capsule of the renal corpuscle appears to be constituted by a parietal layer and a visceral layer with podocytes. The epithelial cells of the parietal layer are flattened and have elongated nuclei. They linning the hole corpuscular teritory and limit with the external surface of the podocytes of the visceral layer. In the center of the corpuscle an agglomeration of basofil mesangial cells, surrounded by many fenestrated capillaries (Fig. 3 a, b & c). In the current study podocytes process was present and larger in Purple Swamphen than in the other studied birds (Fig. 3 d, e & f).

FIGURES

Fig. 1. Allen's Gallinule (*Porphyrio alleni*) **a**, Purple Swamphen (*Porphyrio porphyrio*) **b** and Water Rail (*Rallus aquaticus*) **c**.

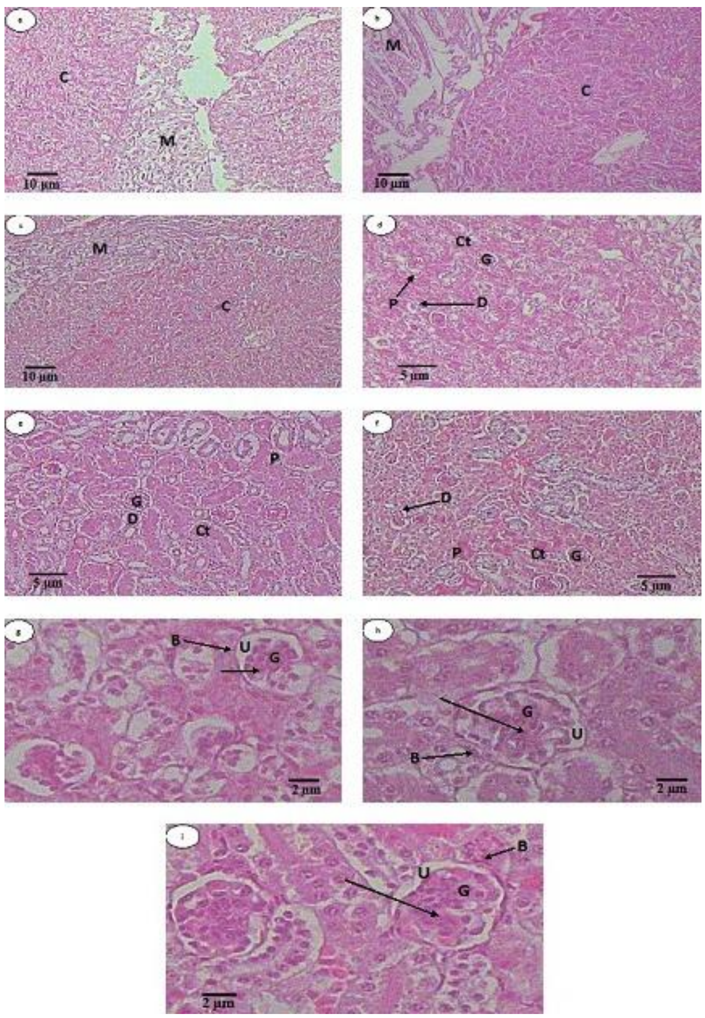

Fig. 2:
Light micrographs of the kidney of Allen's Gallinule (*Porphyrio alleni*), Purple Swamphen (*Porphyrio porphyrio*) and Water Rail (*Rallus aquaticus*).
a: Transverse section of the kidney of Allen's Gallinule (*Porphyrio alleni*) showing large medulla (**M**) and small cortex (**C**). H&E stain, 40 x.

