

## INVESTIGATION OF THE HISTOLOGICAL, HISTOCHEMICAL, AND BIOCHEMICAL CHARACTERISTICS OF THE KIDNEY AND ADRENAL GLAND IN RABBITS (*ORYCTOLAGUS CUNICULUS*) AT VARIOUS STAGES OF DEVELOPMENT

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### ABSTRACT

This study aimed to investigate the development of the kidneys and adrenal glands in rabbits using histological, histochemical, and biochemical techniques. The experiment was conducted on 28 kidney and adrenal gland samples at different ages (one day, seven days, fourteen days, and ninety days). Histological findings revealed that the kidney was covered by a small amount of collagen and reticular fibers and had a thin capsule that thickened with age. The renal corpuscle increased in diameter as the age progressed. The proximal convoluted tubules were the longest, surrounded by cuboidal epithelial tissue, and had a brush border. The adrenal glands were encased in a well-developed capsule, and the cortex was divided into the glomerular, fasciculate, and reticular zones. The medulla was composed of large, pale-staining hexagonal cells arranged in small anastomosing strands held together by reticular fibers and separated by sinusoids. Norepinephrine cells were smaller. Histochemical studies showed that the brush border in the kidney responded positively to PAS, AB, and PAS-AB stains. PAS staining revealed that the basement membranes of the glomerulus and renal tubules were fully developed. Biochemical tests showed significant age-related differences in uric acid levels and each enzyme. As the urea burden increased, more urine was needed because the kidneys' ability to filter urea was limited. The kidneys underwent postnatal developmental changes, reaching maturity three months after birth when the normal adult nephron structure was observed. This indicated that the structure of the kidney and adrenal glands developed after birth with age progression.

**Keywords:** Postnatal, Kidney, Cortex, Medulla, Adrenal, Rabbit

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### INTRODUCTION

Unlike most other organs, the development of kidneys in mammals occurs in three stages, each marked by the emergence of a more

advanced pair of kidneys: pronephros, mesonephros, and metanephros (Sainio et al., 1997; Sainio, 2003). The morphogenesis of the mesonephric nephron begins with mesenchymal cell condensates, which quickly mature into renal vesicles and S-shaped structures. Metanephros undergo similar developmental events. The mesenchymal origin of the various nephron segments is distinguished from the Wolffian

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duct by the collecting duct (Suhett et al., 2023).

The kidneys of domestic animals are large, firm, bean-shaped organs that are reddish-brown in color. They originate in the lumbar region, on either side of the vertebral column, extend forward beneath the last ribs, exit the abdominal region, and are surrounded by fat (Dyce et al., 2010; Marc-André et al., 2011; Baragoth et al., 2014; Kalita and Kalita, 2014; Madrazo-Ibarra and Vaitla, 2020; Dutta et al., 2023; Kaewmong et al., 2023). The functions of the urinary system include waste elimination, ultrafiltration, blood pressure regulation, electrolyte balance, pH control, and the reabsorption of amino acids and glucose. Additionally, the kidneys produce renin, which converts a hormone into angiotensin I, and bradykinin, which acts as a vasodilator during inflammation. The kidneys also have endocrine functions, such as boosting erythropoiesis through erythropoietin. Essential substances like glucose and sodium chloride are preferentially preserved and reabsorbed by the kidney (Mukoyama and Nakao, 2005; Marques-Sampaio et al., 2007; Marco et al., 2009; Maurya et al., 2018).

The structure and function of kidneys vary among different vertebrates, enabling them to survive in diverse environments, including swamps, arid regions, and forests (Abass, 2017). The adrenal glands are a pair of bean-shaped endocrine glands located on top of the kidneys. They produce various hormones, including cortisol, adrenaline, and aldosterone, which regulate sodium and potassium ions, water and electrolyte balance, and protein, carbohydrate, and lipid metabolism (Ekele et al., 2014; Mahmood, 2022).

Currently, limited research has been published on the development of kidneys and adrenal glands in experimental animals. This information is crucial for supporting research in areas like experimental toxicity and teratology. The postnatal development of the kidneys and adrenal glands in rabbits remains unclear, raising the question of which month after birth these structures indicate maturity.

## MATERIALS AND METHODS

### Ethical approval

Ethical approval was obtained from the local Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad (Approval number 2539, dated 15/11/2023), prior to the commencement of this study.

### Study animals:

Twenty-eight samples of the kidney and adrenal gland from local breed rabbits (*Oryctolagus cuniculus*) were used in this experiment, conducted from December 2023 to May 2024. Seven animals were studied at each of the following ages: one day old (newborns), seven days old (nursing bunnies), fourteen days old (young bunnies), and ninety days old (adults).

