The Egyptian Journal of Hospital Medicine (October 2017) Vol. 69 (6), Page 2711-2721

Postnatal Developmental Changes of The Kidneys of The Albino Rat

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ABSTRACT

Background: Being a highly immature organ at birth, the rat kidney is morphologically as fetal when compared with humans. Events that occur during fetal development might determine adult renal diseases.

Aim of work: This study aimed to characterize the postnatal developmental changes of the kidneys in albino rats using light microscope from postnatal day (PND) 2 until PND 70. This may give references to pathologists when evaluating juvenile toxicology studies.

Materials and Methods: Ten healthy pregnant albino rats were used in this study. Twenty-five of their offsprings were obtained and divided according to age into five groups of 5 pups each. Group A: studied at PND 2. Group B: studied at PND 10. Group C: studied at PND 20. Group D: studied at PND 30. Group E: studied at PND 70. Kidneys were removed, processed for light microscopic study and 5 μm thick paraffin sections were obtained and stained with hematoxylin and eosin stain.

Results: Light microscopic examination of the renal cortex at PND 2 revealed subcapsular nephrogenic zone contained immature renal developmental stages, juxtamedullary zone contained formed glomeruli with medullary rays between the two zones. The renal cortex acquired maturation centrifugally with the superficial nephrons was the last to mature by PND 20. The papilla was the most mature region of the kidney and at PND2; it had the structural composition of the inner stripe of outer medulla. The papillary maturation involved a process of tubular elongation and increase in the interstitium until reaching adult structure by PND 20. At PND 2, the medulla was the most immature zone being formed of islets of tubular structures among abundant interstitium with high degree of undifferentiation. Its maturation involved tubular elongation and decrease of the interstitium with the outer medulla was the last to mature as late as PND 30. Consequently, the medulla remained immature for a relatively long postnatal period, in comparison to the other kidney regions.

Conclusion: It was concluded that, rat kidney is immature at birth and kidney sub regions mature at different rates during postnatal development. The papilla was the first to mature (PND 20) followed by the cortex (PND 20) and finally the medulla (PND 30).

Keywords: postnatal development, kidney, rat.

INTRODUCTION

The mammalian kidney is a highly vital organ that eliminates nitrogenous waste, maintains blood volume, composition and pressure and keeps bone density [(1)]. Its development includes three excretory organs, the pronephros, the mesonephros which are transitory and the metanephros [(2)] which is permanent. Development of the metanephros is a two stage processes, the first involves inductive interaction between ureteric bud and metanephric mesenchyme and the second involves nephron development. Renal functional capability begins early with formation of fetal nephron and becomes rapid after birth until reaching adult levels. The morphologic and physiologic characteristics that differentiate the fetal and newborn kidneys from the mature adult kidneys present certain susceptibilities to toxic injury. As renal tubular transport capacities vary with maturation also, the degree of nephrotoxicity may vary with maturation [(3)]. Nephrons number is highly dependent on factors that regulate ureteric bud (UB) growth and nephrogenesis during development [(4)]. Metanephric development begins at 5th week gestation in human and at 10.5 days postcoitum in mouse when the ureteric bud protrudes from the distal portion of the mesonephric duct [(5)]. This process of metanephrogenesis is completed in humans in utero before 36th week of gestation, while in mice and rats it is completed at about 7-10 days postnatal [(6)].

Received:16 /09/2017
Accepted: 25 /09/2017
DOI: 10.12816/0042254

Renal development has been studied in mice, fish and amphibians [7] and also in rabbits [8]. Rats have short gestational periods, high litter sizes and rapid growth during the first few weeks of life [9] . Most newborn rats and mice are at an early stage of kidney development, with only approximately 20% of mature nephrons are present at birth and are ongoing nephrogenesis until PND 10. Consequently, neonatal rats and mice are comparable to premature-born humans regarding the stage of kidney development [10] and postnatal developmental stages in rats might reflect late intrauterine stages in humans [11] .

Under normal conditions, in a healthy newborn and suckling infant ,the renal immaturity is not a hazard, but may be causing problems in cases of certain illnesses, inadequate liquid balance and exogenous pharmacological stress. Besides, such a kidney is more liable to pyelonephritis and calculosis than an adult kidney [12] . On the other hand, the immature kidney could tolerate anoxia to a greater extent than the mature one [13] .This study was designed to get more histological information about the postnatal developmental changes of the kidneys in albino rats using light microscope.

MATERIALS AND METHODS

MATERIALS

The study was conducted on apparently healthy ten pregnant female albino rats (150-200 gm). The rats were obtained from the animal house, Faculty of Medicine, Zagazig University. All animals were housed at room temperature. The dams were allowed free access to food and water throughout the gestational and lactational periods. After delivery 25 of their offsprings were obtained and divided into five groups 5 pups each. Group A studied at PND 2, Group B at PND 10, Group C at PND 20, Group D at PND 30 and Group E studied at PND 70. The 70-day old rats of Group E were used as a control. The 2, 10 and 20 days old albino rats were chosen regardless of sex. However, males only were chosen for the 30 and 70 days old albino rat groups. The female rats were excluded to avoid renal changes resulting from hormonal effects.

METHODS

At the end of the experiment, the rats were anesthetized with ether inhalation, their abdomens were opened and both kidneys were rapidly delivered carefully and prepared for light microscope examination. Each kidney was cut in half across the pelvis along its longitudinal axis to expose the cortex, the medulla and the papilla. The specimens were immediately immersed in 10% neutral buffered formalin for 48 hours to be routinely processed and embedded in paraffin. 5 μm thick paraffin sections were obtained and stained with hematoxylin and eosin stains and examined microscopically [14].

The study was approved by the Ethics Board of ***Zagazig*** University.

RESULTS

Postnatal development of the cortex

The renal cortex of 70-days old rat showed a homogenous appearance as it was densely packed with lobulated glomeruli with no differentiation into zones **(Fig.1A)**. The glomeruli were surrounded by Bowman's space with its visceral layer closely applied to the glomerular capillaries and its parietal layer formed of flat epithelium **(Fig.1B)**.

