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PHYLOGENETIC STUDIES ON THE ARCHITECTURAL FEATURES OF AMPHIBIAN (*BUFO REGULARIS*) AND MAMMALIAN (*ORYCTOLAGUS CUNICULUS*) KIDNEYS

By

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دراسات تشريحية على كلى البرمائيات والثدييات

زينب محمود أحمد الجوهري و حمدي إبراهيم

تهاني عامر و سماح طلعت درويش

من المعروف أن تركيب الكلى يختلف بين سائر الفقاريات تبعاً لطبيعة البيئة التي يستوطنها الحيوان بما يحقق له المقدرة على مواجهة شتى المشاكل البيئية من حيث كمية الماء المتاحة . لذلك عنى هذا البحث بإجراء تجارب لعقد مقارنة تشريحية لكل من الكلية المتوسطة والبعدية اللتان تمثلان طرازى الكلي الفعالة للبرمائيات والثدييات على التوالي ، حيث تقطن تلك الحيوانات بيئات طبيعية مختلفة .

وقد أختبر لهذا البحث ذكور وإناث كل من الضفادع المصرية الرقطاء والأرانب الأهلية حيث أسفرت النتائج على العديد من الإختلافات التشريحية بين كلى الضفادع والأرانب من حيث عدد النفرونات وطبيعتها وحجمها وتركيبها بما يتمشى وطبيعة البيئة لكل منهما .

Key Words: Renal, Mammals, Amphibia, Anatomy

ABSTRACT

The results of the present study revealed that mesonephric and metanephric kidneys of both the anamniotes (*Bufo regularis*) and the amniotes (*Oryctolagus cuniculus*) vertebrates respectively differ morphologically, anatomically and histologically. The structural organization of toad and rabbit kidneys was studied with the light microscope (LM) along with a microdissection technique. In the toad, the kidneys are paired attenuate mesonephric organs and have mesonephric segmental construction. On the other hand, rabbit kidneys are paired bean-shaped structures. Each is composed of elemental units called renal lobules. These lobules are made up of nephrons, three main types of nephron populations were characterized according to the localization of their glomeruli within the cortex. Marked differences between the toad and the rabbit were observed in the number and diameter of renal corpusles and in their size. Also, marked variations were seen in the tubular organization of both species.

INTRODUCTION

The marine origin of vertebrates is a matter of debate but the early movement to freshwater is agreed, and gives the line of gnathostomate evolution, with the characteristic osmotic profile.

The movement to land is associated with the addition of further renal distal segments. Selective specific reabsorption and secretion of sodium, potassium and water is thus possible. The importance of these segments increases from Amphibia to reptiles and birds. With the emergence of mammals, particularly the dominant Eutheria, a further distinctive segment, the loop of Henle, appeared in the nephron. The loop of Henle is capable of

generating concentration gradients within the renal interstitium and augmenting water reabsorption from the urine via the collecting duct.

The kidneys of three orders of Amphibia are regarded as mesonephros morphologically [1, 2, 3]. The structure of the amphibian kidney has been relatively thoroughly investigated in urodeles [1, 4, 5, 6] in apoda [2] and in anurans [3, 7, 8, 9]. In anuran kidneys, a subdivision into a central and a peripheral zone is obvious and the branching patterns of the collecting ducts and the blood supply are similar [10]. The anuran nephron is established by three major convolutions, a proximal, a distal and a third containing the connecting tubule, which are located in the peripheral, central and peripheral zones respectively.

Light and electron microscopic studies of the amphibian renal tubule distinguish generally six segments: a neck segment, a proximal tubule, an intermediate segment, a distal tubule, a connecting tubule and a collecting duct [9, 11].

On the other hand, the mammalian kidney is a bean-shaped organ surrounded by a thin but tough fibrous capsule. The kidney consists mainly of two regions, namely cortex and medulla. The cortical region contains glomeruli, proximal convoluted tubules, distal convoluted tubules, connecting tubules, and initial collecting ducts [12]. The medullary region can be subdivided into outer medulla and inner medulla. The former consists of two regions, namely outer and inner stripes of outer medulla. The outer stripe and the medullary rays have a number of features in common. These regions enable the kidney to elaborate urine which is hypertonic to plasma. They contain proximal straight tubules, thick ascending limbs, and collecting ducts arranged in parallel similar to the arrangement in the deeper portions of the medulla. Three main types of nephron populations (superficial, med-cortical and juxtamedullary) have been described [13, 14, 15] according to their location in the renal cortex, the course of their efferent vessels and the length of their Henle's loops. The morphologic differences observed between nephrons appear to constitute the basis for functional differences. It is well established that the corpuscles of superficial and mid-cortical nephrons are generally smaller in diameter than the juxtamedullary corpuscles, as observed by Bowman [16] and Peter [17]. At least 12 segments differing both in cellular anatomy and function are now more or less routinely studied [18, 19, 20].

The variations in the excretory organs among vertebrates are correlated with problems with which vertebrates have had to cope in adapting themselves to the different conditions under which they have lived. The kidney is a key organ for homeostasis of the internal environment, and the phylogenetic approach in studying the structure of mesonephroi and metanephroi characteristic of anamniotic and amniotic vertebrates respectively is of great importance. Therefore, it was found valuable to perform comparative studies on animals from different habitats as well as with different functional kidneys. The present study presents the anatomical features of the kidney of two different vertebrates with different functional kidneys; mesonephros in toad and metanephros in rabbit.

MATERIALS AND METHODS

1) Experimental Animals

Two different tetrapod animals, an amphibian species; *Bufo regularis* and a mammalian species; *Oryctolagus cuniculus* were chosen for the present investigation to study the structural organization of mesonephros and metanephros; the functional kidney for Amphibia and Mammals respectively. Adult 15 male and 15 female *Bufo* weighing 53 g on the average, and 6 male and 6 female rabbits, weighing 1447 g on the average, were used in the present study.