### Samples collection:

To evaluate variations in the levels of multiple biochemical markers, blood samples (5 ml) were collected from the heart or jugular vein in sterile tubes immediately before slaughter. The samples were centrifuged at 5000 rpm for 1-5 minutes after being allowed to coagulate. The blood samples were allowed to lyse at room temperature to extract blood serum. They were then centrifuged at 40°C for 15 minutes at 706xg. Afterward, the serum was stored in sterile tubes at -20°C. The blood specimens were centrifuged, and blood urea nitrogen (BUN) tests were performed along with serum and urine analyses for urea, uric acid, and ammonia using spectrophotometers. Within two days of collection, BUN levels were assessed in tissue samples and body fluids. The colorimetric method was used to determine serum creatinine levels. In Jaffe's alkaline environment, creatinine reacts without causing deproteinization, resulting in color and intensity changes dependent on the amount of creatinine in each sample. Creatinine levels were then measured using a spectrophotometer. A uric acid (Uricase/PAP) kit was used to measure uric acid levels. Using the colorimetric enzymatic Uricase-POD-PAP technique, the intense red color of the samples indicated high uric acid

content. Sodium, magnesium ( $Mg^{2+}$ ), and potassium levels were measured using flame photometry in samples extracted with 0.1 N nitric acid, as well as in urine and serum samples that had been appropriately diluted with ion-free water. Enzyme levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as kidney function, were assessed by measuring creatinine and uric acid levels using a spectrophotometer (Maurya et al., 2018).

For animals one day of age, euthanasia was performed. For the remaining age groups, an injectable ketamine overdose was used to induce anesthesia. The kidneys and adrenal glands were removed through an incision in the ventral abdominal wall of each animal, with the midline cut to allow access to the abdominal cavity. To facilitate access to the kidneys and adrenal glands, the visceral organs were removed. After formalin fixation, samples from the adrenal glands and various regions of the right and left kidneys (cortex and medulla) were examined histologically (Suvarna et al., 2018).

#### **Histological technique:**

Dehydration was performed through sequential ethyl alcohol concentrations, followed by clearing with xylene. Infiltration and embedding were done using paraffin wax, and sectioning was carried out with a rotating microtome (Suvarna et al., 2018).

**Tissue staining:** stains used; hematoxylin and Eosin (H and E); histological components, Masson's trichrome; demonstrating collagen fibers and smooth muscles (Suvarna *et al.*, 2018).

#### **Histochemical staining:**

Periodic acid-Schiff (PAS) was applied to detect mucopolysaccharides, glycoproteins, and carbohydrates. The sections were immersed in a 1% periodic acid aqueous solution for thirty minutes. After washing the sections to remove any remaining acid, they were exposed to Schiff's reagent for thirty

minutes, followed by immersion in 0.55% potassium metabisulfite for one minute.

For Alcian blue staining at pH 2.5 to detect acidic mucopolysaccharides, the following steps were performed: the material was deparaffinized using xylene, rehydrated through graded ethanol, stained with Alcian blue for thirty minutes, rinsed under tap water for five minutes, and counterstained with nuclear fast red for ten minutes. The slides were then rinsed with water for one minute, followed by dehydration through an ethanol gradient.

The Alcian blue-periodic acid-Schiff (AB-PAS) method was used to compare both neutral and acidic mucopolysaccharides. After deparaffinizing and rehydrating the sections in distilled water, Alcian blue at pH 2.5 was applied. The sections were microwaved for 45 seconds on high power and left to stand for two to five minutes. They were then rinsed under running tap water for five minutes, followed by washing with distilled water.

The sections were placed in 0.5% periodic acid for five minutes and washed with deionized water. Schiff's reagent was applied, and the sections were microwaved for 45 seconds, then left to stand for two to five minutes. The sections were washed under running tap water for five minutes and then rinsed with distilled water (Suvarna et al., 2018).

#### **STATISTICAL ANALYSIS**

The study was analyzed using the One-Ways Analysis of Variance (ANOVA) test at a 5% significance level. Data were processed and analyzed using the statistical program Social Science (Rajathi *et al.*, 2022).

#### **RESULTS**

The kidney of a rabbit exhibited further morphogenesis after birth, with noticeable growth after one week. The glomerular condensation varied from the cortical to the subcortical zones in the renal cortex, with the

peripheral region developing more rapidly than the mid-cortical and subcortical zones. The kidney of the rabbit exhibited further morphogenesis after birth, with noticeable growth after one week. Glomerular condensation varied from the cortical to the subcortical zones in the renal cortex, with the peripheral region developing more rapidly than the mid-cortical and subcortical zones. More renal corpuscles were found in the cortical and subcortical regions than in the juxtamedullary zones. The corpuscles displayed distinct parietal squamous layers and Bowman's capsules. There was greater development in the distal and proximal convoluted tubules. At this age, the medulla displayed collecting ducts lined with cuboidal epithelium, transparent intercellular membranes, slightly stained cytoplasm, and centrally positioned nuclei (Fig. 1, 2).

