

Histological and Biochemical Analysis of Liver and Kidney Development in Rabbits (*Oryctolagus Cuniculus*): Insights into Structural Maturation

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ABSTRACT

Objective: This study aimed to investigate the structural and biochemical development of the liver and kidneys in rabbits (*Oryctolagus cuniculus*) using histological and biochemical methodologies. **Laboratory rabbits** are widely used in biomedical and medical research, necessitating a thorough understanding of their organ development. **Materials and Methods:** The study included 30 liver and kidney samples collected at different postnatal ages: 1 day, 8 days, 15 days, 30 days, and 60 days (n=6 per age group). Histological and histochemical analyses were performed, and biochemical parameters were evaluated to assess age-related developmental changes. **Results:** Histological analysis revealed that the liver was initially encapsulated in a thin capsule, which thickened by 30 and 60 days. The parenchyma comprised radially arranged hepatocytes around a central vein, interspersed by sinusoids and Kupffer cells. Structural dimensions of the liver varied with age. Kidney development showed distinct features, including cortical, midcortical, and juxtamedullary renal corpuscles. The proximal convoluted tubules, lined with cuboidal epithelium and a brush border, were the longest segments. The renal capsule and cortical region initially contained minimal collagen and reticular fibers, which increased with age. Henle's loops were short at 1 day and in adulthood but elongated during intermediate stages. In adulthood, Bowman's capsule displayed more prominent collagen fibers and a larger lumen of nephrons. Histochemical analyses demonstrated that the kidney's brush border reacted positively to PAS, AB, and PAS-AB stains, indicating maturation. Hepatocytes also showed a positive PAS reaction, and renal tubules displayed well-defined basement membranes and cytoplasmic granules. Biochemical analyses revealed significant age-dependent variations in uric acid levels and enzymatic activity. Increased urea load corresponded to reduced filtration capacity, emphasizing the developmental adjustments in renal function. **Conclusion:** The liver and kidneys undergo significant postnatal development, achieving mature structural and functional characteristics by 60 days of age. These findings provide valuable insights into the organogenesis of *Oryctolagus cuniculus*, supporting its use in biomedical research and enhancing understanding of renal and hepatic maturation.

Keywords: Developmental Study, Histochemical, Biochemical, Liver, Kidneys

INTRODUCTION

Laboratory animals are important commercial creatures used in research and clinical trials [1]. The major alimentary gut gives rise to lobes containing hepatocytes divided by circulatory channels that subsequently extend into sinusoids throughout embryonic development, which is how the liver forms. The liver performs essential tasks including metabolism, detoxification, bile secretion, and the synthesis of plasma proteins. It is the primary organ responsible for maintain the metabolic homeostasis [2-5].

The majority of metabolic and synthetic processes in the liver are performed by hepatocytes, which make up 80% of the liver's volume [6]. After hematopoietic stem cells migrate to the bone marrow in the late stage of embryogenesis, the liver becomes the principal organ of metabolism. The liver contains several enzymes that aid in chemical reactions, including aspartate liver enzymes, alanine aminotransminase, and aminotranminase [7-9]. The development of the kidneys in mammals happens in three stages, which are characterized by the creation of a more sophisticated pair of kidneys: the pronephros, mesonephros, and metanephros. This is in contrast to the majority of other organs. The mesonephric nephron's development begins with mesenchymal condensates. Renal vesicles and S-shaped structures rapidly

develop from these condensates. Similar developmental events have been observed in metanephros. The collecting duct separates the different nephron segments' mesenchymal origin from the Wolffian duct (1012). The kidneys of domestic animals are large, firm, bean-shaped organs with a reddish-brown tint. Beginning on either side of the spinal column in the lumber region, they progress forward under the last set of ribs, leave the abdominal region, and are surrounded by more fat [10-12].

The functions of the urine system include blood pressure regulation, pH balance, elimination, ultrafiltration, and the reabsorption of glucose and amino acids after Rennin, which the kidneys also manufacture, converts angiotensin I and Bradykinin, which function as vasodilators during inflammation, from a hormone called an. Additionally, the kidneys perform endocrine duties. The hormone Erythropoietin increases Erythropoiesis. The kidney is where essential substances like salt chloride and glucose are maintained and reabsorbed preferentially [7, 13-16]. Vertebrates may live in swamps, arid areas, and woods because of the structural and functional differences in their kidneys [17-19]. The liver and kidney development of experimental animals is not well studied in published literature. To shed light on rabbit liver

and kidney at different ages was the aim of this study. Especially in the areas of experimental toxicity and experimental teratology, this knowledge is essential for assisting investigative research projects.

MATERIALS AND METHODS

Ethical approval From October 2023 to March 2024, all processes were carried out in accordance with the University of the Sawa's animal care guidelines.