2) Macroscopic and Microscopic investigations

The animals were anesthetized with chloroform, then they were dissected to remove the kidneys. The right and left kidneys were weighed, examined macroscopically, after which they were halved with a razor blade and placed immediately in 10% neutral formalin. The right kidney of each pair was used for histological studies. Each was imbedded in paraffin and 5-um sections were prepared and stained with hematoxylin and eosin for microscopic investigation. To evaluate the general renal architecture, histologic sections were examined for measuring glomerular diameter and volume following the procedure used by Darwisch[21]. For the amphibian kidney, about 20 glomeruli from each of 5 sections were measured. While for mammalian kidney, at least 10 glomeruli of each nephron population were considered.

The left kidneys were used for microdissection. Small blocks of neutral-formallin-fixed renal tissue were macerated in 50% HCl. The nephrons were dissected with thin needles under a dissecting microscope after which the proximal tubules and glomeruli were drawn at a magnification of X 100 with the aid of a camera lucida attached the microscope.

3) Nephron enumeration

In the present study Damadian's technique[22] was employed to determine the number of nephrons from various glomerular populations[23].

4) Statistical Analysis

Statistical evaluation of the present data has been made by using unpaired Student's "t" test and the level of significance was estimated taking the probability ($P<0.05$) as minimal requirement for significance.

RESULTS

1) Renal structural organization and microscopic investigation

Amphibia:

The kidney of the toad (*Bufo regularis*) is an attenuate organ which is lobulated at its median edge. The excretory duct of the toad kidney is the Wolffian duct which extends cranio-caudally on the lateral side of the kidney. The portion of the Wolffian duct

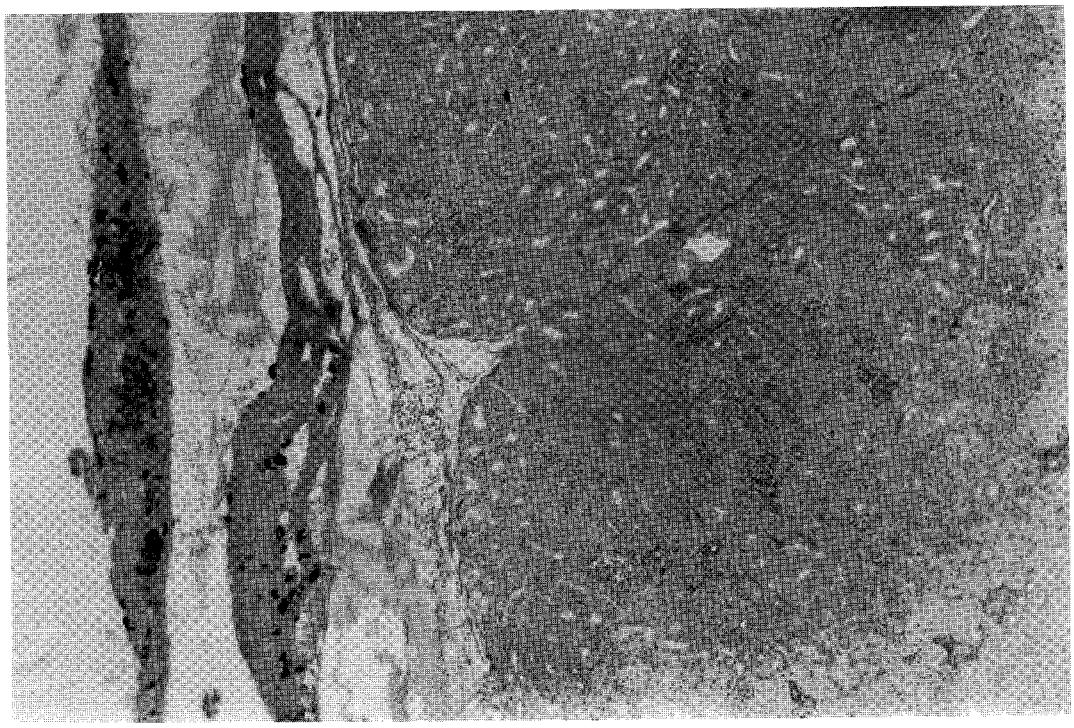

Fig. 1: A photomicrograph of a longitudinal paraffin section of the toad kidney. Note the segmentation at its median edge and the presence of Wolffian duct (arrow). H&E X 100

