



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

Histo- and Ultrastructural Aspects Concerning Renal Corpuscle in *Fulica atra* and *Gallinula angulata* (Aves: Gruiformes)

Lamiaa Elsayed Mokhtar Deef

Department of Zoology, Faculty of Science, Damietta University, New Damietta, Egypt.

Manuscript Info

Manuscript History:

Received: 18 August 2015
Final Accepted: 20 September 2015
Published Online: October 2015

Key words:

renal corpuscle, podocyte,
kidney, histology, medulla,
tubules

*Corresponding Author

Lamiaa Elsayed Mokhtar Deef
lamiaadeef@yahoo.com

Abstract

The avian renal system is quite unique among vertebrate kidneys. Birds have paired kidneys located within a cavity formed by the ventral surface of the synsacrum. Kidneys of coots (*Fulica atra*) and lesser moorhen (*Gallinula angulata*) were removed from the abdomen and subjected to histological and ultrastructure studies. The result showed that coots tend to have a greater medullary thickness than lesser moorhen. The renal corpuscle was smaller in size and larger in number in lesser moorhen than in coot. In lesser moorhen collecting tubules was much larger in size and number than in Coot, they lined by pale cells and cuboidal shape and were intermediate in size between the proximal and distal convoluted tubules. In addition, podocytes process was larger in lesser moorhen than in coot. In this investigation, relatively large medulla found in coot 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 during migration for long distances. On the other hand, differences in kidney histology can be correlated with certain parameters of the water economy in birds.

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INTRODUCTION

Coots and lesser moorhen are medium-sized water birds that are members of the Rallidae family. The avian kidney is unique in structure among vertebrate kidney in having two types of nephrons those with and without a loop of henle (looped and loopless respectively) (Braun and Dartzler, 1972). The loopless nephrons stay within the cortex while the looped nephrons extend from the cortex and into discrete medullary areas called medullary cones with each cone, the number of loops of henle decreases as tip of the cone is approached (Layton, 1986). The two nephrons also called the cortical (reptilian) type in more numerous and lacks a loop of henle, it is located in the cortex, while the other nephrons called medullary (mammalian) type, it has a loop of henle and it is less numerous, which extend into medulla (Bacha and Wood, 1990).

Many detailed studies have been published on the histology of the mammalian kidney but comparatively few have been of the avian kidney. The nephron is the functional unit of kidney and varies greatly in structure amongst different vertebrates. Birds and mammals produce hyperosmotic urine due to the presence of a loop of Henle.

The waste products of the nutrient metabolism, including nitrogenous materials and inorganic salts are actively removed from the body by kidneys and salt glands in different organisms (Holz and Raidal, 2006). The structure of nephrons in different animals tightly correlates with the ability of the animal to conserve ions and water. Understanding the structure of the kidneys and especially the interrelationship of the renal tubules and renal vasculature is essential to appreciate the diverse functions of the kidney (Dellmann and Eurell, 1998).

An understanding of the structure of the kidney and especially the interrelationship of the renal tubules and renal vasculature is essential to appreciate the diverse function of kidney (Dellman and Eurell, 1998). Avian kidneys normally consist of three divisions' cranial, middle and caudal lobe and each lobe consist of smaller lobes (Johnson and Skodhauge, 1975; Richardson *et al.*, 1991). The purpose of this research was to provide some basic information about light and electron microscopic features of the kidney in *Fulica atra* and *Gallinula angulata* and to discriminate renal corpuscle structure between them due to vital function of kidneys.

MATERIAL AND METHODS

Experimental birds

Coot (*Fulica atra*) and Lesser morhen (*Gallinula angulata*) are water birds sometimes called marsh hens which belong to one order Gruiformes and one family Rallidae. Coot is a migratory species while Lesser morhen is a resident one. These birds were caught using a mist net or picked up by some volunteers. The two birds were sacrificed; kidneys were removed from the abdomen and examined for their gross features.