b: Transverse section of the kidney of Purple Swamphen (*Porphyrio porphyrio*) showing large cortex (**C**) and small medulla (**M**). H&E stain, 40 x.
c: Transverse section of the kidney of Water Rail (*Rallus aquaticus*) showing large cortex (**C**) and small medulla (**M**). H&E stain, 40 x.
d: Enlarged part of a transverse section of the kidney of Allen's Gallinule (Porphyrio alleni) showing, (G) glomerulus, (P) proximal convoluted tubule, (D) distal convoluted tubule and (Ct) collecting tubule. H&E stain, 100 x.
e: Enlarged part of a transverse section of the kidney of Purple Swamphen (*Porphyrio porphyrio*) showing, (**G**) glomerulus, (**P**) proximal convoluted tubule, (**D**) distal convoluted tubule and (**Ct**) collecting tubule. H&E stain, 100 x.
f: Enlarged part of a transverse section of the kidney of Water Rail (*Rallus aquaticus*) showing, (**G**) glomerulus, (**P**) proximal convoluted tubule, (**D**) distal convoluted tubule and (**Ct**) collecting tubule. H&E stain, 100 x.
g: A higher magnified section through cortex of the kidney of Allen's Gallinule (*Porphyrio alleni*) showing, (**B**) Bowman's capsule, (**G**) glomerulus, (**U**) urinary space and (**arrow**) mesanglia cell. H&E stain, 400 x.
h: A higher magnified section through cortex of the kidney of Purple Swamphen (*Porphyrio porphyrio*) showing, (**B**) Bowman's capsule, (**G**) glomerulus, (**U**) urinary space and (**arrow**) mesanglia cell. H&E stain, 400 x.
i: A higher magnified section through cortex of the kidney of Water Rail (*Rallus aquaticus*) showing, (**B**) Bowman's capsule, (**G**) glomerulus, (**U**) urinary space and (**arrow**) mesanglia cell. H&E stain, 400 x.

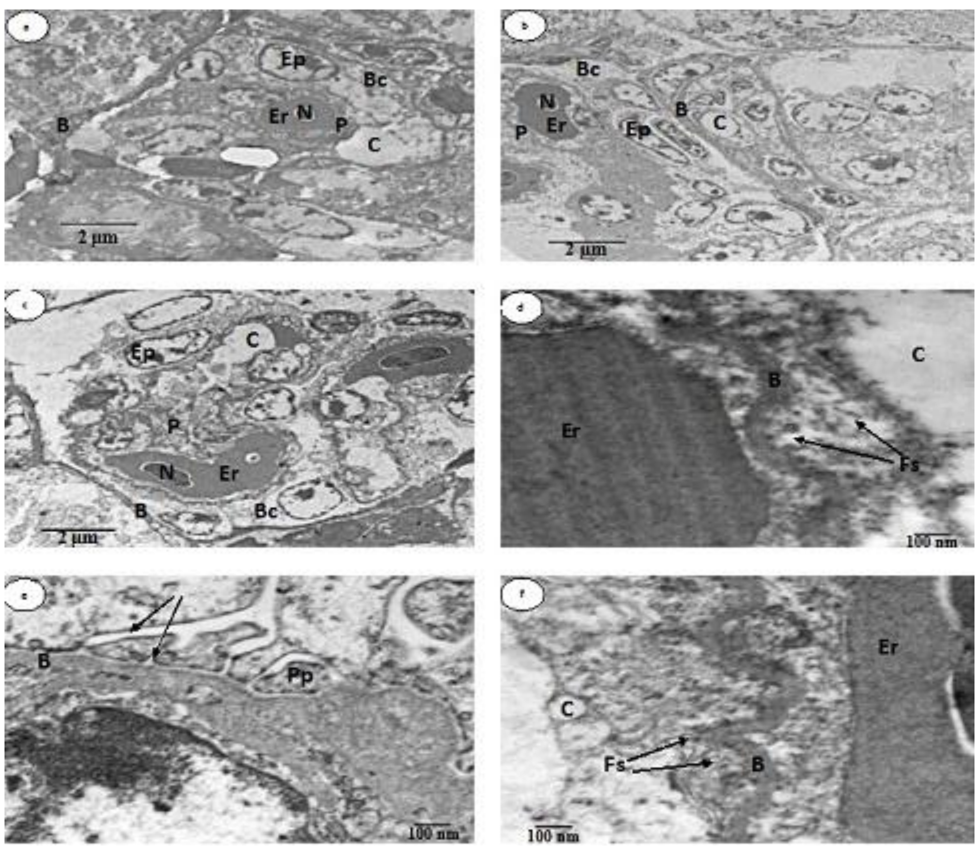

Fig. 3:
Transmission electron micrograph of the kidney of Allen's Gallinule (*Porphyrio alleni*), Purple Swamphen (*Porphyrio porphyrio*) and Water Rail (*Rallus aquaticus*).
a: Transmission electron micrograph of the kidney of Allen's Gallinule (*Porphyrio alleni*) showing component of the glomerulus, (**P**) podocytes, (**C**) caplliary, (**Bc**) bowman's capsule, (**B**) basement membrane, (**N**) nucleus, (**Er**) erthrocytes and (**Ep**) epithelial cell.