The kidney exhibited increased morphogenesis and renal structural development at two weeks of age. Clear Bowman's capsules and a parietal squamous layer were visible. Each nephron component had distinctive histology by this time. The brush border of the proximal convoluted tubule epithelium was well-developed, and the loops of Henle were visible. Renal lobes were formed by renal pyramids in the medulla, while the medullary rays, collecting ducts, and tubules extended toward the subcortical and cortical zones. The proximal tubules displayed a narrow lumen and few cells. The circular apex of the medullary pyramid extended from the base to form the renal papilla, which was lined with low

cuboidal epithelium in the collecting tubules and cuboidal epithelium in the collecting duct (Fig. 3, 4).

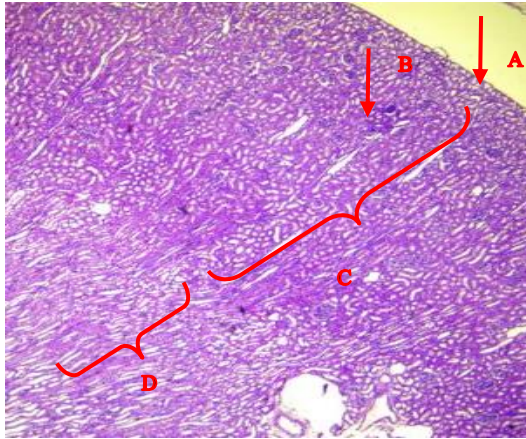
The kidney of a three-month-old rabbit exhibited a mature structure of the renal parenchyma. The collagen fibers in the interstitial tissue capsule comprised the majority of the stroma. The normal morphology of the renal corpuscles was formed from the parietal and visceral layers of Bowman's capsule, which are separated by the characteristic Bowman's gap around the glomeruli. Additionally, the renal loops elongated, and the collecting tubules and ducts occupied the denser medulla. A collagenous fibrous tissue capsule enveloped the kidney in adults (Fig. 5), which was thicker than in other ages, with significant differences ( $P > 0.05$ ) (Table 1). To control the shape and size of the kidney according to the age of the animal, the present investigation referred to a connective tissue capsule. The glomerulus was positioned subcapsularly at various stages of development, appearing invaginated in Bowman's capsule. The diameter of Bowman's space varied (Fig. 5).

The cortex was characterized by several features, including a urinary pole and a vascular pole, complex nephron tubules, and medullary rays—parallel arrays of tubules extending into the cortex and forming the core of the lobules. It also contained straight tubules, collecting ducts, and more mid-cortical nephrons than juxtamedullary nephrons near Henle's loops.

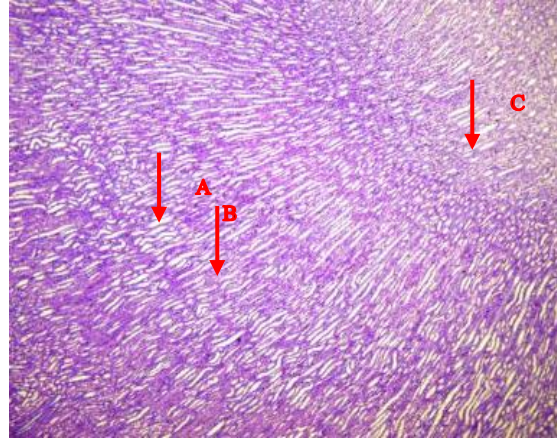
**Table 1:** Measurement of kidney and adrenal gland at different ages,  $\mu\text{m}$  ( $\bar{X} \pm \text{S.E}$ )

Measure Age	Capsule of kidney	Capsule of gland	Distal tube	Proximal tube	Glomerulus	Collecting duct
1 day	28.3±0.7 A	26.2±0.3 C	71.4±2.6 a	49.1±0.3 a	94.1±1.4 A	15.2±0.1B
7 day	34.6±1.1 A	30.5±1.1 C	75.3±1.1a	55.6±0.1 a	122.4±1.3 A	18.3±0.2B
14 day	36.8±0.1 A	32.2±0.4 C	81.4±2.3a	62.2±0.4 a	131.3±1.2 A	20.4±0.1B
90 day	42.5±1.2 A	36.6±1.1 C	92.5±2.4a	70.1±0.1 a	142.6±1.1 A	23.6±0.3B

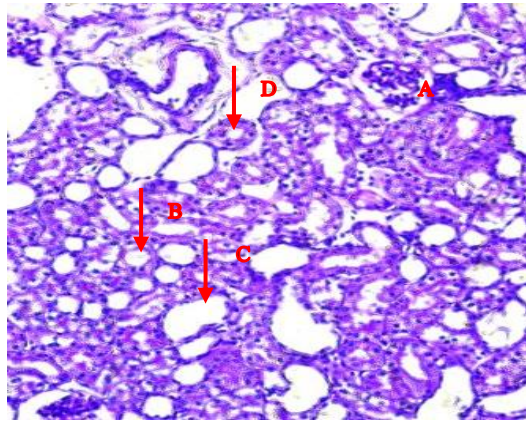
The values column with capital letters in the same column denote to significant difference ( $P > 0.05$ ) whereas column values with small letters denote to the nonsignificant differences ( $p < 0.05$ ).