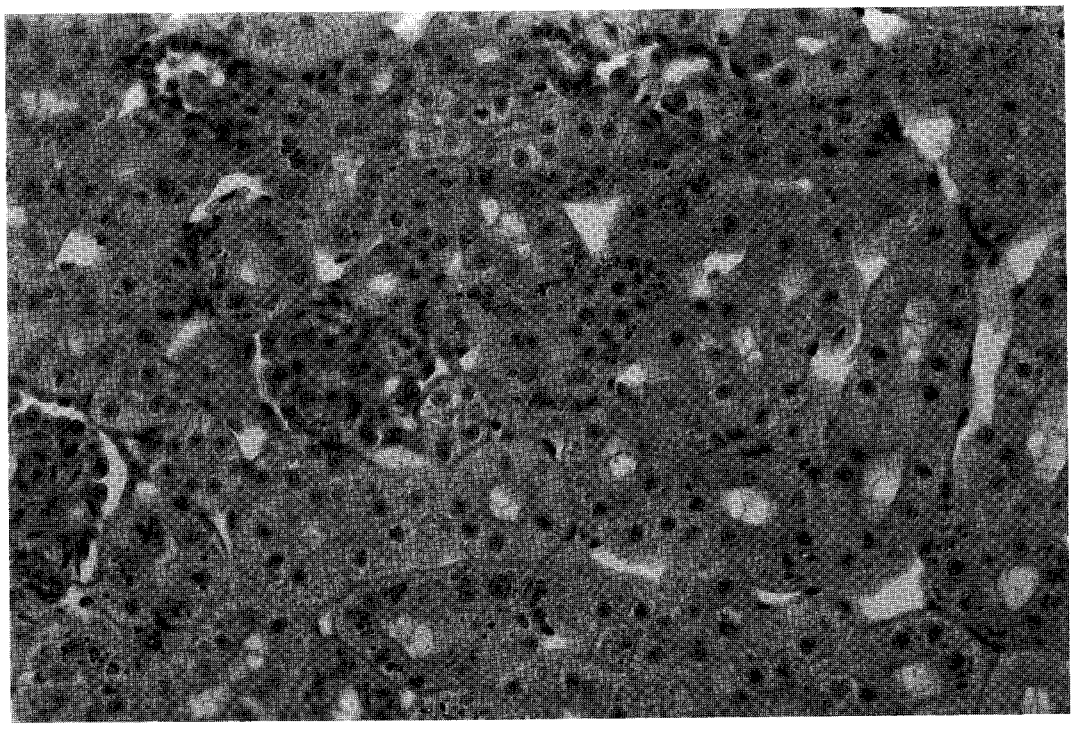

Fig. 2: A photomicrograph of a transverse section of the toad kidney showing the dorso-lateral (DL) and ventro-medial (VM) zones and the distribution of the renl corpuscles (Arrow). H&E X 400

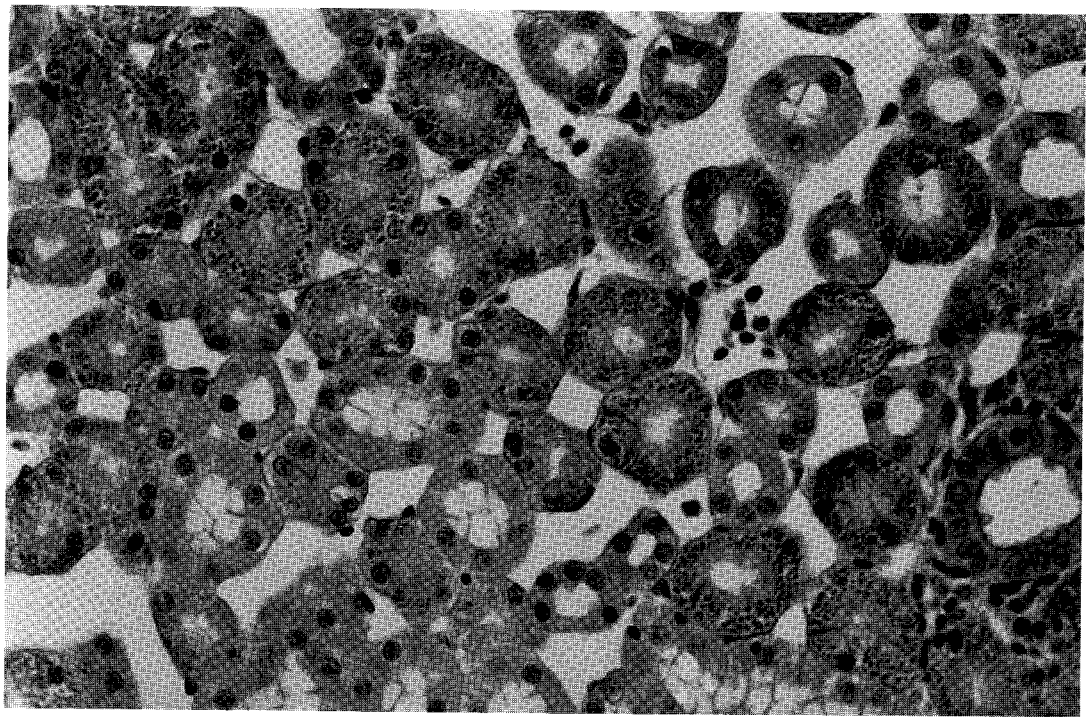

Fig. 3: A photomicrograph of a transverse section of the toad kidney showing the central zone containing distal tubules only. H&E X 400