Study Animals

In this experiment, which took place between October 2023 and March 2024, thirty samples of liver and kidney from local rabbits, *Oryctolagus cuniculus*, were used. Six animals at each age range were included in the study: one day old as a freshly born, eight days old as pre-weaning, fifteen days old as weaning, thirty days old as young, and sixty days old as an adult (1).

Samples Collection

Blood samples (5 ml) were drawn from heart or jugular vein in sterile tubes shortly before killing in order to assess differences in the levels of certain biochemical tests. After allowing the samples to clot for one to five minutes, they were centrifuged at 5000 cycles. Samples of blood were left to lyse and extract blood serum at room temperature for a short while. After that, samples were centrifuged for 15 minutes at 40C and 706xg. Following that, the serum was stored at 20 degrees Celsius in sterile vials. Using spectrophotometers, blood urea nitrogen test readings were obtained along with serum and urine samples, urea, uric acid, and ammonia after the blood specimens were centrifuged. Tissue samples and body fluids were evaluated for urea nitrogen levels in the bloodstream two days after collection. The serum's creatinine level was ascertained by the Colorimetric technique. The amount of creatinine in each sample determines the color and intensity changes that emerge from the interaction between creatinine and Jaffe's alkaline environment without leading to deproteinization. Following that, a spectrophotometer was used to test and analyze each sample's creatinine level. The level of uric acid was measured using the uric acid (Uricase/PAP) kit. The sample evidence's bright red hue suggests a higher degree of uric acid content, according to data analysis using the colorimetric enzymatic Uricase-POD-PAP procedure. Salt, magnesium dioxide (Mg²⁺), and potassium were measured using flame photometry on samples that were extracted with 0-1 N nitric acid and on urine and serum samples that were suitably diluted with ion-free water. A spectrophotometer was used to measure the levels of creatinine and uric acid in order to determine the presence of an enzyme known as Alanine Aminotransaminase (ALT) and Aminotransaminase (AST), as well as renal function [20, 21]. After killing in one day of life, an overdose of injectable ketamine was administered to induce anesthesia for the remaining years. From each

animal's ventral abdominal wall, the liver and kidney were removed. The midline had been severed from the abdominal cavity. The internal organs were removed to make the liver and kidneys easier to reach. Samples from the left and right kidneys (cortex and medulla) as well as the liver lobes were histologically explored [22].

Histological technique: sectioning using a rotating microtome, xylene for clearing, paraffin wax for infiltration and embedding, and dehydration with sequential spiraling ethyl alcohol. The following stains are utilized in tissue staining: Verhoeff stain to elastic tissue; hematoxylin Eosin (H and E); Masson's trichrome and Mallory trichrome to histological components; these stains show the collagen fibers and smooth muscles [23].

Histochemical Staining

Glycoproteins, and mucopolysaccharides were treated by Periodic Acid Schiff (PAS). Sections were immersed in the aqueous solution 1% periodic acid for 30 minutes. The slices were subject to Schiff's reagent and 1 minute of potassium metabisulfite 0.55%. Alcian blue pH 2.5: Perform to acidify mucopolysaccharides: deparaffinize by xylene. Rehydrate in graded ethanol after thirty minutes of alcian blue, five minutes of water, and ten minutes of nuclear fast red, water for a minute. applying a gradient of an ethanol. The neutral and acidic mucopolysaccharides were compared by Alcian Blue plus Periodic Acid Schiff (AB-PAS), involved deparaffinizing and rehydrating, alcian blue; pH 2.5. to five minutes and forty-five seconds to use maximum power. distilled water after five minutes. Work with 0.5% periodic acid for five minutes. When washing, use de-ionized water. Schiff's Reagent and Microwave to five minutes to rest after 45 second of vigorous exercise [24].

Statistical Analysis

The study is analyzed by using the one-ways analysis of variance (ANOVA) test at a 5% level of significance. Data are processed and analyzed with using the statistical program social science.

Results and Discussion

The parenchyma, which included hepatocytes, was surrounded by a capsule that had simple squamous epithelium. The major vein that ran through these cells was split by sinusoids. Around the central vein, hepatocytes were radially raised, and Kupffer cells protruded next to the endothelial cells that surrounded the hepatic sinusoids (Fig. 1,2). Table 1 illustrates the considerable age differences in the hepatic duct and central vein capsule sizes and thicknesses; the adult age was greater than the other ages. The liver is separated into lobules with little connective tissue septa at thirty days of age. The liver also exhibited enhanced morphogenesis and hepatic cells' structural establishment.

