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Normal Histological Developments of The Liver of Newborn Rats For Days (1, 3, 5, 7, and 10)

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Abstract

THE LIVER is a vital organ in the body responsible for a variety of functions that support metabolism, immunity, digestion, toxin removal, and vitamin storage, among others. Therefore, the aim of this study was to determine the most important histological changes in liver cells of laboratory-bred white rats after birth for the days (1, 3, 5, 7, 10). Additionally, it aimed to assess the activity of liver cells during the same period using the MTT assay. This study was conducted at the animal facility of the College of Veterinary Medicine, Tikrit University, Iraq. Eight rats were used in the experiment, divided into five females for breeding purposes and three males for mating. Subsequently, tissue sections of selected organs (liver parts) were prepared using hematoxylin and eosin staining. The results of these sections showed clear histological developments and changes in those cells, along with their high activity during the developmental process, consistent with the extent of development occurring during that period.

Keywords: Rats, Liver, Histological changes and developments in newborns.

Introduction

The liver is considered one of the most important organs in the body, playing a vital role in maintaining the physiological balance of mammals. It is also responsible for many metabolic processes, including fat metabolism, detoxification, bile acid synthesis, and sugar breakdown[1]

The basic units of the liver are repeated anatomical units called liver lobules. Additionally, the presence of liver cells divided into parenchymal cells, constituting (60%) of liver cell composition and (80%) of liver mass, and non-parenchymal cells [2].

While non-parenchymal cells (NPCs), including cholangiocytes (biliary epithelial cells), liver endothelial cells (LECs), hepatic stellate cells (HSCs), Kupffer cells, and other immune cell populations, constitute 20% of the remaining liver mass [3, 4]

Previous studies have utilized various rodent models to investigate liver structures, functions, and composition, assuming similarities between human, rat, and mouse livers[5].

Rats can be considered one of the most suitable laboratory animals for research and studies in mammals due to several reasons. Firstly, their high fertility rate, frequent births, and short gestation period. Additionally, the similarity in tissue structures between humans and rats during embryonic development, especially in the early stages [6].

Aim of the study: The aim of this study was to demonstrate the histological developments of liver tissues as well as showing the activity of cells during that period.

Material and Methods

In this study, laboratory albino rats of the (*Norvegicus rattus*) species were used. These rats were obtained from the animal house at the College of Veterinary Medicine, University of Tikrit, the same location where the experiment took place. They were healthy and untreated with any substances prior to the study. The study was conducted from the ninth month (September) to the end of the twelfth month (December) of the year 2023.

The total number of animals used in this study was eight (8 animals), distributed among five (5

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females) and three (3 males) for mating purposes only. Initially, suitable conditions were provided for them, and special plastic cages with dimensions of (50×30×15) cm were prepared. The room temperature was maintained at (25) degrees Celsius, with moderate lighting provided for 12 hours and darkness for the remaining 12 hours to facilitate mating **[**7**].**

Water and food were available throughout the study period. The appropriate feed was provided, consisting of wheat (34%), barley (20%), corn (25%), dry protein (10%), powdered milk (10%), table salt (1%), and vitamins and minerals (1 g) per kilogram. This feed was ground, mixed with a little water and oil to form a cohesive dough, then shaped into small pieces and placed in the appropriate location **[**8**]**.

In order to ensure that all animals were not pregnant, they were isolated for a period of twenty-five (25) days. Afterward, two females were placed with one male overnight for mating, with observation for the appearance of the vaginal plug to confirm mating and determine the day of conception (Gestation zero, G0). The following day was considered the first day of pregnancy **[**9**]**.

The females were distributed over five days after birth, including days (1, 3, 5, 7, 10 day). Histological sections were taken and the cell activity test (MTT assay) was conducted on female offspring for those days.

The care process for the female rats continued until the births occurred, starting from the twenty-second (22^{nd}) day of pregnancy and lasting until the twenty-fifth (25^{th}) day. Afterward, the newborns were taken and anesthetized using chloroform. They were then placed on a dissecting table, where an incision was made in the abdominal cavity to extract the fetal livers for conducting tests on them**[**10**]**.

The tissue sections were prepared according to the protocol established by the world **[**11**].** Initially, the required samples (portions of the liver) were fixed in 10% neutral buffered formalin for approximately 24 hours at room temperature. Subsequently, the samples were washed with running tap water to remove the formalin. Then, they underwent a dehydration process using sequential ethyl alcohol gradients starting from (50%, 60%, 70%, 80%, 90%, to 100%) for two hours each concentration. Next, the samples were filtered twice using xylene for half an hour each time. Afterwards, the tissue samples were embedded in paraffin wax at a temperature of (56 - 58 degrees Celsius) for two hours, repeated twice for each step. Then, they entered the embedding phase where a thin layer of Mayer's egg albumen solution was applied to fix the section strip onto glass slides. The tissue slides were dried using a hot plate at a temperature of (40 degrees Celsius) and left for 24 hours. Subsequently, they were passed through a descending series of ethyl alcohol, starting with (100%, 90%, 80%, 70%, 60%, and finally 50%).