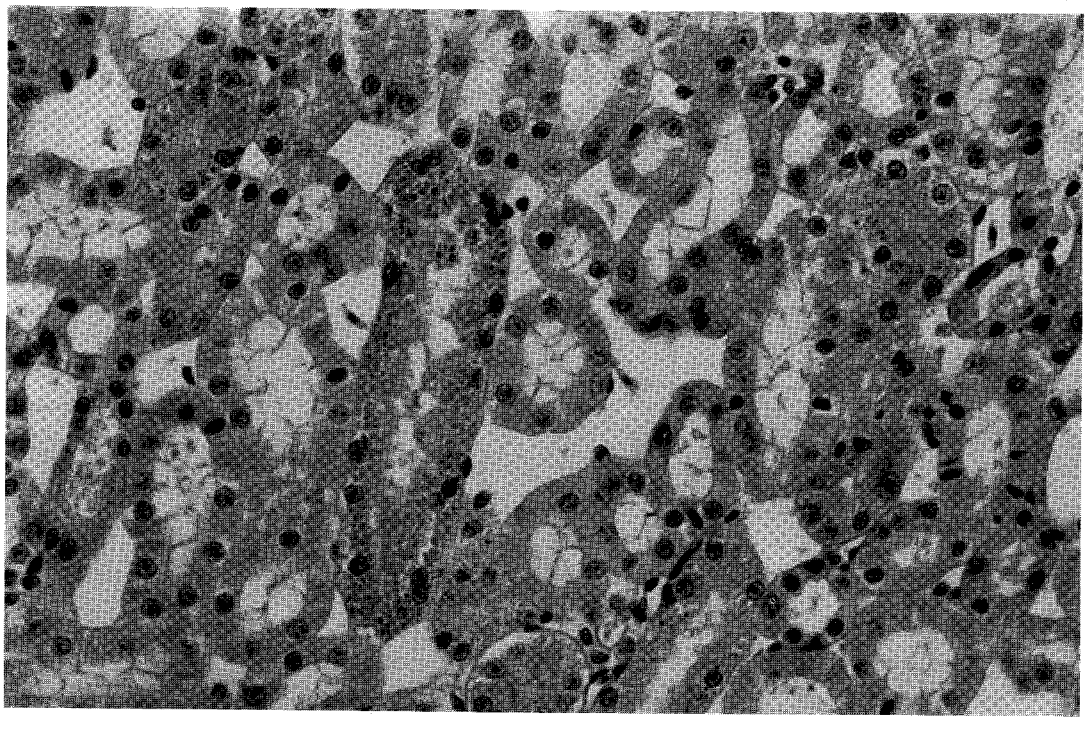

Fig. 4: A photomicrograph of a transverse section of the toad kidney showing the pripheral zone which contains proximal and collecting tubules. H&E X 400

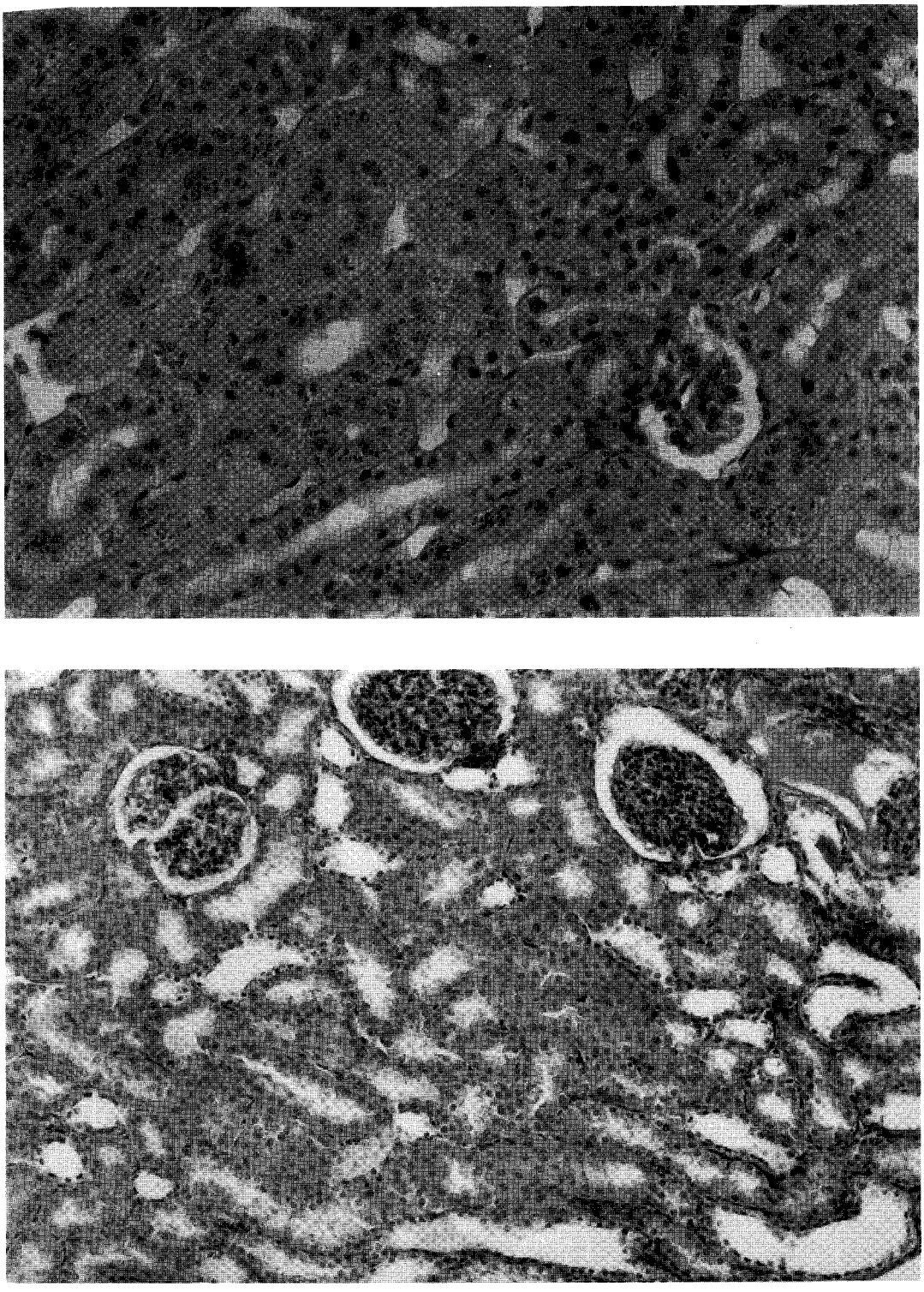

Fig. 5: A photomicrograph of a transverse section of the toad kidney (A) and rabbit kidney (B). Note that the filter mechanism is thinner and less structured in rabbit than in toad. H&E X 400