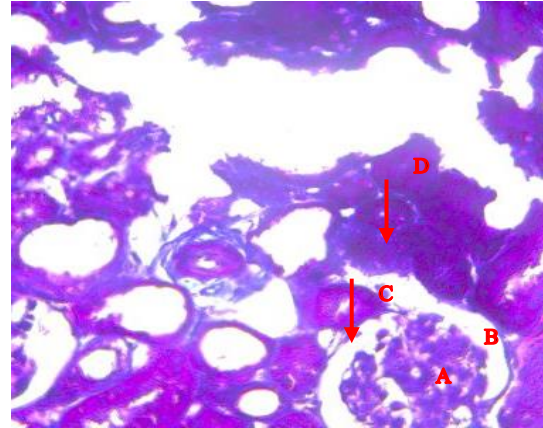
**Fig. (1):** Cross section of kidney, at 1 day age; capsule (A), glomerulus (B), cortex (F), medulla (G), Masson 100X.



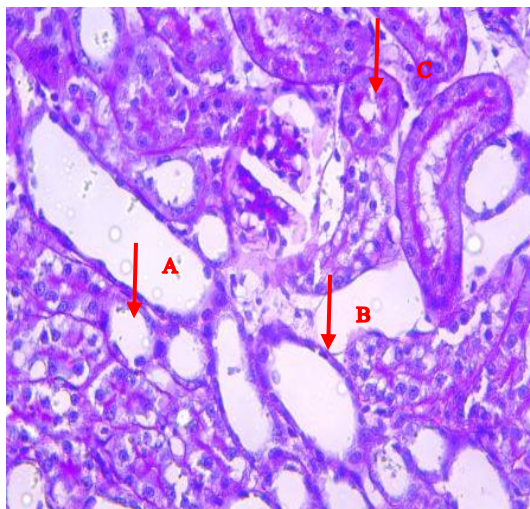
**Fig. (2):** Cross section of kidney, at 7 day age; proximal tube (A), distal tube (B), medulla (C), Masson, 200X.



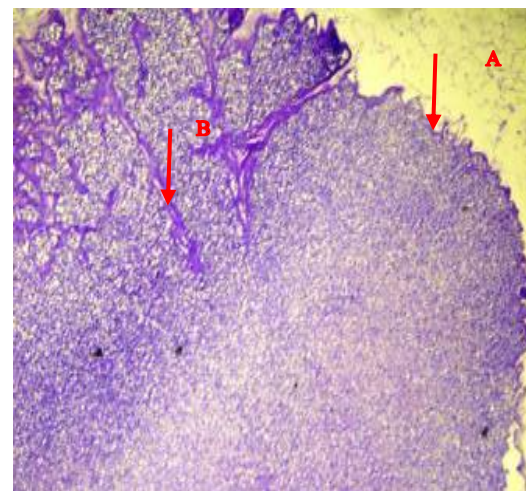
**Fig. (3):** Cross section of kidney, at 14 day age; glomerulus (A), proximal tube (B), distal tube (C), collecting duct (D), Masson, 200X.



**Fig. (4):** Cross section of kidney, at 90 day age; glomerulus (A), renal space (B), Bowman capsule (C), Juxtaglomerular apparatus (D), PAS, 400X.



**Fig. (5):** Cross section of kidney, at 90 day; proximal tube (A), distal tube (B), collecting duct (D), AB, 400X.



**Fig. (6):** Cross section of adrenal gland, at 1 day; capsule (A), trabecule of capsule to cortex (B), H@E 100X.

The proximal tubules, finer structures of the nephron, were located close to the renal corpuscles and were surrounded by cuboidal epithelium. Their luminal side had brush borders, and their spherical nucleus was contained within. The afferent arteriole penetrated the renal corpuscle and formed the glomerulus, which is encircled by Bowman's capsule (Fig. 4-5).

The glomerulus, renal tubules, and the brush border of the proximal tubules had well-developed basement membranes, as demonstrated by the PAS stain. In the renal morphology at three months of age. The podocytes surrounding the glomerular capillaries are conspicuous, and the distal tubules with a large lumen are located at the vascular pole of the renal corpuscles (Figs. 4, 5).