The renal cortex of 2-days old rats revealed three zones. The nephrogenic zone which appeared subcapsular and showed all immature renal developmental stages. The seconed zone was juxtamedullary and showed maturing glomeruli and convoluted tubules. A third zone; the medullary rays extended from the medulla to the capsule across the two previous zones **(Fig.1C)**.

In the subcapsular nephrogenic zone, the ureteric buds were observed as straight tubules with characteristic fork like bifurcation and ending with swollen ampullae among the metanephric mesenchyme. Some hemispherical glomeruli in the capillary loop stage were seen in the deep part of the nephrogenic zone close to the ureteric bud **(Fig.1D)**. The ureteric bud was lined with cuboidal cells with centrally placed nuclei. The glomeruli in the capillary loop stage showed columnar cells lining the visceral layer of Bowman's space and flat endothelial cells lining the parietal layer. Some cuboidal cells in the parietal layer were seen at the lower pole of the glomerulus **(Fig.1E).**

Aggregations of the mesenchymal cells were also seen in close association with the upper parts of the ureteric buds forming caps **(Fig.1F).**Spherical clusters of the mesenchymal cells were observed at the sides of the lower part of the ureteric bud ampullae forming peritubular condensates. These condensates formed the renal vesicles **(Fig.1G).**Immature forms of renal

developmetal stages including the renal vesicle, comma-shaped and S-shaped bodies were observed in the nephrogenic zone close to the capsule **(Fig.1H).**The renal vesicle had a central lumen and was lined by columnar cells **(Fig.1I)**. The Comma-shaped bodies contained a single cleft **(Fig.1J).** The S-shaped bodies appeared to be formed of three parts; an upper limb, lower limb and middle segment with an upper and lower clefts **(Fig.1K).**The S-shaped bodies were found close to the branched ureteric bud with connection of their upper portions connected to bud branches**(Fig.1L).**

At postnatal day 10, interruption of the nephrogenic zone by the growing glomeruli and tubules was observed **(Fig.1M)**. In other rats, the nephrogenic zone disappeared, although the ureteric buds were still present. The superficial glomeruli were compact and small while the juxtamedullary glomeruli appeared larger and lobulated **(Fig.1N)**. At postnatal day 20, the cortex acquired full maturation; the superficial glomeruli increased in size, became no longer compact and acquired lobulations **(Fig.1O)**. At Postnatal day 30, no further changes were reported.

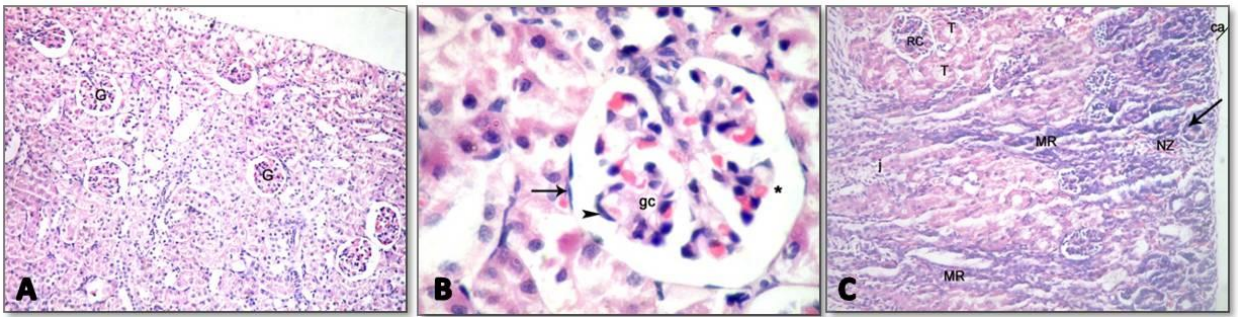

Fig. 1.A-C: A: a photomicrograph of a section in the renal cortex of a 70-days old rat showing a homogenous appearance as it is densely packed with glomeruli (G).(H&E X 200). **B:** a higher magnification of A showing the visceral layer (arrowhead) of Bowman's space (*) closely applied to the glomerular capillaries (gc) and the parietal layer (arrow) lined with flat epithelium. (H&E X 1000). **C:** a photomicrograph of a section in the renal cortex of a 2-days old rat showing the nephrogenic zone (NZ) containing immature forms of renal developmental stages (arrow), the juxtamedullary zone (j) containing formed glomeruli (RC) and the convoluted tubules (T). The medullary rays (MR) appear extending from the medulla towards the capsule (ca) across the two previous zones.(H&E X 200).