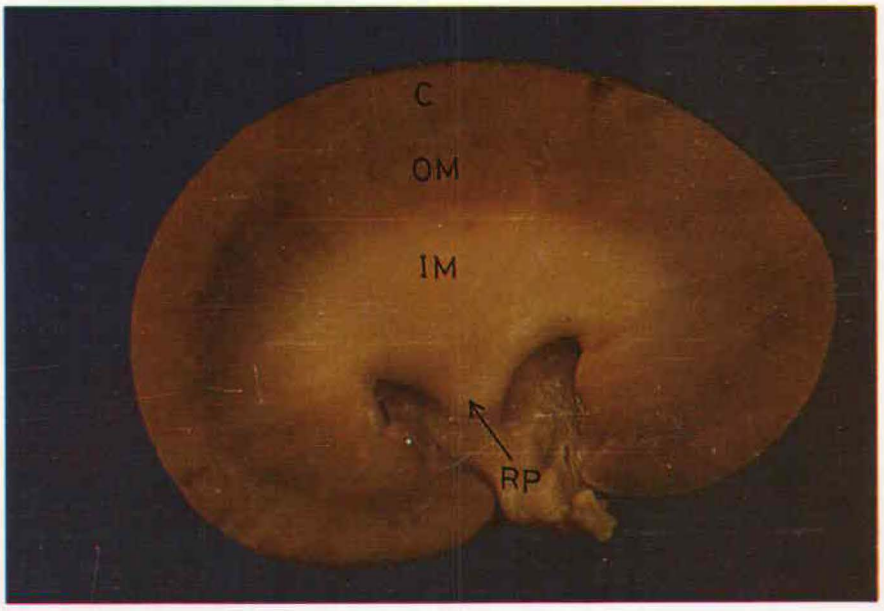

Fig. 6: A photomicrograph of *saggital* section through a unipapillary kidney of rabbit, showing the main renal regions; cortex (c), outer medulla (OM) and inner medulla (IM). The inner medulla is massively developed and has a short renal papilla (RP). Without any stains X 3

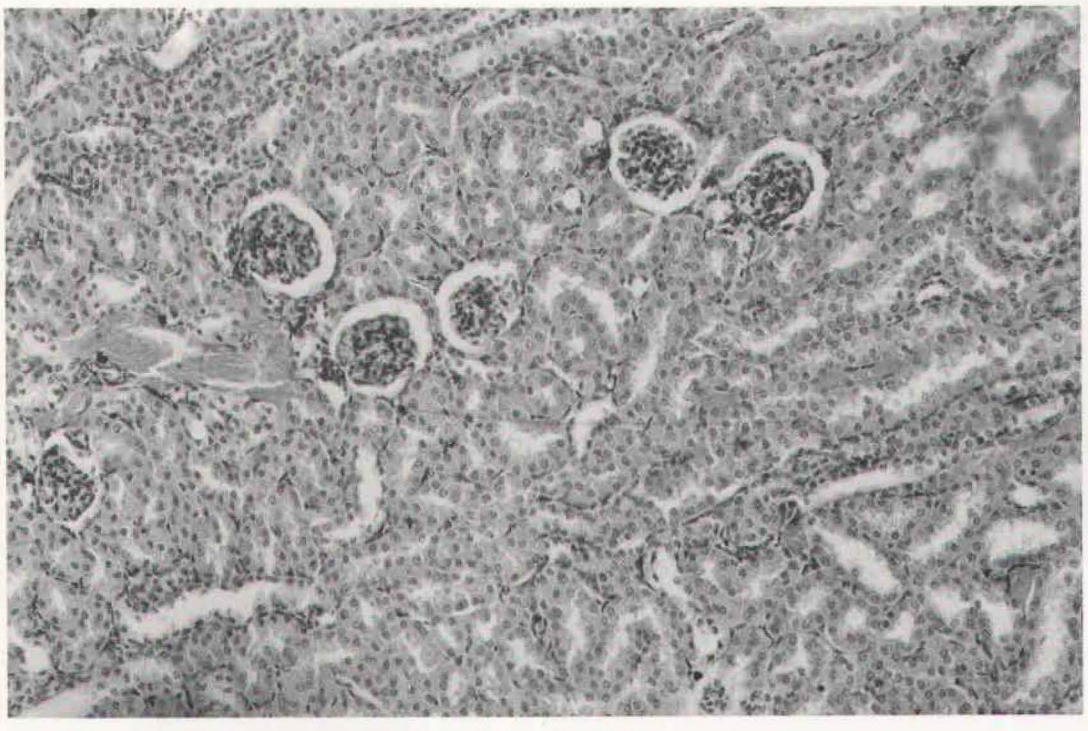

Fig. 7: A photomicrograph of a transverse section of toad kidney, showing the cortical region which contains renal corpuscles, proximal tubules and distal tubules. H&E X 200