Table 1: Measurement of Liver and Kidney at Different Ages, μm ($\bar{X} \pm \text{S.E}$)

Measure Age	Capsule Of Liver	Capsule Of Kidney	Proximal Tube	Distal Tube	Glomerulus	Central Vein	Hepatic Duct
1 Day	26.1+0.2 A	23.4+0.2 A	38.1+2.1 A	66.1+0.1 A	82.3+1.2 A	194.20.4 A	94.30.1 A
8 Day	32.41. 3a	31.51.4 A	44.2+1.3A	75.70.5 A	91.4+0.3 A	217.40.2 A	117.6+0.1 A
15 Day	34.70.4 A	37.5+0.6 A	50.4+2.6A	82.2+0.6 A	141.7+1.5A	262.6+0.2 A	122.50.2 A
30 Day	46.61.5 A	42.31.6 A	55.6+2.5A	87.5+0.3 A	148.4+1.3A	296.6+0.3 A	126.6+0.4 A
60 Day	51.51.2 A	48.41.1 A	63.5+2.8A	94.4+0.1 A	162.51.9 A	359.50.1 A	149.50.6 A

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

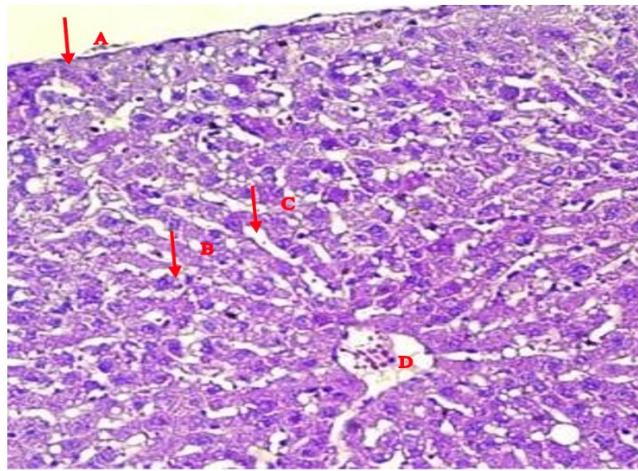


Figure 1: Cross section of Pup's Liver, At 1 Day Age; Capsule (A), Hepatic Cells (B), Sinusoids (C), Central Vein (D), PAS 200X.

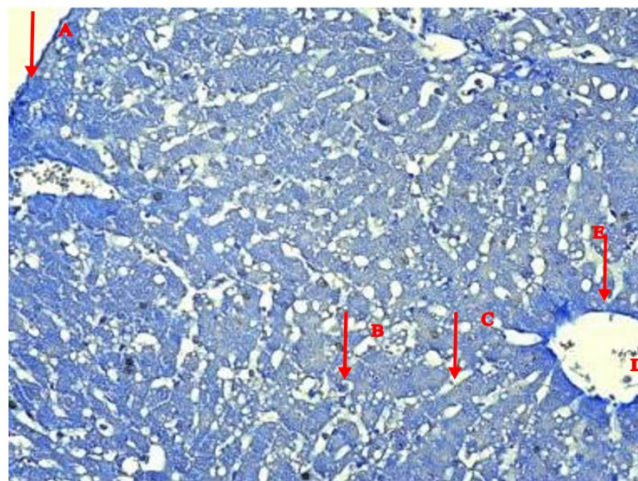


Figure 2: Cross section of Liver, At Pups 8-Day Age; Capsule (A), Hepatic Cells (B), Sinusoids (C), Central Vein (D), Endothelium (E), AB 200X.

Within the lobules, central veins are dispersed erratically. The polyhedral cells that made up hepatocytes had rounded nuclei. The hepatic artery, bile duct, and portal vein make up the portal triads, which are situated in the angles of the hepatic lobules (Fig. 3-7). The hepatocyte's cytoplasm is visibly vacuolized, and its nucleus and cytoplasm are homogeneous and may be either dark heterochromatic or light euchromatic. Because the hepatic arteriole and portal venule have smaller diameters than the liver, the adult liver has a broader portal triad than livers of other ages. This is consistent with observations from [25-27]. This may be due to elevated metabolic activity as well as hepatocyte functions, which are correlated with age-related variations in surface area and size that support the maintenance of body homeostasis. The liver's morphology is influenced by tension, stress, the atmosphere, and embryonic development, as evidenced by the way animals adapt to their surroundings, according to a series of research on the liver. The physiological role of Kupffer cells is defense, which is demonstrated by their consumption of foreign bodies and toxic substances that enter through the portal artery. A rabbit's kidney continued to grow after birth and began to demonstrate further morphogenesis around eight days of age. The perimeter of the renal cortex developed more quickly than the mi cortical and subcortical zones, indicating a variation in glomerular condensation from the cortical to the subcortical zones. In comparison to the juxtamedullary zones, the kidney's cortical and