The adrenal glands were encased in a highly developed capsule that occasionally contained smooth muscle fibers as well as dense, irregular connective tissue. Slender trabeculae in the capsule penetrate the cortex but extend less deeply into the medulla (Figs. 6–12). The parenchymal cells that make up the adrenal cortex are located between the medulla and the capsule. This cortex consists of the glomerulosa, fasciculata, and reticularis (Fig. 6–8). The fibrous capsule of the gland directly covers the glomerulosa (Fig. 7). The lowest layer, called the reticularis, consists of a network of short, anastomosing secretory cell cords, with sinusoids separating them. Compared to other

cortex, the mature structure of the renal components was observed, where the renal tubules encircled the renal corpuscles. The glomerular capillaries were surrounded by the parietal and visceral layers of Bowman's capsule, with noticeable podocytes surrounding the glomerular capillaries. The spaces among the renal corpuscles were filled with renal tubules that varied in breadth and diameter. Distal tubules with a broad lumen, as part of the juxtaglomerular apparatus, were located at the vascular pole of the renal corpuscles. According to the current study, the kidney of an adult rabbit shows normal zones, these specific cells were frequently stained more strongly. Their small cells were arranged erratically into clusters and cords, with connective tissue and capillaries dividing them (Fig. 7). The largest layer of the cortex, the zona fasciculata, was made up of long, straight cords of giant polyhedral secretory cells situated between the zona reticularis and the zona glomerulosa. The cells are arranged in columns facing the medulla radially (Fig. 6).

The biochemical analysis of all measurements, including serum urea, creatinine, sodium, and potassium levels, revealed significant differences between age groups. Serum creatinine levels were significantly higher in adult rabbits ( $P < 0.05$ ). Blood from younger ages showed significantly lower AST and ALT levels than adult blood, with a probability threshold of 0.05 (Table 2).

**Table 2:** The renal biochemical parameters at different ages; Mg-dl

Measure Age	ALT	AST	Uric acid	ammonia	urea	Creatinine	Na	K	Mg <sup>2+</sup>
1 day	10.4±0.6 a	11.2±0.1A	1.42±0.3A	42.3±0.1A	29.1±0.3 B	1.1±0.2A	116.2± 1.3 b	2.3±0.23 a	1.1±0.04 a
7 day	13.6±0.2a	13.4±0.3A	2.18±0.2A	46.4±0.2 A	31.1±0.8 B	1.7±0.1A	120.4± 1.6 b	3.1±0.06 a	1.6±0.07 a
14 day	16.3±0.1 a	15.6±0.1A	2.48±0.4A	51.6±0.5 A	34.1±0.2 B	2.5±0.6A	131.2± 1.4 b	4.1±0.13 a	1.8±0.03 a
90 day	18.1±0.3 a	19.2±0.8A	3.77±0.6A	54.2±0.1 A	38.1±0.9B	2.9±0.1A	142.4± 1.5 b	7.1±0.04 a	1.9±0.09 a

The value with capital letter in same column denotes to significant difference ( $P > 0.05$ ); whereas value with small letter denote to the nonsignificant differences ( $p < 0.05$ ).

The physiological and biochemical results from blood samples collected at different ages are often correlated with the effectiveness and adaptation of the animals (Table 2). It also indicates that the levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in adult blood increased significantly compared to blood samples taken at earlier ages.

## DISCUSSION

The kidney of a rabbit exhibited further morphogenesis after birth, with noticeable growth after one week. At this age, the medulla displayed a collecting duct lined by cuboidal epithelium and transparent intercellular membranes. This is similar to the findings of Sainio (2003) and Al-Rawi and Kalaf-Allah (1980). Consequently, this finding was linked to changes in tubule activity concerning water quality. Proximal tubules were also filled with delicate secretions to increase the surface area of the tubule lumen and the amount of fluid consumed, as well as to retrieve a higher number of components from the lumen into the blood. Nearly all minerals, vitamins, ions, and plasma proteins are also absorbed by the proximal tubule cells, which reabsorb 65% of the water filtered inside the corpuscle (Rossi et al., 2023).

The kidney showed increased morphogenesis and renal structural establishment at two weeks of age. The proximal tubules displayed a limited lumen and few cells. The circular apex of the medullary pyramid extends from the base to form the renal papilla, which is lined with low cuboidal epithelium in the collecting tubules and a collecting duct. This is similar to a previous study (Sainio et al., 1997).