Light Microscopy

After making transverse sections, the left kidney pieces were placed in 10% neutral buffered formalin. The fixation solution was refreshed after 24 hours. The fixed tissues were washed by water and dehydrated in an ascending series of graded concentration of alcohol (70%, 80%, 90%, 100%) then cleared with xylene, infiltrated with paraffin wax and embedded in paraffin block. Sections (5 µm) were prepared using a microtome were stained with hematoxylin and eosin, dehydrated and cover slipped using permount as the mounting medium and viewed under alight microscope (Luna, 1968).

Electron Microscopy

The left kidneys from Coot and Lesser morhen were processed for transmission electron microscopy. Samples from the kidneys were fixed in 2.5% gluteraldehyde at 4 °C in 0.1 M Na cacodylate buffer at pH 7.4 for about 2 - 3 hrs. The tissues were then washed in cacodylate buffer several times for about 30 min. The samples were post fixed for 2 hours in 1% osmium tetroxide at pH 7.4. The samples were then washed in the buffer several times overnight at 4 °C and then passed through increasing concentrations of ethanol, rinsed with 100% propylene oxide for 1 hr. and embedded in Araldite epoxy resin. Ultrathin sections were cut at 50 - 60µm thickness, mounted on copper grids, and stained with uranyl acetate and lead citrate. Photographs were taken with a JEOL electron microscope at an accelerating voltage of 80 kV Electron Microscopy Unit in Faculty of Science, Alexandria University.

RESULTS

Light Microscopy

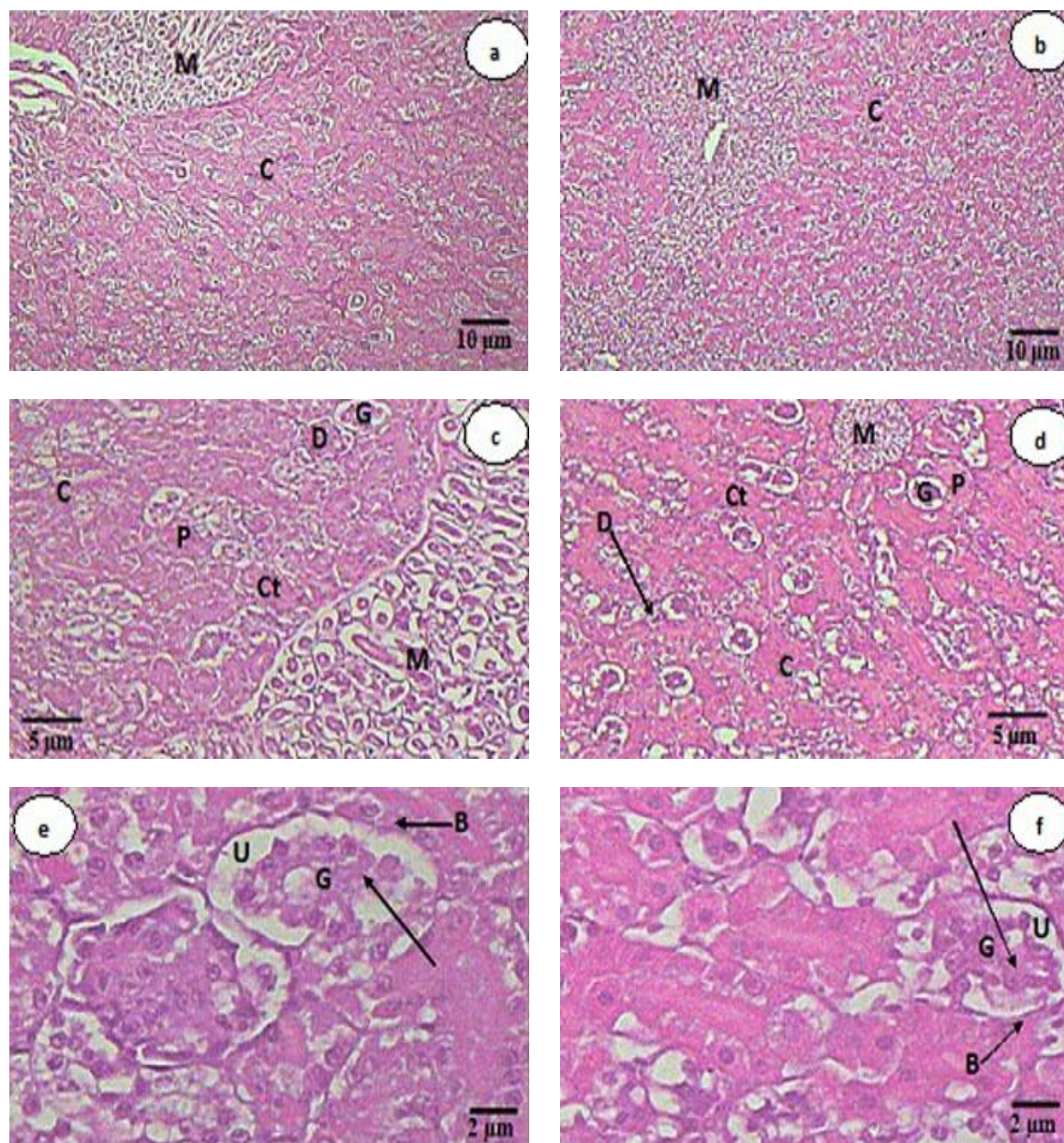
General histology of the kidneys of two studied birds consisted of two zones, the cortex and medulla. The cortex made up the largest area of the kidney with only a small portion being medulla. The cortex and the medulla were arranged in cones of different lengths, which were distributed randomly within the kidney (Fig. 1 a, b). In the current study coots tend to have a greater medullary thickness than lesser moorhen (Fig. 1 a, b).