subcortical regions had more renal corpuscles. Bowman's capsules and a distinct parietal squamous layer were visible in the corpuscles. The proximal and distal convoluted tubules had more development. At this stage of development, the medulla showed a collecting duct bordered with cuboidal epithelium, clear intercellular membranes, cytoplasm that was faintly stained, and centrally located nuclei (Fig. 8,9). It's comparable to [28]. As such, variations in tubule activity with respect to water quality were associated with this. Delicate secretions were also injected into the proximal tubules in order to enhance the lumen's surface area, augment fluid intake, and draw a greater number of components into the bloodstream. The proximal tubule cells also absorb nearly all of the vitamins, minerals, ions, and plasma proteins; additionally, they receive 65% of the filtered water within the corpuscle [29, 30]. At fifteen and thirty days of age, the kidney displayed enhanced morphogenesis and renal structural establishment. The parietal squamous layer and Bowman's capsules were evident. At this point, every nephron component has developed its unique histology. Proximal convoluted tubules have a well-developed brush border, and the Henle loops are well evident. Renal pyramids comprise the kidney lobe, and tubules, collecting ducts, and medullary rays were seen rising toward the subcortical and cortical zones. There was a little lumen seen in the proximal tubules. The renal papilla, which is bordered with low cuboidal epithelium in collecting tubules with cuboidal epithelium, is formed when the circular apex of the

medullary pyramid falls from the base (Fig. 10). This reminds me of [30]. A recorded developed renal parenchyma structure was found in the kidney of a sixty-day-old rabbit. The bulk of the stroma is made up of collagen fibers found in the interstitial tissue capsule. It was noted

that the parietal as well as the visceral layers of Bowman's capsule, that are divided from one another by the distinctive Bowman's gap surrounding the glomeruli, are what give renal corpuscles their typical form.

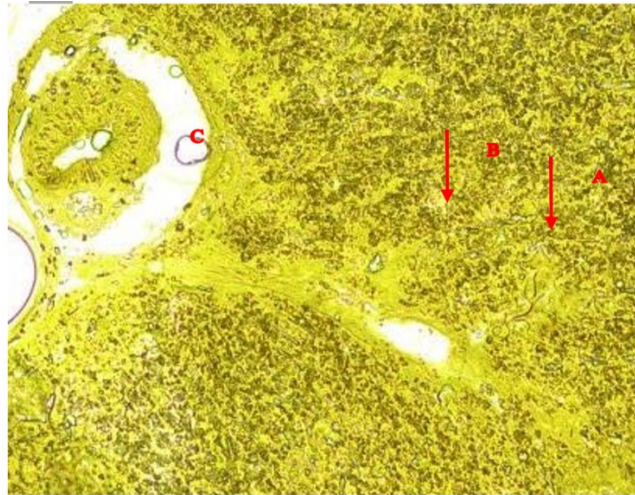


Figure 3: Cross section of Pup's Liver, At 15-Day Age; Hepatic Cells (A), Sinusoids (B), Central Vein (C), Verhoeffs Stain 2 00 X.

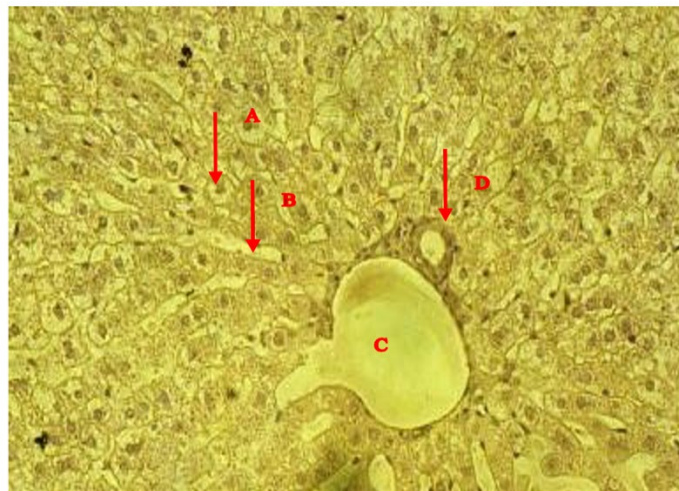


Figure 4: Cross section of Rabbit Liver, At 30-Day Age; Hepatic Cells (A), Sinusoids (B), Central Vein (C), Bile Duct (D), Verhoeffs Stain 2 00X.