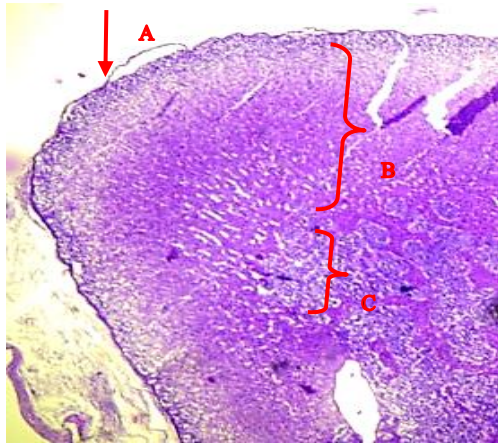
The kidney of a rabbit that was three months old exhibited a mature structure of the renal parenchyma. The distinctive eosinophilic cytoplasm of the lining cells of the proximal tubules indicates differentiation. A collagenous fibrous tissue capsule enveloped the kidney in adults, which was thicker than in other ages, with significant differences

( $P > 0.05$ ), and there was substantial blood flow in the kidney, responsible for filtering the circulating blood. These findings were comparable to those of Batah and Mirhish (2019) and Nawata and Pannabecker (2018). The glomerulus is positioned subcapsular at varying stages of development and appears to be invaginated in Bowman's capsule. The diameter of Bowman's space varied. These results were confirmed by Kaewmong et al. (2023) and Pereira-Sampaio et al. (2004). According to Seema et al. (2017) and Yang et al. (2023), the afferent arteriole penetrates the renal corpuscle and forms the glomerulus

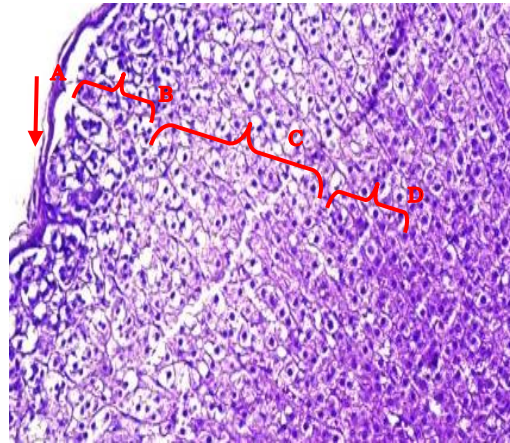
As part of the juxtaglomerular apparatus, distal tubules with a large lumen are located at the vascular pole of the renal corpuscles, and the podocytes surrounding the glomerular capillaries are conspicuous. This is consistent with the results of Marques-Sampaio et al. (2007). This structure establishes an osmotic gradient between the cortex and medulla, driving water reabsorption from the urine, which is essential for the function of the metanephric kidney. The macula densa acts as a vasoconstrictor and is very sensitive to ion concentration and fluid volume in the tubule, conveying chemical signals that trigger the release of the renin enzyme into circulation. The mesangial cells have phagocytic functions in these processes (Ebeid et al., 2023).

The adrenal glands were encased in a highly developed capsule that occasionally contained smooth muscle fibers as well as dense, irregular connective tissue. This observation was confirmed by Quaggin and Kreidberg (2008). The capsule is composed of dense connective tissue and smooth muscle cells, a finding consistent with the results of Al-Jebori et al. (2014).

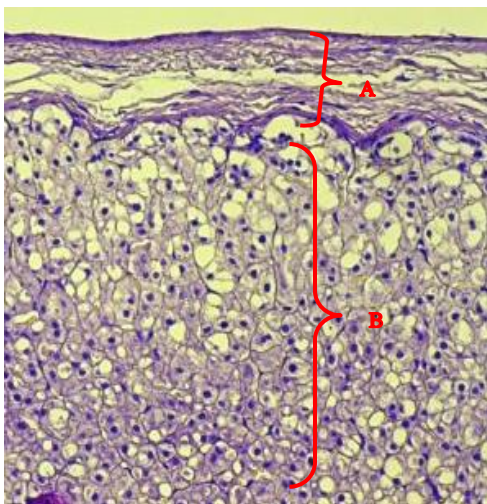
The glomerulosa, fasciculata, and reticularis make up the adrenal cortex, which is comparable to the findings of Al-Jebori et al. (2014). Mineralocorticoid hormones, which control the body's  $\text{Na}^+$  and  $\text{K}^+$  ion balance as well as water and electrolyte balance, are released by the cells in this zone (Kalita and Kalita, 2014). In the zona fasciculata, the cells release glucocorticoid hormones, which



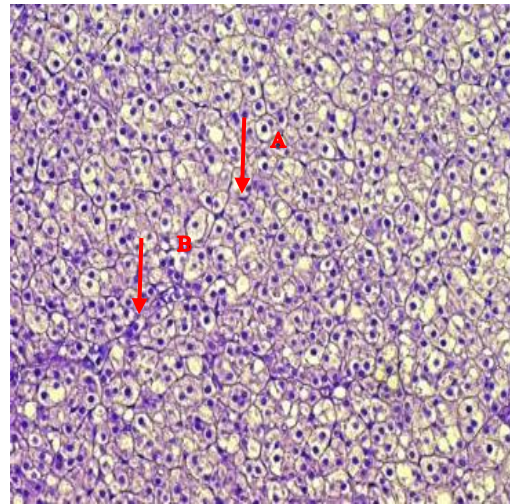
**Fig. (7):** Cross section of adrenal gland, at 7 day; capsule (A), cortex (B), medulla (C), H@E 100X.