The result showed 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. The cortical nephrons have smaller renal corpuscles than the medullary nephrons, the large renal corpuscles of medullary nephrons lie close to the medulla.

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. 1 c, d). In this study, the renal corpuscle was smaller in size and large in number in lesser moorhen than in coot (Fig. 1 c, d).

In both lesser moorhen and coot the proximal convoluted tubules were lined by simple low cuboidal epithelium .The distal convoluted tubules were also lined by simple cuboidal epithelium , the lumen of the distal convoluted tubules were more clearly defined (Fig. 1 e, f).

Collecting tubules were occurred in the peripheral part of the cortex, in Lesser moorhen collecting tubules was much larger in size and number than in coot, they lined by pale cells and cuboidal shape and were intermediate in size between the proximal and distal convoluted tubules (Fig. 1 e, f).

FIGURES**Figure 1:**

Light micrographs of the kidney of Coot (*Fulica atra*) and Lesser Moorhen (*Gallinula angulata*).

a: Transverse section of the kidney of Coot (*Fulica atra*). Large (C) cortex and small (M) medulla. H&E stain.

b: Transverse section of the kidney of Lesser Moorhen (*Gallinula angulata*). Large (C) cortex and small (M) medulla. H&E stain.

c: Enlarged part of a transverse section of the kidney of Coot (*Fulica atra*). (G) glomerulus, (C) cortex, (M) medulla, (P) proximal convoluted tubule, (D) distal convoluted tubule and (Ct) collecting tubule. H&E stain.

d: Enlarged part of a transverse section of the kidney of Lesser Moorhen (*Gallinula angulata*). (G) glomerulus, (C) cortex, (M) medulla, (P) proximal convoluted tubule, (D) distal convoluted tubule and (Ct) collecting tubule. H&E stain.

e: A higher magnified section through cortex of the kidney of Coot (*Fulica atra*) showing, (B) Bowman's capsule, (G) glomerulus, (U) urinary space and (arrow) mesangia cell. H&E stain.

f: A higher magnified section through cortex of the kidney of Lesser Moorhen (*Gallinula angulata*) showing, (B) Bowman's capsule, (G) glomerulus, (U) urinary space and (**arrow**) mesanglia cell. H&E stain.