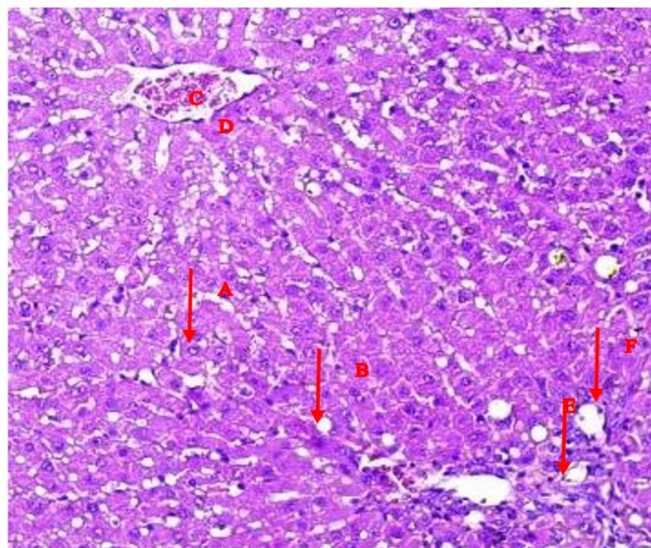


Figure 5: Cross Section of Pup's Liver, At 60-Day Age; Hepatic Cells (A), Sinusoids (B), Central Vein (C), Bile Duct (D), PAS -AB 200X.

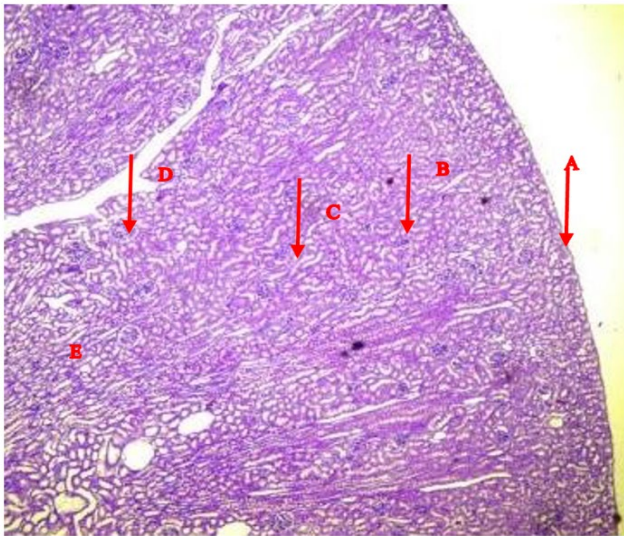


FIGURE 6: Cross Section of Rabbit Liver, at 60 Day Age; Hepatic Cells (A), Sinusoids (B), Central Vein (C), Bile Duct (D), Hepatic Artery (E), Portal Vein (F), Masson 200X.

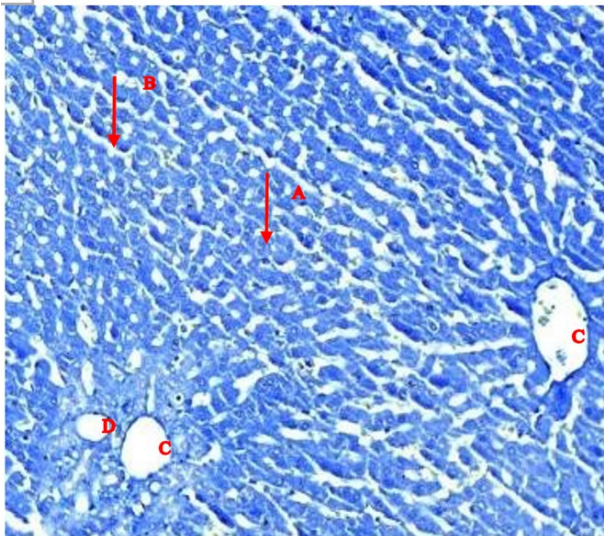


FIGURE 7: Cross section of Rabbit Liver, At 60-Day Age; Hepatic Cells (A), Sinoids (B), Central Vein (C), Endothelium (D), Bile Duct (E), Portal Vein (F), Masson 200X.

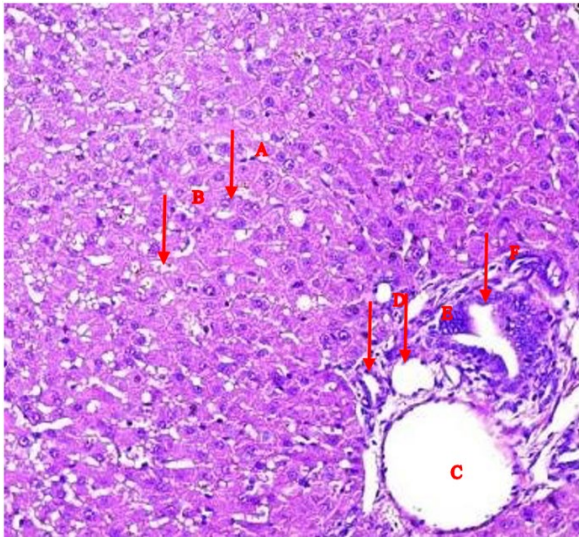


FIGURE 8: Cross Section of Pups Kidney, At 1 Day Age; Capsule (A), Proximal Tubule (B), Distal Tubule (C), Glomerulus (D), Medulla (E), H@E,100X.