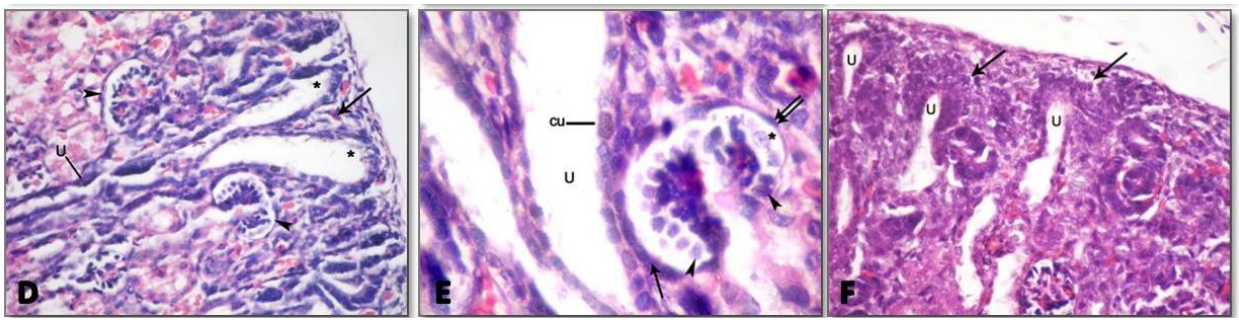

Fig. 1.D-F: D: a photomicrograph of a section in the renal cortex of 2-days old rat showing the ureteric bud (U) as a straight tubule with fork like bifurcation and ending with swollen ampullae (*) among the metanephric mesenchyme (arrow) in the nephrogenic zone. Arrowheads point to renal corpuscles in the capillary loop stage with hemispherical glomeruli in the deep part of the nephrogenic zone. **(**H&E X 400). **E:** a higher magnification of **D** showing cuboidal cells (cu) with central nuclei lining the ureteric bud (U). The glomerulus in the capillary loop stage shows columnar cells forming the visceral layer (arrow head) of Bowman's space (astrix) and flat endothelial cells lining the parietal layer (double arrow). Some cuboidal cells in the parietal layer are seen at the lower pole of the glomerulus (arrow**).**(H&E X 1000). **F:** a photomicrograph of a section in the renal cortex of a 2-days old rat showing aggregations of the mesenchymal cells forming caps (arrow) in close association with the upper parts of the ureteric buds (U). (H&E X 400).

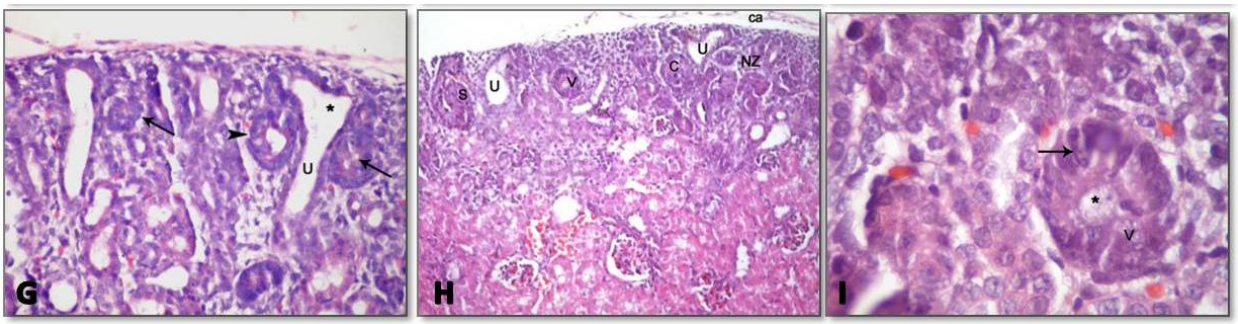

Fig. 1.G-I: G: a photomicrograph of a section in the nephrogenic zone of the renal cortex of a 2-days old rat showing spherical clusters of the mesenchymal cells forming peritubular condensates (arrow) at both sides of the lower part of the ampulla (astrix) of the ureteric bud (U). Notice, the renal vesicle (arrow head).(H&E X 400). H: a photomicrograph of a section in the renal cortex of a 2-days old rat showing the renal vesicle (V), comma-shaped body (C) and S-shaped body (S) arranged close to the capsule (ca). Notice, ureteric buds (U) in the nephrogenic zone (NZ). (H&E X 200). I: a higher magnification of H showing the renal vesicle (V) with a central lumen (astrix) and columnar cell lining (arrow). (H&E X1000).

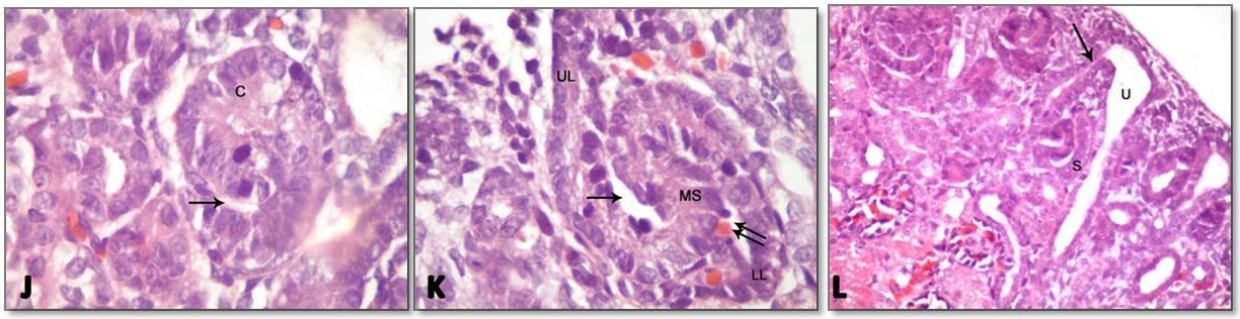

Fig. 1.J-L: J: a higher magnification of H showing the comma-shaped body (C) with a single cleft (arrow). (H&E X 1000). K: a higher magnification of H showing the S-shaped body formed of an upper limb (UL), lower limb (LL) and middle segment (MS) with an upper cleft (arrow) and lower cleft (double arrow). (H&E X 1000). L: a photomicrograph of a section in the renal cortex of 2-days old rat showing the connection (arrow) of the upper portion of the S-shaped body (S) to the ureteric bud (U). (H&E X 400).