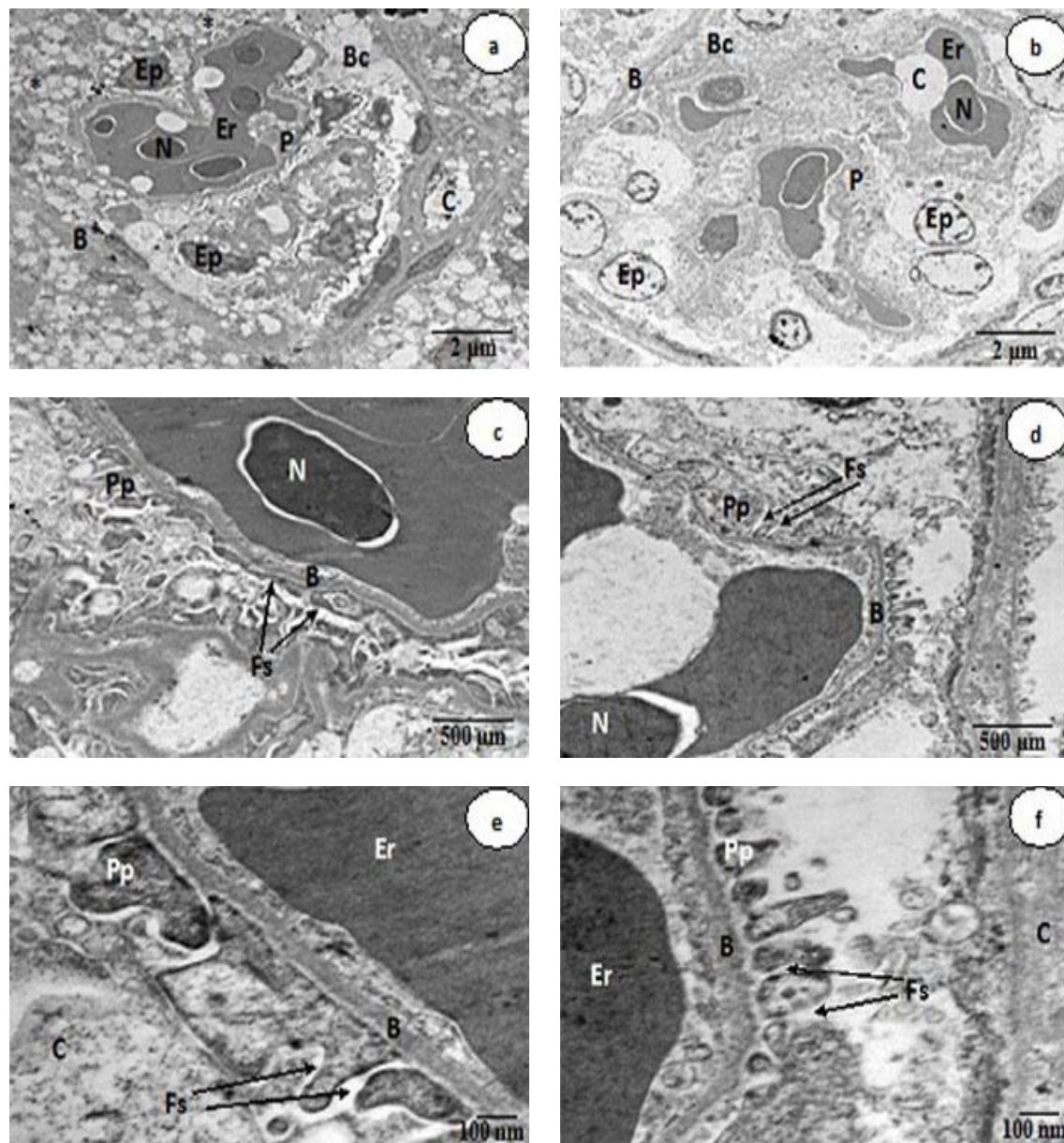


Figure 2:

Transmission electron micrograph of the kidney of Coot (*Fulica atra*) and Lesser Moorhen (*Gallinula angulata*).

a: Transmission electron micrograph of the kidney of Coot (*Fulica atra*) showing component of the glomerulus, (P) podocytes, (C) capillary, (*) multiple vesicle, (Bc) bowman's capsule, (B) basement membrane, (N) nucleus, (Er) erythrocytes and (Ep) epithelial cell.