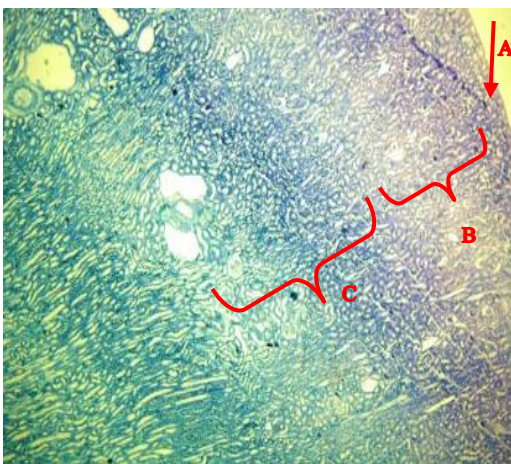
**Fig. (8):** Cross section of adrenal gland, at 14 day; capsule (A), glomerularis (B), fascicularis (C), reticularis (D), PAS 100X.



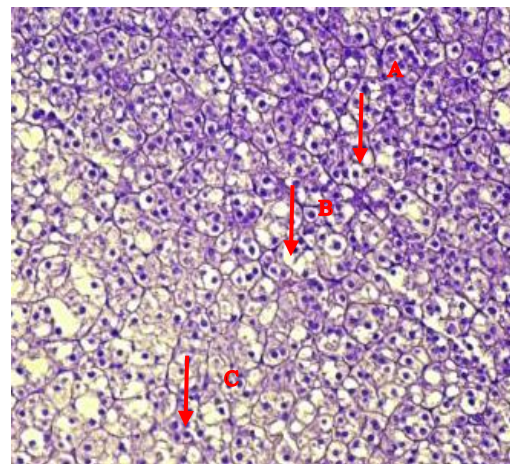
**Fig. (9):** Cross section of adrenal gland, at 90 day; Capsule (A), cells of glomerulosa zona (B), AB 200X.



**Fig. (10):** Longitudinal section of adrenal gland, at 14 day; Cells of glomerulosa zona (A), cells of fasciculate zona (B), PAS 400X.



**Fig. (11):** Longitudinal section of adrenal gland, at 14 day; Capsule (A), glomerulosa zona (B), fasciculate zona (C), PAS-AB400X.



**Fig. (12):** Longitudinal section of adrenal gland, at 90 day; Cells of glomerulosa zona (A), cells of fasciculate zona (B), cells of fasciculate zona (C), PAS 400X.



affect the metabolic processing of proteins, fats, and carbohydrates (Ebeid et al., 2023). The cortical zones interacted with one another, and the arrangement of the cells in this region resembles glomeruli, aligning with the results of Baragoth et al. (2014), who found that the cells in this zone exhibited a glomerulus-like structure. This layout differs from that of horse, canine, and pig cells, which are organized in arcs with convexity oriented toward the periphery (Marc-André et al., 2011).

The biochemical analysis of all measurements, including serum urea, creatinine, sodium, and potassium levels, revealed significant differences between age groups. The results also suggested significant differences in the amount of urea in the bloodstream and a wide range in the types of nitrogenous compounds produced by various metabolic processes. Serum creatinine levels were significantly higher in adult rabbits ( $P < 0.05$ ). Additional research has shown that uric acid and creatinine levels are elevated in several circumstances when urea accumulates in the blood. Blood from younger animals was shown to have significantly lower AST and ALT levels than adult blood at a probability threshold of 0.05. Blood proteins play a crucial role in the synthesis of hormones, enzymes, and antibodies, in addition to regulating osmotic pressure. The capsular fluid, which resembles plasma in composition, is where blood urea is reabsorbed and reintroduced into circulation. A few molecules are discharged from tubule cells, along with secretory chemicals supplied by the blood; these include the concentration and release of urea in the loop of Henle, where the cells actively absorb water. The percentage of urea in blood is influenced by the amount of nitrogen ingested, and the difference in urea clearance is proportional to the amount of nitrogen consumed, the percentage of renal filtration, and the concentration of urea in plasma. Protein catabolism results in the production of ammonia, a hazardous chemical that must be removed from all tissues. The organism

transforms ammonia into urea. Osmotic diffusion allows urea to be present in most body fluids and tissues (Suhett et al., 2023).