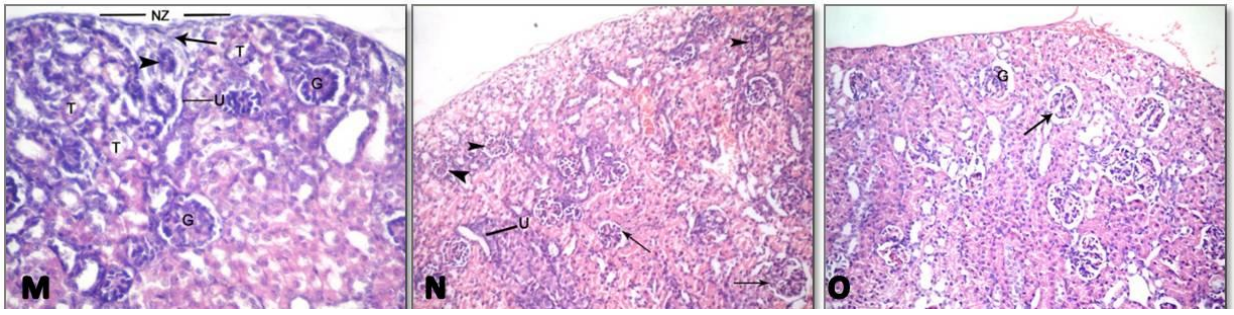

Fig. 1.M-O: M: a photomicrograph of a section in the renal cortex of a 10-days old rat showing the interruption of the nephrogenic zone (NZ) by the developing tubules (T) and the glomeruli (G). Notice, ureteric bud (U), condensate (arrowhead) and mesenchymal cells (arrow) in the nephrogenic zone.(H&E X 200). **N**: a photomicrograph of a section in the renal cortex of a 10-days old rat showing the absence of the nephrogenic zone and persistence of the UB (U). The glomeruli in the superficial cortex are small and compact (arrowhead), while those of the deeper cortex appear larger and lobulated (arrow).(H&E X 200). **O:** a photomicrograph of a section in the renal cortex of a 20-days old rat showing the cortex is densely packed with glomeruli (G). The superficial glomeruli show increased size as compared to P10 and acquire lobulations (arrow).(H&E X 200).)

Postnatal development of the medulla

Light microscopic examination of the adult medulla at postnatal day 70 showed two zones, an outer medulla (OM) and an inner medulla (IM) **(Fig.2A).** The outer medulla was differentiated into an inner stripe (IS) and an outer stripe (OS) with clear distinction between them **(Fig.2B)**. The medulla of postnatal day 2 rat was extremely disorganized; it was formed of islets of tubular structures among abundant interstitium **(Fig.2C)**. The tubular structures showed heterogenicity regarding number of the lining cells and shape of their nuclei (**Fig.2D).** The interstitial cells showed different sizes and shapes denoting high degree of undifferentiation **(Fig.2E).** In the region of the future outer medulla, extensions of medullary rays separated by abundant connective tissue were recognized **(Fig.2F)**. At postnatal day 10, the medulla showed beginning of differentiation into outer and inner zones **(Fig.2G)**. The inner zone was studded by the collecting ducts (lined by cuboidal epithelium) and the thin loops of Henle (lined by squamous epithelium with bulging nuclei into its lumen) **(Fig.2H).** The outer medullary zone contained abundant interstitium and was not differentiated into OS and IS. However, elongated proximal tubules (straight part) could be seen extending into the medulla **(Fig.2I).** The collecting ducts, thin descending and thick ascending limbs of Henle's loop(lined by cuboidal epithelium with indistinct borders) were seen among the excessive connective tissue; a structure of the inner stripe **(Fig.2J)**. At postnatal day 20- rat, most of the interstitium of the outer medulla disappeared and the OS was observed **(Fig.2K).** The OS appeared as a zone containing the thick descending limb of Henle's loop (the straight part of the proximal tubule) lined by cuboidal epithelium and its lumen is occluded by the brush border , thick ascending limb of Henle's loop (straight part of the distal tubule) and the collecting ducts **(Fig.2L)**.

In 30-days old rats, as compared to P20, medullary expansion with marked tubular growth and increased size was clear **(Fig.2M).** The interstitium in the OM nearly completely disappeared with sharp demarcation between the IS and the OS. The OS became clearly recognized and the medulla acquired an adult morphology **(Fig.2N).**

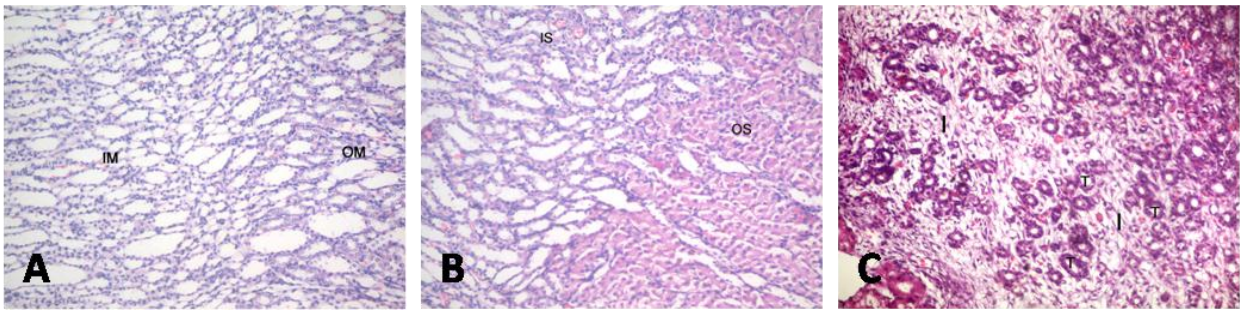

Fig. 2.A-C: A: a photomicrograph of a section in the renal medulla of a 70-days old rat showing the boundary between the inner medulla (IM) and the outer medulla (OM).(H&E X 200). **B:** a photomicrograph of a section in the renal outer medulla of a 70-days old rat showing a clear distinction between the inner stripe (IS) and the outer stripe (OS). (H&E X 200). **C**: a photomicrograph of a longitudinal section in the renal medulla of a 2-days old rat showing islets of tubular structures (T) among abundant interstitium (I). (H&E X 200).