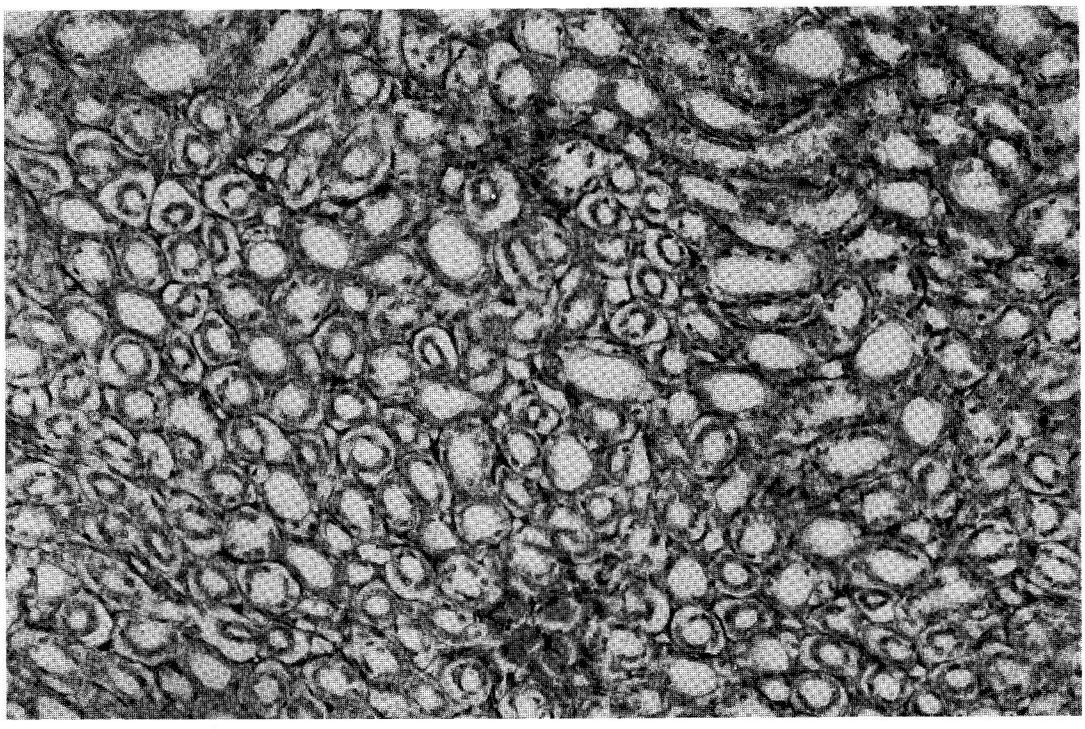

Fig. 8: A photomicrograph of a transverse section of toad kidney showing the medullary region which contains loop of Henle and collecting tubules. H&E X 200

Fig. 9: A photomicrograph of a longitudinal thick ascending limb of Henle's loop of the rabbit kidney. H&E X 400

adjacent to the kidney is enlarged and has a glandular epithelium (Fig. 1).

In cross sections of the toad kidney, the renal tissue can be subdivided into dorsolateral and ventromedial zones, separated by the renal corpuscles which lie essentially in a planar distribution between the two zones (Fig. 2). The central zone contains only distal tubules (Fig. 3). The peripheral zone contains all the other segments of the renal tubules (Fig. 4). The glomerulus of the toad is enclosed by a very large and quite irregular - shaped Malpighian corpuscle. The glomerular capsule is lined on its inner surface by a simple squamous epithelium with nuclei which protrude into the capsular space. The glomerular tufts of toads are relatively small in relation to the size of the entire renal corpuscle. Therefore, the filter mechanism of the glomerulus is thicker and more structured than that of rabbit (Fig. 5).

Visual identification of proximal and distal tubule segments was achieved on the basis of the smaller diameter and relatively greater transparency of the distal nephron. Based on its epithelial structure, the renal tubule is subdivided into six segments: a ciliated narrow segment, a proximal tubule, an intermediate segment, a distal tubule, a connecting tubule and a system of collecting ducts.

In the rabbit the metanephric kidneys were asymmetrically placed in the body cavity, the right kidney being anterior to the left. Each kidney is a bean-shaped organ, and from the indentation on its inner side issues the metanephric duct (ureter). In cross-section, the rabbit kidney showed distinct cortical and medullary regions (Fig. 6). The former contains principally the renal corpuscles and the convoluted portions of the tubules (Fig. 7). The medulla was mainly composed of long, straight collecting tubules and loops of Henle (Fig. 8); it was these which give a straight appearacne to the pyramids (Fig. 9). The medullary rays are bundles containing parallel-oriented proximal straight tubules, thick ascending limbs, and collecting ducts that extend far into the cortex from the outer medulla.

2) Number of nephrons and glomerular volume

The number of nephrons per whole kidney as well as per gram kidney weight for left kidneys of both male and female toads and rabbits are cited in Table (1). The number of nephrons per gram kidney weight was respectively lower in male (24095) and female (8862) toads than corresponding male (218989) and female (32364) rabbits.

The data concerning the glomerular diameters and total glomerular volume (which may be considered as an index of the filtering surface area) are given in Tables (2,3).

3) Microdissection of nephrons

A generalized diagram of the dissected neprhons from the median portion of the toad kidney is shown in Figure (10). The amphibian nephron is characterized by three major convolutions, a proximal, a distal, and a third containing the connecting tubule, which are located respectively in the peripheral, central and peripheral zones. The various nephron populations of the rabbit kidney are presented in Figure (11). Note the variable locations

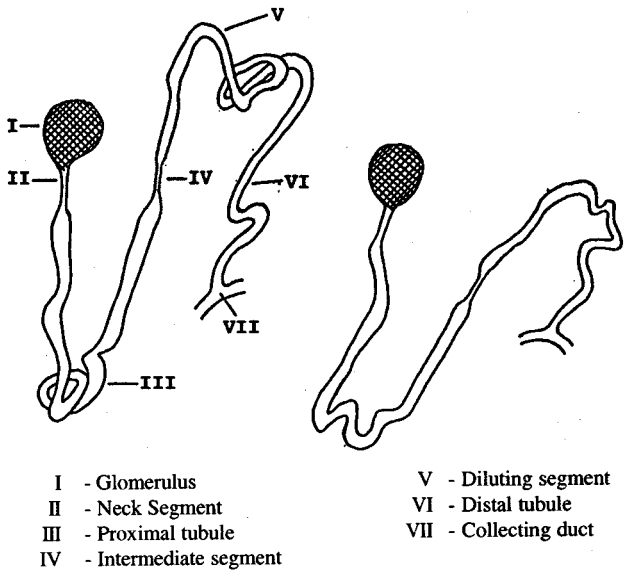

Fig. 10: A simplified representation of camera lucida drawings of nephrons dissected from a toad kidney.