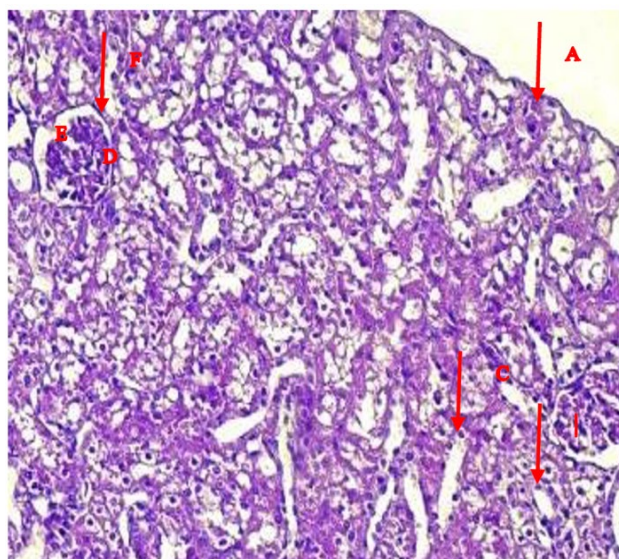


FIGURE 9: Cross section of Pups Kidney, At 1 Day Age; Capsule (A), Proximal Tubule (B), Distal Tubule (C), Glomerulus (D), Renal Space (E), Bowmann Capsule (F), PAS,100X.

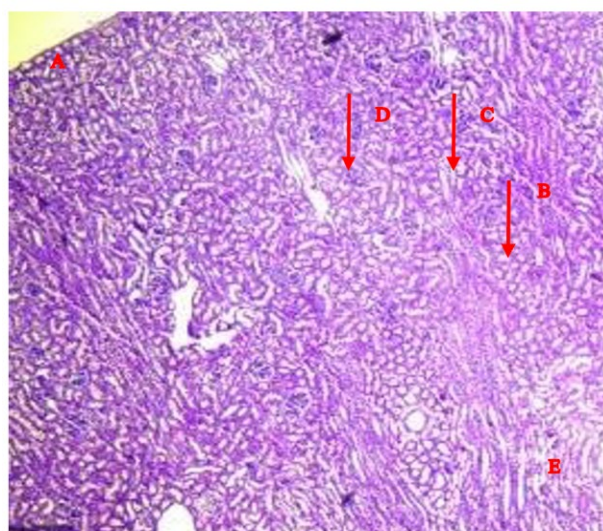


FIGURE 10: Cross section of Pups Kidney, At 8 Day Age; Capsule (A), Proximal Tubule (B), Distal Tubule (C), Glomerulus (D), Medulle (E), H@E,100X.

The lining cells of proximal tubules has a characteristic eosinophilic cytoplasm that signifies differentiation. the denser medulla was home to collecting tubules, ducts, and renal loop elongation. The adult kidney, which is responsible for filtering blood that circulates, was encased in a collagenous fibrous tissue capsule (Fig. 11), that was thicker than in other ages showing significant differences ($P>0.05$). The kidney also has a large blood flow. These warnings were similar [31, 32].

The current study used a connective tissue capsule to regulate the kidney's size and shape in relation to the animal's age. The glomerulus seems to be invaginated within Bowman's capsule and is positioned subcapsular at different developmental stages. The Bowman's gap's diameter varied (Fig. 11–14). This was verified by [33]. The cortex was divided into cortical labyrinths; its features included a urinary and vascular pole, complex tubules of the nephrons, medullary rays, parallel arrays of the tubules that entered the cortex and formed core of lobules, straight tubules, and collecting ducts, and more mid-cortical nephrons beside the Henle's loops than

juxtamedullary nephrons. Situated near the renal corpuscles, the proximal tubules are the finer components of the nephron. Surrounded by cuboidal epithelium, they receive ultrafiltrate from space. They have a confined spherical nucleus and brush borders on their luminal side. The glomerulus, which is surrounded by Bowman's capsule, is formed by the afferent arteriole penetrating the renal corpuscle, as per. (Fig. 11). This is consistent with the findings of other researchers, who found that higher metabolic activity and a larger surface area cause faster glycogen depletion. Histochemical analysis of the liver showed lower amounts of carbohydrates, glycogen, and activity of alkaline within the cytoplasm of rabbit liver, compared to weak positive responses for PAS in the cytoplasm of liver in other ages (Fig. 12–14).

Thus, the disparity might have resulted from the animal's diet or from environmental variables. The liver is thought to be a highly reliable sign of nutritional illness due to its function in the metabolism of materials in the digestive system. PAS stain revealed that the basement membranes of glomerulus, renal tubules, and brush border

of proximal tubules were all well-developed. The mature organization of the renal components was seen where the renal tubules surrounded renal corpuscles in renal cortex, parietal and visceral layers of the Bowman's capsule were seen to encircle the glomerular capillaries. Wide- and narrow-diameter renal tubules filled the gaps between renal corpuscles.