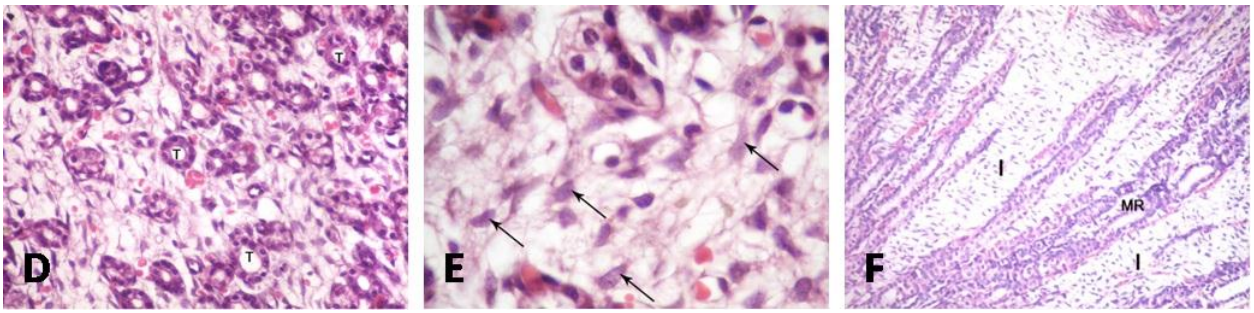

Fig. 2.D-F: D: a higher magnification of **C** showing the tubular structures (T) with heterogenicity regarding the number of lining cells and the shape of their nuclei (H&E X 400).. **E:** a higher magnification of figure D showing the interstitial cells (arrow) with different sizes and shapes denoting undifferentiation (H&E X 1000). **F:** a photomicrograph of a section in the outer medullary region of a 2-days old rat showing extension of the medullary rays (MR) which are separated by abundant interstitium (I). (H&E X 200).

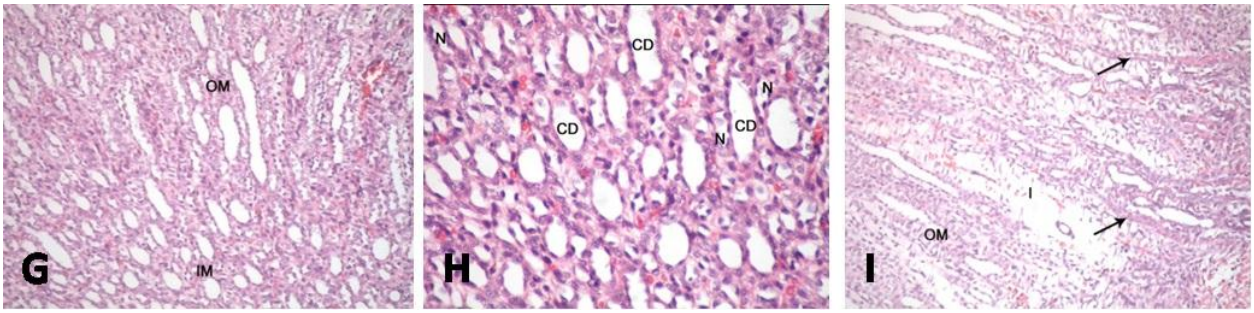

Fig.2G-I: G: a photomicrograph of a section in the renal medulla of a 10-days old rat showing differentiation into an inner zone (IM) and an outer zone (OM).(H&E X 200). **H:** A higher magnification of figure I showing the inner zone of the renal medulla studded by the thin loop of Henle (N) and the collecting ducts (CD).(H&E X 400). **I:** A photomicrograph of a section in the outer medulla (OM) of a 10-days old rat showing abundant interstitium (I) and lack of differentiation into inner and outer stripes. However, proximal tubules (straight part) are seen extending into the medulla (arrow). (H&E X 400).

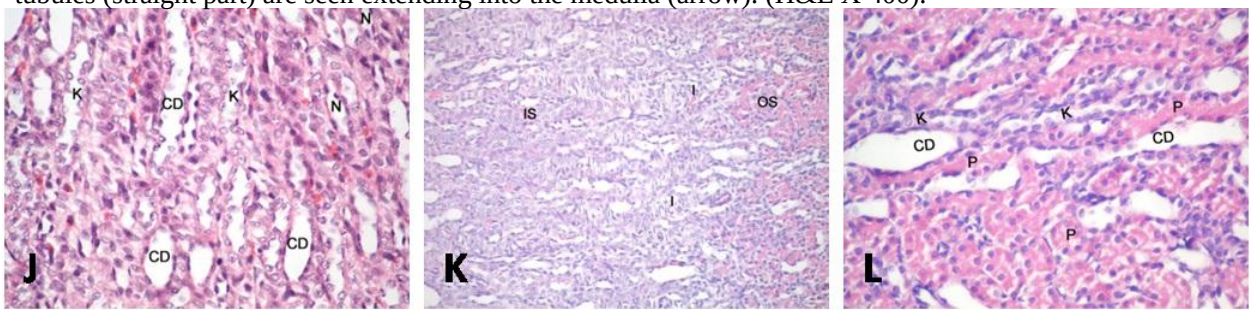

Fig. (2.J-L): J: a photomicrograph of a section in the outer medulla of a 10-days old rat showing the collecting ducts (CD), thin descending (N) and thick ascending (K) limbs of Henle's loops; a structure of the inner stripe**(**H&E X 400).**K:** a photomicrograph of a section in the outer renal medulla of a 20-days old rat showing a small amount of the interstitium (I). The outer medulla is differentiated into inner stripe (IS) and outer stripe (OS). (H&E X 200). **L:** a higher magnification of K in the region of the outer stripe of the outer medulla showing the collecting duct (CD), the thick ascending limb of Henle's loop (K) and the straight part of proximal tubules (P).(H&E X 400).