As for the slide staining process, only hematoxylin and eosin stains were used. They were fixed by adding a quantity of (DPX), followed by placing a cover slip on the section strip mounted on glass slides and covering it.

After completing the tissue sectioning stages, and the tissue slides became ready, they were examined using a compound light microscope of the (Japan, Olympus) type. Imaging was conducted using a German-made imaging system of the (Kruss) type, equipped with a digital camera of the (4DCE-50B) model. Additionally, a regular camera was used to capture some external visible changes in young rats at an advanced stage of age.

Examination of liver cell activity using the MTT assay

This examination relied on the use of the tetrazolium dye (4,5-diphenyltetrazolium bromide) (MTT assay). Young rats were used after birth for the days (1, 3, 5, 7, 10 day). The steps were as follows, according to the source[12].

1- Preparation of the MTT dye by adding the stocked dye to neutral phosphate-buffered saline solution in a ratio of (1:9 %) (adding 10 microliters with 90 microliters of neutral solution).

2- Preparation of dimethyl sulfoxide (DMSO) with the chemical formula (C2H6OS).

3- Preparation of sodium dodecyl sulfate (SDS) with the chemical formula (NaC12H25SO4).

4- Preparation of the plate for the ELISA apparatus, then placing the prepared MTT dye inside the well with a volume of (100 microliters), followed by placing the liver tissue taken from the young rats directly on top of the dye.

5 - Placing the plate containing the dye with the organ inside the incubator at a temperature of (37 °C) for one hour.

6 - Then, removing the plate from the incubator and adding (DMSO + SDS) solution with a volume of (100 microliters) and leaving it for ten minutes. After that, removing the organ from the well and placing it in the ELISA apparatus to read the results, with the reading being at a wavelength of (690 nm).

Results

After the births and the tissue sections were performed, the results of this study for days (1, 3, 5, 7, 10 day) postpartum showed that the liver was uniformly shaped, smooth, reddish-brown in color with clear lobules, located in the upper part of the abdomen. On the first day (Day 1), the results for the young rats were normal, without any congenital abnormalities. The total weight of the first pup was (5.5 grams), with a length of (6.7 cm), while the second pup weighed (5.5 grams) with a length of (6 cm). Additionally, the tissue sections for the first day showed hepatocytes with regular polygonal shapes, spherical nuclei, with some cells having more than one nucleus. The sinusoids contained numerous Kupffer cells, and many sinusoidal areas were filled with disintegrated blood, continuous with the central vein at their ends (Figs. 2 , 3).

On the third day (Day 3) of rat life, the observable results for the young rats were normal. The first pup weighed (8 grams) with a length of (6 cm), while the second pup weighed (6.5 grams) with a length of (6) cm. The tissue sections showed the fetal liver parenchyma containing clustered hepatocytes, some with spherical nuclei, some darkly stained, and others with faintly stained nuclei, along with the presence of binucleated cells. The sinusoids and channels contained some Kupffer cells, as observed in (Fig. 4).

On the fifth day (Day 5) postpartum, the results showed that the young rats were normal, with a total weight of (11.7 grams) and a length of (8 cm). The second pup weighed (11 grams) with a length of (7.5 cm). The tissue sections revealed the central vein surrounded by an expanded sinusoidal network, lined with multiple polygonal hepatocytes, some enlarged and surrounded by a network of blood sinusoids with excessive Kupffer cells. Additionally, the portal area showed portal vein branches filled with blood and surrounded by leukocytes, and around the bile duct branches, the sinusoidal channels contained enlarged Kupffer cells and hepatocytes found in stacked clusters, some with faintly stained nuclei and acidic cytoplasm, as seen in (Figs. 5 , 6).

On the seventh day (Day 7) postpartum, the results showed that the total weight of the first pup was (12.5 grams) with a length of (6 cm), while the second pup weighed (11 grams) with a length of (7 cm). The liver tissue on this day contained clusters of interwoven hepatocytes, appearing polygonal with faintly stained spherical nuclei surrounded by acidic cytoplasm with some small gaps. The vascular network of the sinusoidal channels was wide with numerous darkly stained Kupffer cells, continuous with the central vein, as shown in (Fig. 7, 8).