b: Transmission electron micrograph of the kidney of Lesser Moorhen (*Gallinula angulata*) showing component of the glomerulus, (P) podocytes, (C) capillary, (Bc) bowman's capsule, (B) basement membrane, (N) nucleus, (Er) erythrocytes and (Ep) epithelial cell.

c: A higher magnified part of podocyte ultrastructure of the kidney of Coot (*Fulica atra*), showing, (B) basement membrane, (N) nucleus, (Fs) Filtration slits and (Pp) podocyte process.

d: A higher magnified part of podocyte ultrastructure of the kidney of Lesser Moorhen (*Gallinula angulata*), showing, (B) basement membrane, (N) nucleus, (Fs) Filtration slits and (Pp) podocyte process.

e: A higher magnified part of kidney of Coot (*Fulica atra*) showing structure relation establish between podocyte and capillary, (B) basement membrane, (C) capillary, (Fs) Filtration slits, (Er) erthrocytes and (Pp) podocyte process.

f: A higher magnified part of kidney of Lesser Moorhen (*Gallinula angulata*), showing structure relation establish between podocyte and capillary, (B) basement membrane, (C) capillary, (Fs) Filtration slits, (Er) erthrocytes and (Pp) podocyte process.

Electron Microscopy

Electron microscopy study showed that, 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 lining the whole corpuscular territory and limit with the external surface of the podocytes of the visceral layer, the capsular space. In the center of the corpuscle an agglomeration of basophilic mesangial cells, surrounded by many fenestrated capillaries (Fig. 2 a, b).

In the studied species it was observed that there is a relation between the podocytes and the capillary wall. Primary podocytes processes that surround the capillary wall can be observed. 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 and the distal end is wider. In the cytoplasm of the two extremities of the secondary podocytes processes, especially at the origin, a series of phagosomes and pinosomes can be distinguished, along with an electron-concentrated, granular material. Podocytes process was larger in lesser moorhen than in coot (Fig. 2 c, d).

The nucleus of the podocytes is oval, euchromatic and has obvious nucleoles. Sometimes is a little deformed, generating the appearance of a reduced indentation. The presence of processes of different sizes creates the flattened aspect of the cell that has only one prominent single central area, the one occupied by the nucleus. The number of filtration slits was larger in Lesser moorhen than in Coot (Fig. 2 c, d, e & f).

DISCUSSION

The avian renal system is quite unique among vertebrate kidneys. Birds have paired kidneys located within a cavity formed by the ventral surface of the synsacrum. The avian kidneys consisted of two areas, the cortex and medulla. The cortex represented the largest area of the kidney with only a small portion being medulla. In the current study coots tend to have a greater medullary thickness than lesser moorhen this feature is similar to other species. It would appear that the avian medullary cones are structurally similar (analogous) to the outer medulla of mammal kidneys (Casotti *et al.*, 2000). This character 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 during migration for long distances.

Instead, there is a tendency for bird 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).

In this study, the renal corpuscle was smaller in size and large in number in lesser moorhen than in coot. In both lesser moorhen and coot the proximal convoluted tubules were lined by simple low cuboidal epithelium. The distal convoluted tubules were also lined by simple cuboidal epithelium, the lumen of the distal convoluted tubules were more clearly defined. Nabipour (2009) agreed with these results, who observed that proximal and distal convoluted tubules of kidney in rock, collard dove and owl consisted of a cuboidal epithelium and the luminal surface area of the proximal convoluted tubules enhanced by a thick layer of microvilli forming a brush borders.

The medullary collecting ducts continued into a distal papillary duct which consisted of a columnar epithelium. The result similar to Nicholson (1982), who was reported that, the collecting tubules in the kidney of starling bird lined by cuboidal cells and connected to collecting duct. Also, it was obvious that in lesser moorhen collecting tubules was much larger in size and number than in Coot.

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 nephrons tightly correlate with the ability of animal to conserve ions and water besides, the amount of waste product eliminated by kidneys (Samuelson 2007).

In conclusion, relatively large medulla found in coot 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 during migration for long distances. On the other hand, 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. Also, differences in kidney histology can be correlated with certain parameters of the water economy in birds.

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