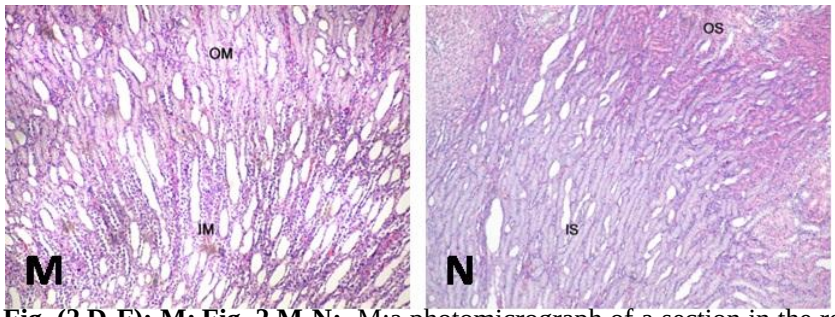

Fig. (2.D-F): M: Fig. 2.M-N: M:a photomicrograph of a section in the renal medulla of a 30-days old rat at the junction between inner medulla (IM) and outer medulla (OM) showing medullary expansion with marked tubular growth as compared to P20. (H&E X 200). **N:** a photomicrograph of a section in the outer renal medulla of a 30-days old rat showing complete disappearance of the interstitium with sharp demarcation between the inner stripe (IS) and the outer stripe (OS).(H&E X 200).

Postnatal development of the papilla

Light microscopic examination of hematoxylin and eosin stained sections in the 70-days old rat renal papilla showed that, the renal papilla was formed of the papillary surface epithelium with the openings of large ducts of Bellini and abundant interstitium among the tubular structures **(Fig.3A).** These tubules included the collecting ducts (CDs) and the thin limbs of Henle's loop **(Fig.3B).** The papilla of a 2-day old rat was studded with the CDs, the thin limbs and the thick ascending limbs of Henle's loops and had small amount of papillary interstitium **(Fig.3C, D).**

The papilla of a 10-day old rat showed increased interstitium. The tubules were elongated and showed numerous bifurcations of the collecting ducts **(Fig.3E)**.

In addition to the CDs, the papilla contained the thin limbs of Henle's loop within the interstitium. However, no thick ascending limbs were observed **(Fig.3F).**

The 20–day old rat papilla acquired adult configuration; it contained abundant interstituim between the collecting ducts and thin limbs of Henle's loop like that revealed at postnatal day70. The openings of large ducts of Bellini into the surface epithelium were observed **(Fig.3G,H).**

The transitional type of the surface epithelium was first recognized **(Fig.3I).** No further changes were reported at postnatal day 30.

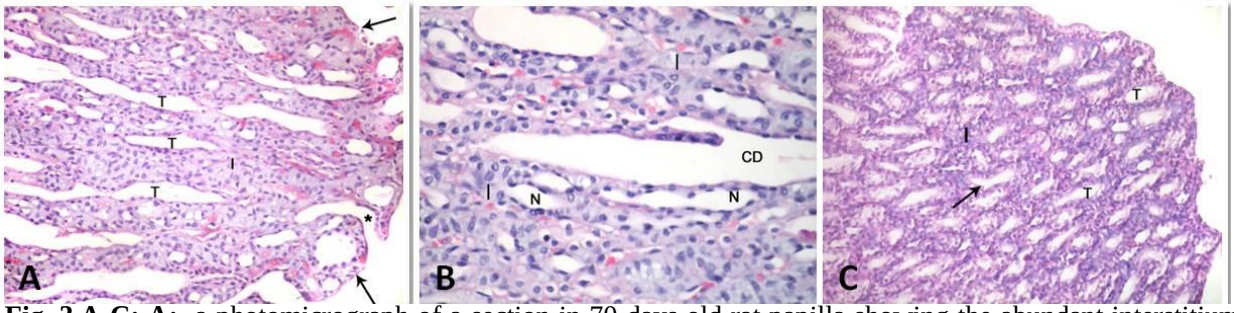

Fig. 3.A-C: A: a photomicrograph of a section in 70-days old rat papilla showing the abundant interstitium (I) among the tubular structure (T) and the papillary surface epithelium (arrow) showing the opening of the large duct of Bellini (astrix). (H&E X 200). **B**: a higher magnification of **A** showing the collecting ducts (CD), thin limb of Henle's loop (N) and the interstitium (I).(H&E X 400). **C:** a photomicrograph of a section in 2-days old rat papilla which is studded with tubules (T) and has a small amount of interstitial tissue (I). Arrow points to the bifurcation of the collecting duct.(H&E X 200).

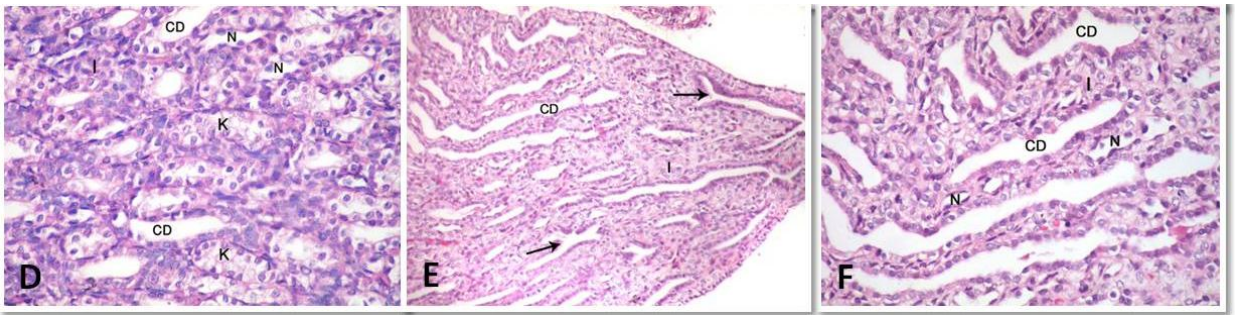

Fig. 3.D-F: D: a higher magnification of **C** showing the thin limbs of Henle's loop (N), the thick ascending limb of Henle's loop (K), the collecting duct (CD) and small amount of interstitium (I).(H&E X 400). **E:** a photomicrograph of a section in a 10-days old rat papilla showing increased interstitium (I). The collecting ducts (CD) are elongated and show numerous bifurcations (arrow). (H&E X 200). **F**: a higher magnification of **E** showing the collecting ducts (CD), thin limbs of Henle's loop (N) within the interstitium (I).(H&E X 400).