Distal tubules with a wide lumen were seen at vascular pole of renal corpuscles as a component of the juxtaglomerular apparatus (Fig. 11). This agrees with the findings of. This structure is essential to the function of the metanephric kidney because it generates an osmotic gradient between the cortex and medulla, which is what drives the extraction of water from the urine.

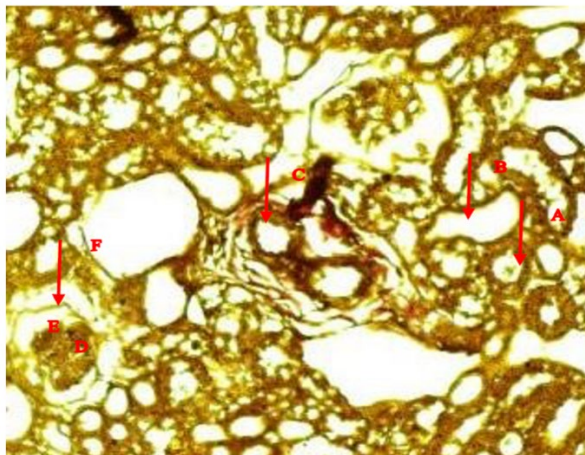


FIGURE 11: Cross section of Pups Kidney, at 15 Day Age; Proximal Tubule (A), Distal Tubule (B), Collecting Duct (C), Glomerulus (D), Renal Space (E), Bowman's Capsule (F), Verhoeff, 400X.

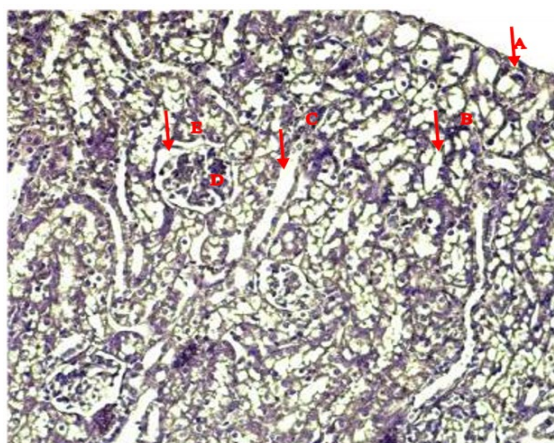


FIGURE 12: Cross Section of Rabbit Kidney, At 30-Day Age; Capsule (A), Proximal Tubule (B), Distal Tubule (C), Glomerulus (D), Bowman's Capsule (E), Mallory, 400X.

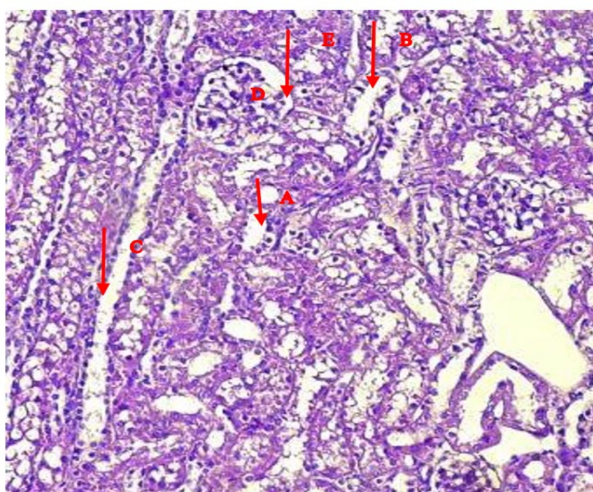


FIGURE 13: Cross section of Rabbit Kidney, At 30-Day Age; Proximal Tubule (A), Distal Tubule (B), Henle Loop (C), Glomerulus (D), Bowman's Capsule (E), PAS, 400 X.

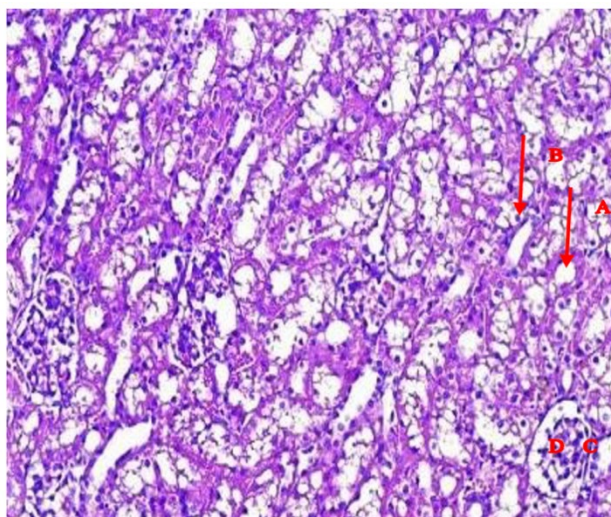


Figure 14: Cross section of Rabbit Kidney, At 60-Day Age; Proximal Tubule (A), Distal Tubule (B), Glomerulus (C), Bowmann Capsule (D), PAS,400X.