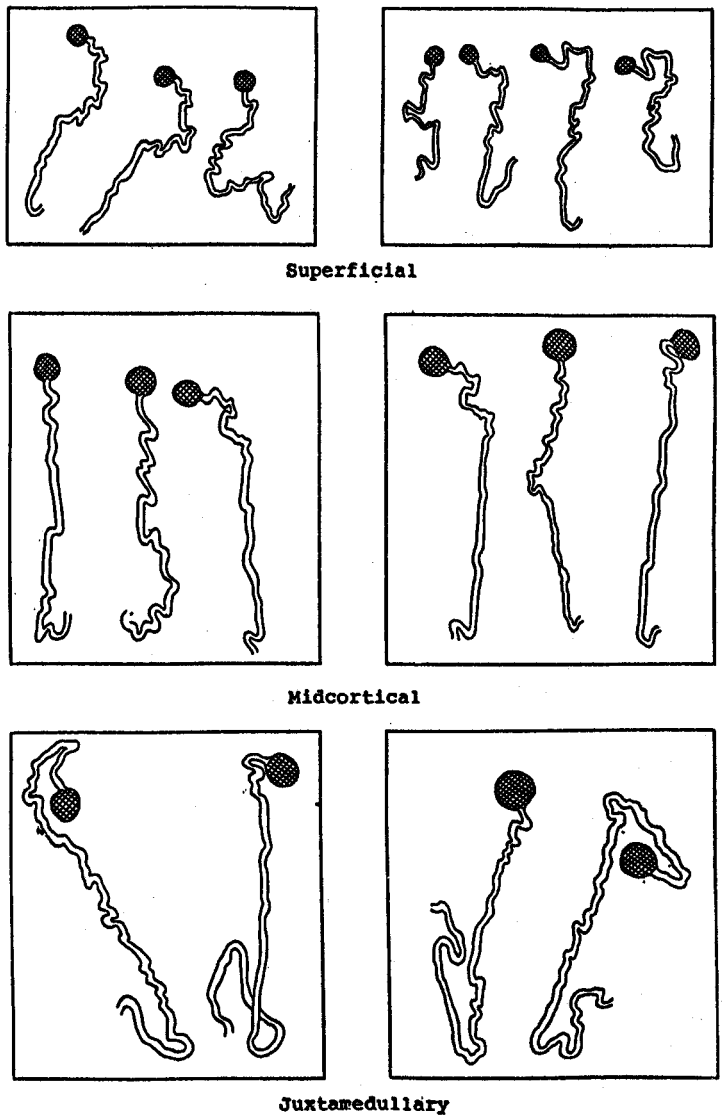

Fig. 11: A simplified representation of camera lucida drawings of nephrons dissected from a rabbit kidney.

Table 1

Body weight (g.), Kidney weight (g) and Number of nephrons in left kidney of both male and female rabbits (*Oryctolagus cuniculus*) and toads (*Bufo regularis*). All the data are represented as means ± standard errors. [Numbers in parentheses are the number of the experimental animals]

Experimental Animals	Body Weight (g)	Kidney Weight (g)	Number of nephrons	
			Per Whole Kidney	Per g. Renal Tissue
a) Male rabbit (6)	1576 ± 29.93	6.56 ± 0.81	158382 ± 5751	21898 ± 795
b) Female rabbit (6)	1318 ± 119.43	5.52 ± 0.67	203456 ± 5692	32364 ± 3488
c) Male toad (15)	31.27 ± 0.72	0.06 ± 4.82^{-3}	1451 ± 75	24095 ± 1182
d) Female toad (15)	75 ± 01	0.16 ± 0.01	1201 ± 28	8862 ± 493
Probability				
a v b	> 0.05 (N.S.)	> 0.05 (N.S.)	<0.005 (S)	<0.05 (S.)
c v d	< 0.005 (S.)	> 0.005 (S)	. .	< 0.005 (S)
a v c	. .	. .	. .	> 0.05 (N.S.)
b v d	. .	. .	. .	< 0.005 (S)

N.S: No significant S: Significant

Table 2

Glomerular diameter (mm). Glomerular size and their Total volume [filtering surface area (mm^3)] of both male and female toads (*Bufo regularis*). All the data are represented as means ± standard errors. [Numbers in parentheses are the number of the experimental animals]

Experimental Animals	Glomerular Diameter (mm)	Glomerular Size (mm^3)	Total Volume (mm^3)
a) Male toad (15)	0.0625 ± 0.002	0.1306 ± 0.006	189.50 ± 9.92
b) Female toad (15)	0.0875 ± 0.002	0.1671 ± 0.007	200.74 ± 4.71
Probability			
a v b	<0.005 (S)	< 0.005 (S)	>0.05 (N.S.)

N.S: No significant S: Significant

of the different nephron populations within the cortical region as well as the relative size of the various glomeruli and length of the renal tubules.

DISCUSSION

The integration of morphological and structural observations continues to provide valuable new insight into the mechanisms by which the kidney performs its varied and intricate tasks. In many respects the Amphibia span the conceptual evolutionary gap between aquatic and terrestrial vertebrates; indeed the basic renal characteristics of contemporary forms may reflect certain phenomena associated with the evolution of amniote vertebrates.

In the present study, similarities and dissimilarities exist in the gross external morphology and internal organization of the kidneys of amphibia, *Bufo regularis* and Mammals, *Oryctolagus cuniculus*. The external morphology of the kidneys of the toads used here varied more than that those of the rabbits. This is undoubtedly due to the extreme variation in the nature of the functional kidney of each studied species.

Toads have attenuate mesonephric kidneys. The paired mesonephric kidneys are placed dorsally within the abdominal cavity. Amphibian kidneys in general have neither renal medulla nor do their nephrons possess a loop of Henle. The toad nephron is differentiated into a glomerulus and a renal tubule. The renal tubule consists of a narrow segment which connects the glomerular capsule with the long proximal segment. A short connecting segment leads to the distal nephron, which is joined to the collecting duct.

The filter mechanism of the toad glomerulus is thicker and more structured than that of the rabbit, such findings are in agreement with the description of Clothier et al.[24]. The anatomical organization of the toad kidney is similar to the findings previously observed in other amphibia by Stoner[25] in *Rana pipiens*, and a salamander, Clothier *et al.*[24] on *Amphiuma means*, a urodele amphibian, and by El Gohary[3] on *Bufo viridis*.