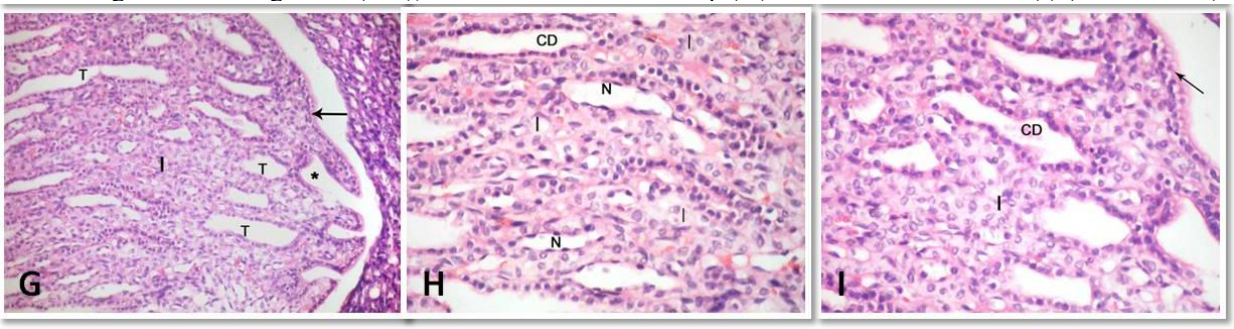

Fig. 3.G-I: G: a photomicrograph of a section in a papilla of 20 days old rat showing abundant interstitium (I) between the tubules (T). Opening of the large ducts of Bellini (astrix) are seen on the surface epithelium (arrow).(H&E X 200). **H:** a photomicrograph of a section in a papilla of 20 days old rat showing abundant interstitium (I) between the tubules (T). Opening of the large ducts of Bellini (astrix) are seen on the surface epithelium (arrow).(H&E X 200).). **I:** a higher magnification of **G** showing the transitional surface epithelium (arrow). Notice, papillary interstitium (I) and the collecting ducts (CD). (H&E X 400).

DISCUSSION

The current study made an investigation to the postnatal developmental changes of the rat kidney using light microscope. This study might be helpful for other fields of research such as experimental toxicology. In the present work, the renal cortex of 2 days old rat revealed three zones; the nephrogenic zone contained immature forms of renal developmental stages (ureteric bud, cell condensate, renal vesicle, comma and S shaped bodies), the juxtamedullary zone contained formed glomeruli and the medullary rays extending among the two previous zones. The ureteric buds were observed as straight tubules with characteristic fork like bifurcation and ending with swollen ampullae among the metanephric mesenchyme. Mesenchymal condensates formed caps closely related to the upper portion of the advancing ureteric bud. Other condensates appeared in close relation to its lowerportion forming the peritubular aggregates with one forming a renal vesicle. These results coincide with **Márquez *et al.* [15], Şimşek *et al.*[16] and El-gammal *et al.* [17]**.

Previous investigation has suggested that, the tip of the ureteric bud could induce surrounding nephron progenitor of the metanephric mesenchyme to survive, proliferate, condense and epithelialize to form a renal vesicle [18]. Such mesenchymal-to-epithelial transformation process is strictly controlled [19]. Also, several polarity genes induction might play a role in renal vesicle epithelial lumina formation [20]. Moreover, cell aggregates induced apicobasal polarity could establish a hollow epithelial structure. Proteins such as Cadherins and Crumbs might initiate such processes [21].

Georgas *et al* [22] and El-gammal *et al.*[17] observed that , the epithelial renal vesicle (Stage I nephrons) acquires a lumen and begins to "unwind" to form comma-shaped and S-shaped bodies (Stage II nephrons), vascularization occurs at the proximal end of S-shaped body to form a capillary loop (Stage III) and finally mature nephron (Stage IV)

Hartman *et al.* [23] suggested that, after birth, the ampulla of the UB, the site of dichotomous branching, become thinned and scalloped with each concavity being the location of an attaching newly formed nephron. The previous authors added that, loss of the ampulla, in the interval between birth and PND3 suggested the end of branching morphogenesis and by PND3 there was complete loss of the capping mesenchyme.

In the current study, at posnatal day 2 renal cortex, S-shaped body was observed close to the ureteric bud and connected by its upper portion (future distal tubule) with that of the ureteric bud(future collecting duct) and the lumina were continuous. Similar observations have been recognized by many investigators [24, 25, 26].

A role of the renal vesicle in the fusion of the two parts of the nephron to form a patent uriniferous tubule has been suggested by **Georgas *et al.*[22]** who mentioned that, the ureteric epithelial basement membrane between the renal vesicle and ureteric tip was lost as the distal renal vesicle region expanded into the ureteric tip. This was accompanied by an increased rate of distal renal vesicle proliferation. A continuous basement membrane and a completely patent lumen linking the early nephron with the ureteric epithelium were clear by late comma/early S-shaped body stage

In the present work, at postnatal day 2 renal cortex, crescent shaped glomeruli (the capillary loop stage) were seen in the deep part of the nephrogenic zone. Similar developmental structures were noticed by **Neiss and Klehn [27] and Nobakht *et al.* [28]** in the newborn rats and by Syed ***et al.*** [29] in the deep aspects of the superficial cortex in humans.

In the current study, at 10 PND, the rat renal cortex showed interruption of the nephrogenic zone by the growing glomeruli and tubules .In other rats; the nephrogenic zone disappeared, although the ureteric buds were still present. Similar observations were recorded by **Eguchi *et al.*[30]** and **Abrahanson [31].**

Márquez *et al.* [15] revealed immature renal developmental stages until PND 5**. Hartman *et al.*** [23] identified early stage nephrons such as vesicles in the kidneys of PND 7 mice. On the other hand, the nephrogenic zone disappeared within the first postnatal week [32] and glomerulogenesis has been completed by PND 10 [33] . In humans, the nephrogenic zone disappeared by 36 weeks of gestation [34] and was interrupted after 32 weeks of gestation [29].