The release of the renin enzyme is initiated by chemical signals carried by the macula dense cells, which function as vasoconstrictor and are highly responsive to the fluid volume and ion concentration in the tube. In these procedures, the mesangial cells perform phagocytic tasks. A substantial difference in the ages, including serum urea, creatinine, sodium, and potassium. Additionally, they proposed that the body creates nitrogenous substances as a consequence of

different metabolic processes, and that there are notable variations in the quantity of urea in the blood. Adult rabbits had considerably higher serum creatinine levels ($P < 0.05$). Further studies have demonstrated that when there is a buildup of urea in the blood, there are certain situations in which the levels of creatinine and uric acid are high. Blood from young people had considerably lower levels of AST and ALT than blood from adults at a probability threshold of 0.05 (Table 2).

Table 2: The Biochemistry Parameters in Liver and Kidney at Different Ages; Mg-dl

Measure Age	ALT	AST	Uric Acid	Ammonia	Urea	Creatinine	Na	K	Mg ²⁺
1 Day	12.4±0.2 A	13.4±0.1A	2.41±0.3 A	46.1±0.2A	30.1±0.2 B	1.6±0.3a	119.4± 1.2 B	2.8±0.21 A	1.3±0.06 A
8 Day	14.6±0.4a	15.8±0.3A	3.19±0.2A	49.4±0.1 A	32.1±0.7 B	1.9±0.5 A	124.2± 1.4 B	3.4±0.07 A	1.9±0.05 A
15 Day	17.3±0.3 A	17.7±0.1A	3.49±0.4A	53.5±0.4 A	35.1±0.1 B	2.4±0.4A	136.3± 1.6 B	4.6±0.12 A	2.3±0.02 A
30 Day	19.3±0.8 A	20.4±0.8A	4.79±0.6A	57.4±0.1 A	39.1±0.5 B	3.1±0.3A	148.4± 1.2 B	6.2±0.03 A	2.5±0.04 A
60 Day	21.5±0.7 A	22.3±0.8A	5.11±0.6A	59.1±0.2 A	41.1±0.4 B	3.8±0.5A	152.7± 1.7 B	7.9±0.01 A	2.9±0.08 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 ($p < 0.05$).

Table (2) displayed the biochemical findings from blood samples obtained at various ages, which are frequently associated with the animal's adaptability and efficacy. Furthermore, compared to blood samples obtained at previous ages, it demonstrated a considerable rise in the number of enzymes in adult blood. Since the influence of tissues on internal organs is assessed using enzyme measurements. The kidneys are severely affected by the illness, with the liver and skeletal muscles following in severity. These findings were in line with reference [30], which showed that measuring the levels of enzymes in internal organs reveals the extent of tissue degradation. This increase could be attributed to enzymatic processes that aid in the removal of hazardous substances released during the digestion of meals high in chemicals. increased levels of urea in the blood serum. This relates to the animal's capacity to tolerate adversity and challenging surroundings. Because of its ability to hold onto water, it might also be involved in the body's water balance.

In addition to controlling osmotic pressure, blood proteins are essential for the creation of hormones, enzymes, and antibodies. In heel loop, the cells are actively absorbing water, urea is concentrated and released, among other secretory chemicals given by the blood and a few molecules released from tubule cells. The amount of nitrogen has an impact on the blood's urea percentage, and the variation in urea clearance is directly correlated with nitrogen levels. Ammonia, a dangerous molecule that must be eliminated from all tissues, is produced as a result of protein catabolism. The organism changes to

become urea. Urea can be found in most body fluids and tissues due to osmotic diffusion. Protein influences the blood urea content because it has big renal glomeruli. Regarding the relationship between the development of the renal pelvis, the volume of urine, and the effects of urea, the kidney is responsible for maintaining mg dioxide equilibrium. While magnesium dioxide is reabsorbed transcellularly in the distal tubule due to mela statin's temporary binding potential, it is reabsorbed paracellularly in the proximal tubule. Additionally, rabbit tubules show a higher percentage of Mg²⁺ reabsorption, allowing for more accurate control over Mg²⁺ excretion, but there are significant differences between species in this regard.

Conclusion

The current study indicates that at sixty days of age, the kidney of an adult rabbit exhibits the typical morphology.

Ethical Consideration

The current study complied with the ethical conduct guidelines that the University of Sawa in Iraq has established.

Acknowledgements

We sincerely thank our hardworking team for their invaluable contribution to the study. The histology department at Sawa University in Iraq deserves special recognition.

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