b: Transmission electron micrograph of the kidney of Purple Swamphen (*Porphyrio porphyrio*) showing component of the glomerulus, (**P**) podocytes, (**C**) caplliary, (**Bc**) bowman's capsule, (**B**) basement membrane, (**N**) nucleus, (**Er**) erthrocytes and (**Ep**) epithelial cell.
c: Transmission electron micrograph of the kidney of Water Rail (*Rallus aquaticus*) showing component of the glomerulus, (**P**) podocytes, (**C**) caplliary, (**Bc**) bowman's capsule, (**B**) basement membrane, (**N**) nucleus, (**Er**) erthrocytes and (**Ep**) epithelial cell.
d: A higher magnified part of podocyte ultrastructure of the kidney of Allen's Gallinule (*Porphyrio alleni*) showing (**B**) basement membrane, (**C**) capillary, (**Fs**) Filtration slits, (**Er**) erthrocytes .
e: A higher magnified part of podocyte ultrastructure of the kidney of Purple Swamphen (*Porphyrio porphyrio*) showing structure relation establish between podocyte and capillary, (**B**) basement membrane, (**Fs**) Filtration slits and (**Pp**) podocyte process.
f: A higher magnified part of podocyte ultrastructure of the kidney of Water Rail (*Rallus aquaticus*) showing (**B**) basement membrane, (**C**) capillary, (**Fs**) Filtration slits, (**Er**) erthrocytes.

DISCUSSION

Undoubtedly, the kidneys play numerous vital roles in birds. One primary role of the kidney is elimination of metabolic wastes. The kidneys also aid the liver in detoxification. Because the kidneys are responsible for eliminating numerous metabolites, tissue concentrations of antibiotics (apramycin and ciprofloxacin) and toxins (lead and cadmium) are often highest in renal tissue (Afifi and Ramadan 1997).

The results of this study reported differences in the amount of cortex and medulla. This feature is similar to other species. It would appear also that the avian medullary cones are structurally similar (analogous) to the outer medulla of mammal kidneys (Casotti et al 2000). In Water Rail and Purple Swamphen the cortex made up the vaster area of the kidney than in kidney of Allen's Gallinule. These findings are similar to those obtained by (Warui 1989) who reported that the kidney of mallard duck consists of a very large cortex and relatively small medulla. Possible differences in the urinary concentrating ability of species from different zones may be the result of differences in the proportion of cortex and medulla. Previous studies have demonstrated that arid zone birds have a high volume of renal medulla than mesic zone birds (Casotti 2001).

Instead, previous studies on kidney morphology in both birds and mammals have examined differences related to habitat. In birds, there is a tendency for species inhabiting arid environments to have a greater medullary thickness (Braun 1985); amongst mammals, those inhabiting an arid environment tend to have the longest medullary papillae (Braun 1985; Beuchat 1996). For both birds and mammals, those species that live in arid habitats tend to be better at concentrating their urine than those inhabiting freshwater habitats, although there are many exceptions (Braun 1985; Beuchat 1996).

The proximal convoluted tubules were lined by simple low cuboidal epithelium in the three birds; this result was agreement with Nabipour et al 2009 whom demonstrated that proximal and distal convoluted tubules of kidney in rock, collard dove and owl consisted of a cuboidal epithelium and the luminal surface. On the other hand, the findings of this study agreed with Casotti et al 2000 who showed that the avian kidney has a similar parallel arrangement of the loops of henle and collecting ducts in the medullary cones and these structures were lined by cuboidal epithelium.

The electronic microscopy images highlight best the relations between the podocytes and the capillary wall. Primary podocytes processes that surround the capillary wall can be observed. From this level, secondary podocytes processes are very numerous, thin and short, perpendicularly attached to the basement membrane of the capillary. At origin they have a conic aspect, which gradually becomes thinner. The distal end is wider, in order to enlarge the area of contact with the basement membrane. The differences in the podocytes and fenestration and also the structure of nepherons tightly correlate with the ability of animal to conserve ions and water besides, the amount of waste product eliminated by kidneys (Samuelson 2007).

In summary, relatively large medulla found in Allen's Gallinule seems to be the adaptive advantage of species which have to mitigate scarcity of water or excessive evaporative water loss by maximum renal water conservation. On the other hand, differences in kidney histology can be correlated with certain parameters of the water economy in birds.

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