The course and segmentation of the renal tubule of the amphibian nephron is established by three major convolutions, a proximal, a distal and a third containing the connecting tubule,

Table 3

Diameter (mm)., Size and Total volume (filtering surface area) of the different glomerular populations (mm^3) of both male and female rabbits *(Oryctolagus cuniculus)*.
All the data are represented as means ± standard errors.
[Numbers in parentheses are the numbers of the experimental animals]

Experimental Animals	Glomerular Diameter (mm)			Size of Different Glomerular Populations (mm^3)			Total Volume (mm^3)
	S.	Mc.	Jm.	S.	Mc.	Jm.	
a) Male rabbit (6)	0.025 ± 0.00	0.063 ± 0.005	0.1 ± 0.005	0.104 ± 0.00	0.261 ± 0.02	0.418 ± 0.02	41337.88 ± 1501.12
b) Female rabbit (6)	0.025 ± 0.00	0.069 ± 0.003	0.106 ± 0.003	0.104 ± 0.00	0.287 ± 0.01	0.444 ± 0.01	55930.82 ± 1587.92
Probability							
a v b	>0.05 (N.S.)	>0.05 (N.S.)	>0.05 (N.S.)	>0.05 (N.S.)	>0.05 (N.S.)	>0.05 (N.S.)	<0.005 (S.)

N.S: Not significant S: Significant S: Superficial Mc: Midcortical Jm: Juxtamedullary

all of which are located in the peripheral zone (1,2,26). Light and electron microscopic studies of the amphibian renal tubule generally distinguish six segments: a neck segment, a proximal tubule, an intermediate segment, a distal tubule, a connecting tubule and a collecting duct in Urodela[1,4,6], Anura[9] and in Apoda[2,27].

In the present study the distal nephron of the toad kidney was shown to be bipovrite- the earliest part of the distal nephron and the distal tubule[26]. Also, the distal renal tubule was composed of two segments with homogeneous and heterogeneous epithelia as pointed out by Taugner et al.[9]. The first part of the distal nephron of the amphibian kidney may be considered to correspond to the diluting segment (thick ascending limb of Henle's loop; straight part of the distal tubule) in the rabbit kidney, on the basis of the similar appearance of their epithelia.

The thick ascending limb of Henle' loop of the rabbit nephron is the segment in which lumen fluid is diluted by absorption of NaCl in excess of water. Such transport property is important for both urinary dilution and concentration[28]. The second, distal, part of the distal nephron of the amphibian kidney is quite different from the distal convoluted tubule in the rabbit kidney. It contains two cell types both of which resemble to some extent the two cell types occurring in the connecting tubule (which follows the distal convoluted tubule) in the mammalian nephron[29]. Thus, from this point of view, the second part of the distal nephron of the toad kidney is comparable to the connecting tubule of the rabbit kidney.

Further support for this concept comes from the study of Stoner[25] who reported that the diluting segment of the nephrons of, *Rana pipiens*, and *Ambystoma tigrinum*, exhibited lumen-positive voltages, however, lumen-negative voltages have been reported for the distal tubule of (various) other amphibian species[30,31]. It seems reasonable, therefore, to postulate that functional heterogeneity of the distal nephron is a general trait of the amphibia[25]. A similar heterogeneity, at least with respect to voltage polarity, has already been established for the mammalian distal nephron. The voltage polarity of the rabbit thick ascending limb[28,32] is lumen-positive while that of cortical collecting tubule[33,34] is lumen-negative. In addition, the rate of sodium transport in the diluting segments of frog and salamander is very nearly equal to that of the rabbit thick ascending limb at 37°C[25].

In general, rabbit kidneys are composed of elemental units called renal lobules. A renal lobule can be established according to two different criteria. Oliver[35] considered the collecting ducts of a medullary ray to be the center of the lobule; all nephrons draining into these ducts establish the renal lobule. In contrast, other authors[29,36] considered the axis of the lobule to be formed by the interlobular artery; all the nephrons irrigated by this artery and their related collecting ducts constitute the lobule. In either case, nephrons arrange radially surrounding the axis of the lobule.

In the present study, three main types of nephron populations were identified based on their location in the renal cortex of the rabbit kidney. Such observations are consistent with the findings of Bankir and Farman[37], Rouffignac and Bonvalet[38], Beeuwkes[13] and Bulger and Dobyan[14] on various mammalian species. In addition, the arcuate nephrons have been described[15] as a constant population of nephrons in the rabbit kidney, where they constitute between 2 and 3% of all juxtamedullary nephrons. They differ from other juxta-medullary nephrons by their morphologic characteristics, location and spatial relationships.

The morphologic differences observed between nephrons appear to constitute the basis for functional differences. Thus, knowledge of the precise architecture of the mammalian kidney appears to be basic to a better understanding of the functional significance of the nephrons.

The present findings concerning the whole number of nephrons per gram renal tissue as well as the average volume (size) of the glomeruli revealed that toads have more nephrons and larger glomeruli. Comparable results have been presented by Altschuler et al.[39] who demonstrated fewer number of neprhons in the pocket mouse, a species with a high urine concentrating ability than species with moderate ability. In the same vein, Dewey et al.[40] reported that mice living in a damp environment had nearly twice as many nephrons (13,700 vs. 8,600 nephrons) as mice of a similar body size living in an arid zone, resulting in a doubling of the filtration surface area. Thus it would appear that variations in nephron number and size of glomeruli, and in the filtration surface area could be correlated with the different habitats and thereby to a water economy. In contrast, Wake[41] found that *Gymnopis proxima*, a typical terrestrial live-bearer has significantly larger glomeruli than *Tylonectes compressicauda*, a totally aquatic live-bearer amphibian.

Therefore, the larger size of glomeruli and fewer nephrons per gram kidney weight in toads, coupled with their unusual habitat, allows speculation that the amphibian kidney may be modified for efficient excretion of great quantitities of dilute urine.

On the other hand, the relatively smaller size of the renal corpuscle (glomerulus) of the rabbit nephron in combination with a simple glomerular architecture, probably limits the volume of ultrafiltrate produced. Such suggestion agrees with the view that mammals are able to form a urine hyperosmotic to plasma.

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