Findings in the present work revealed that, at PND 20, the renal cortex appeared fully mature as it was closely packed with fully developed glomeruli. These results are in accordance with that obtained by **Speller and Moffat** [35] who observed less prominent

nephrogenic zone at PND 10 and its beginning to disappear by PND 14. **Márquez *et al* [15]** said that, in rat, the ureteric bud disappeared at PND15 and the cortex reached full maturation at weaning.

In the current work, the medulla was the most immature zone at PND2. Its maturation involved tubular elongation and decrease of the interstitium with the inner medulla matured first and the outer medulla was the last to mature as late as postnatal day 30. The medulla of 2 days old rat kidney was extremely disorganized with high degree of undifferentiation**.** At PND 10, the medulla appeared differentiated into outer (OM) and inner medulla(IM). However, the OM showed no differentiation into IS and OS. At PND 20, the outer medulla was differentiated into IS and OS. At PND 30, sharp demarcation between the IS and OS was revealed. Similar observations were reported by Speller **and Moffat [35]**, **Neiss and Klehn [27] and Márquez *et al.*[15]** .

The lack of separation of the medulla into OM and IM in the newborn rat and the mechanism of this later differentiation was explained by Kim ***et al*.** **[36]** who mentioned that, the newborn rat the kidney lacks the thin ascending limb of Henle's loop and the thick ascending limb is present throughout the renal papilla. However, during the first 2 weeks of life, the cuboidal epithelium of the thick ascending limb in the renal papilla is gradually transformed into the squamous epithelium of the thin ascending limb by a process that starts just before the bend of the loop and proceeds toward the OM. Accordingly, the IM develops as this epithelial transformation occurs in ascending direction from the papillary apex.

In the present study, differentiation of the outer medulla into IS and OS at PND 20 might be explained by **Neiss and Klehn [27]** who said that, before maturation of the OS, all nephrons display a differentiated proximal tubule epithelium with convoluted part and short straight part and a differentiated distal tubule which are connected to each other by a primitive tubule segment with low squamous epithelium. This immature segment forms a major portion of Henle's loop. The differentiation of the OS is accomplished by descending transformation of the primitive squamous limb of Henle in the medullary rays and later OS into straight proximal tubule epithelium. As a result differentiated pars recta of the PTs of all nephrons elongate toward the medulla to reach the boundary between IS and OS.

Cha *et al.* [37] recorded that, the kidney medulla proliferate through mitotic activity in loops of Henle that peak around PND 14 and is completed in the fourth week. Loops of Henle undergo considerable elongation to reach the adult conformation in the second and third postnatal weeks. This occurred through mitotic activity and apoptosis in the descending and ascending limbs. **Fischer *et al.*[38]** postulated that during postnatal growth, the elongation of medullary collecting ducts (CDs) was achieved by mitosis that was aligned with the long axis of the duct. There was little cell migration or intercalation, so that longitudinally oriented cell division led to CD elongation without a change in diameter. **Şimşek *et al*. [16]** observed that, the proximal and distal convoluted tubules were still developing until PND 20.

In the current work, the papilla was the most mature region of the kidney at birth and at PND 2; the papilla contained the CDs, thin loops of Henle and thick ascending limbs and had a small amount of interstitium. At Postnatal day10, the papilla showed increased interstitium and the thick ascending limb of Henle's loop was not seen. By PND 20, abundant interstitium was observed between the collecting ducts and thin loops of Henle. Also, the papillary surface transitional epithelium with the opening of the large ducts of Bellini was recognized and they acquired an adult morphology. These findings are in agreement with **Speller and Moffat [35], Neiss and Klehn [27] and Márquez *et al.* [15]**.

Neiss and Klehn ([27] explained that, the papilla of the newborn rat contains the tip of the Henle's loop of the juxtamedullary nephrons. The descending limbs of these loops in the papillla are seen lined by low squamous, strongly basophilic epithelium. The turning points of the latter loops and the entire ascending limbs are lined by cuboidal distal tubule epithelium.

In the current work, the first recognition of the papillary surface transitional epithelium was at PND 20. **Krause [39]** mentioned that, the transitional epithelium is continuous with the epithelium of the papillary ducts, thus providing a complete epithelial lining that prevents escape of urine into the neighboring tissues. The previous author added that, the transitional epithelium also forms a barrier to the diffusion of salt and water into and out of the urine.

In the present study, the maturation of the papilla involved increase of the interstitium that was abundant at PND 20. **Knepper et *al.*** [40] mentioned that, urine concentration involves in part removal of water by the accumulation of solutes in the papillary interstitium. **Dwyer and Schmidt-Nielsen** [41] added that, negative interstitial pressures develop and tend to move water from the epithelial cells of the CDs in the papilla into the interstitium.

It was reported that, a rapid and progressive increase in concentrating capacity develops during the third postnatal week, in particular just prior to weaning [42]. Linking this fact to the observations in the present study; at PND 20, all the medullary zones have developed and the papilla reached adult structure with abundant interstitium.

CONCLUSION

It was concluded that in rats, at PND 2, only the juxtamedullary nephrons were developed and the renal cortex acquired maturation in a centrifugal manner with the superficial nephrons were the last to mature by PND 20.

The papilla had the structural composition of the IS of OM at PND 2, and its maturation involved a process of tubular elongation and increase in the interstitium until reaching adult structure at PND 20.

The medulla was the most immature zone at birth. Its maturation involved tubular elongation and decrease of the interstitium with the inner medulla matured first and the outer medulla was the last to mature as late as PND 30.

Accordingly, the cortex and the papilla acquired maturation and adult morphology before the medulla did. Consequently, the medulla remained immature for a relatively long postnatal period, in comparison to the other the kidney zones.

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