The current results showed that the physiological and biochemical findings from blood samples collected at different ages are often correlated with the effectiveness and adaptation of the animal. The results also indicated that the levels of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in adult blood increased significantly compared to blood samples taken at earlier ages. Since enzyme measurements are used to evaluate the effect of tissues in internal organs, this condition places a significant burden on the kidneys, followed in intensity by the liver and skeletal muscles. These results are consistent with those of Dyce et al. (2010), which demonstrated that evaluating enzyme levels indicates the degree of tissue degradation within internal organs. On the other hand, this increase may be related to enzymatic processes that help eliminate dangerous compounds produced when chemical-containing meals are digested, which elevate blood serum urea concentrations. This is related to the animal's ability to withstand stress and harsh environments and may also play a role in the body's water balance due to its capacity to retain water (Kalita and Kalita, 2014).

Proteins impact the renal glomeruli, thereby influencing blood urea levels. The kidneys play a key role in maintaining magnesium balance. Additionally, there is a relationship between urea levels, urine volume, and the development of the renal pelvis. In the proximal tubule, magnesium is reabsorbed paracellularly, while in the distal tubule, the transient receptor potential melastatin (TRPM) facilitates the transcellular reabsorption of magnesium. Moreover, rabbit tubules exhibit a greater proportion of  $Mg^{2+}$  reabsorption, which facilitates more precise regulation of  $Mg^{2+}$  excretion, albeit with notable variations among animals (Rajathi et al., 2022).

## CONCLUSION

The structure of the kidney changes with age. The kidneys undergo postnatal developmental modifications, reaching maturity three months after birth, at which point the normal adult nephron structure is established. To support the current findings, an electron microscopic study of the kidneys and adrenal glands at various ages will be conducted in the future.

### Funding

This research does not receive external funding; the author contributes to support this work in a self-supporting manner

### Availability of data and materials

Data from the current study area are available upon - reasonable request.

### Competing interest

There is no conflict of interest between the authors.

### Ethical consideration

The current research follows the accepted principles of ethical conduct of the University of Tikrit, Iraq.

### Author contributions

All author contributes equally.

## ACKNOWLEDGMENTS

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## NOVELTY STATEMENT

**The novelty of this study lies in its** comprehensive investigation of kidney and adrenal gland development in rabbits, employing a combination of histological, histochemical, and biochemical techniques. By detailing these developmental processes, the study paves the way for enhanced utilization of laboratory rabbits in medical, biomedical, and scientific research.

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## دراسة الخصائص النسيجية والكيميائية النسيجية والكيميائية الحيوية للكلية والغدة الكظرية في الأرانب (*Oryctolagus cuniculus*) في مراحل مختلفة من النمو

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هدفت هذه الدراسة إلى دراسة تطور الكلى والغدة الكظرية في الأرانب ؛ حيث استخدمت تقنيات الأنسجة والكيمياء النسيجية والكيمياء النسيجية والكيمياء الحيوية. أجريت التجربة على (٢٨) عينة من الكلى والغدة الكظرية، سبع عينات في أعمار مختلفة (يوم واحد، سبعة أيام، أربعة عشر يوماً، تسعين يوماً). كشفت النتائج النسيجية عن أن الكلية كانت مغطاة بكمية قليلة من الكولاجين والألياف الشبكية، وكانت ذات كبسولة رقيقة تزداد سماكة مع تقدم العمر، وكان قطر الكلية يزداد مع تقدم العمر، ويمكن تصنيف الكلية إلى قشرية أو قشرية وسطية أو قشرية مجاورة، وكانت الأنابيب الملتفة القريبة هي الأطول، وكانت محاطة بنسيج طلائي مكعب، ولها حدود فرشاة. غطت كبسولة متطورة جيداً الغدة الكظرية، وكانت القشرة مقسمة إلى مناطق الكبيبات والحشوية والشبكية، وكان النخاع مكوناً من خلايا سداسية كبيرة شاحبة اللون ومرتبطة في سلاسل صغيرة متلاصقة متماسكة معاً بواسطة ألياف شبكية ومقسمة بواسطة الجيوب الأنبوبية، وكانت الخلايا التي تفرز الأدرينالين كبيرة. أما الخلايا التي تفرز الأدرينالين فهي الخلايا الأصغر حجماً. الدراسات الكيميائية النسيجية؛ أظهرت أن حدود الفرشاة في الكلية استجابت بشكل إيجابي لكل من PAS و AB و PAS-AB. كشفت صبغة PAS أن الأغشية القاعدية للكبيبات والأنابيب الكلوية قد تشكلت بالكامل. أظهرت الاختبارات الكيميائية الحيوية اختلافات كبيرة مرتبطة بالعمر في كميات حمض اليوريك وكل إنزيم. مع ارتفاع عبء اليوريا، تزداد الحاجة إلى المزيد من البول لأن قدرة الكلى على تصفية اليوريا محدودة. خضعت الكلى لتغيرات في النمو بعد الولادة، حيث وصلت إلى مرحلة النضج بعد ثلاثة أشهر من الولادة عندما لوحظ وجود نفرون طبيعي للبالغين.