The results of the tenth (Day 10) postpartum showed the newborn in its normal form, with the weight of the first newborn being (13 grams) and a length of (8.5 centimeters). As for the second newborn, its weight was (12 grams) with a length of (8 centimeters). The liver tissue exhibited regularity in hepatocytes, which were polygonal with spherical nuclei surrounded by acidophilic cytoplasm. The liver cells around the central vein appeared like honeycomb cells, surrounded by a widespread network of sinusoids containing Kupffer cells. The central vein was free from blood, surrounded, and continuous with the sinusoids at its ends, as depicted in (Fig. 9). Additionally, the portal area of the rat liver on this day contained a wide-open portal vein filled with blood and some white blood cells inside its lumen, along with branches of the bile duct lined with cuboidal cells. Moreover, the portal area contained a large influx of white blood cells surrounded from the outside by widely dispersed and stacked hepatocytes, as observed in (Fig. 10).

As for the MTT assay cell activity test for days (1-3-5-7-10 day) after birth, there was an increase in the level of hepatic cell activity for those days, indicating the level of development occurring within the liver. The results were as follows, as illustrated in (Table 2) and (Fig 1).

Discussion

The results on the first (day1) after birth showed in the histological sections of the liver cells, polygonal cells with regular borders. The cytoplasm had spherical nuclei, each containing more than one nucleus, and some cells had binucleation as shown in (fig 2 and 3). As for the histological sections on the third, fifth, and seventh (days 3, 5, 7) after birth, the liver tissue appeared to contain stacked hepatic cells, some with spherical nuclei, some with dark-stained nuclei, and others with faintly stained nuclei. The cytoplasm retained binucleation in some cells, as depicted in (figures 4, 5, 6, 7, 8). By the tenth day (Day10) after birth, hepatic cells became more

uniform, with polygonal nuclei surrounded by acidophilic cytoplasm. Hepatic cells around the central vein appeared as honeycomb-like structures, as shown in (figure 9). This finding is consistent with researchers Li el al [13] who demonstrated that hepatic cells organize within liver lobules, forming a structural pattern within the liver. Additionally, this study aligned with researchers Bofarda and Anware [14],[15] regarding the developmental changes in cells, such as multinucleation, explaining the increased mitotic activity, continuing until the fifteenth day, where no cell division was observed at three months of age.

Regarding the hepatic sinusoids, they contained numerous Kupffer cells, and many sinusoidal areas were filled with continuously decomposed blood, connecting to the central vein at its ends, starting from the first day after birth . This expansion in sinusoids continued until they formed pockets and channels by the tenth day after birth (10), remaining continuous with the central vein and becoming more organized and stable. This study observed that hepatic sinusoids expanded widely from the first day after birth to form continuous pockets with the central vein. This observation was in agreement with researchers McCuskey et al [16]، who confirmed that one of the most noticeable morphological developments during the lactation period is the extensively vacuolated sinusoidal network. With age progression after birth, the hepatic sinusoids remained closely associated with the central vein, becoming more expanded and straighter to accommodate the increasing blood volume. Additionally, an increase in Kupffer cell activity was observed after birth and until the fourth or fifth days in rats and mice. The sinusoidal and peri-sinusoidal structures were almost fully formed, despite minor morphological differences from those in adult livers.

As for the Kupffer cells, an increase in the activity of these phagocytic cells was immediately observed after birth, leading to an increase in their numbers within the sinusoids from the first day after birth until the tenth day after birth. This study noted a significant increase in the number of Kupffer cells within the sinusoids, consistent with researchers Liang et al [17], who demonstrated an increase in Kupffer cell numbers due to a hybrid appearance of phagocytic and parenchymal cells.

Regarding blood during this study, it was decomposed immediately after birth inside the cavities, becoming more abundant, and the proportion of blood formation inside the liver decreased with age. This study agreed with researchers Al-bayaa and Sasaki et al [18],[19]، who demonstrated the formation of separate foci consisting of small dark-stained cells distributed between the hepatic cell cords, confirming that blood-forming cells remain in the liver for up to two weeks after birth. Additionally, this study aligned with researcher Bofarda [14]، who confirmed that after birth, the blood-forming cells appeared clearly as scattered clusters throughout the livers of newborn rats, being more concentrated around the foci within the liver and around the pseudo-sinusoidal spaces, characterized by their dark staining.

Regarding the central vein during this study, it appeared wide and filled with decomposed blood immediately after birth, continuing to expand and remain continuous with the hepatic sinusoids, filled with blood, until the tenth day after birth, becoming lined with honeycomb-like cells. This study differed from researchers Ody et al [20] who demonstrated that the portal vein is undeveloped after birth, being lined with undifferentiated cells, and after three days, these cells begin to differentiate and accumulate around the cavity, forming two separate layers of perpendicular smooth muscle cells.

During this study, the results of the cell activity test showed an increase in cell activity levels, indicating mitochondrial activity after birth. This finding concurred with researcher David [21], who found an increase in mitochondrial size during the development of hepatic cells after birth from the first day to the fourteenth day, decreasing around the sixth month of age in rats. Additionally, the number of mitochondria also increased after birth.

Conclusion

-There is a clear transition in the developmental process of liver tissues in newborn rats immediately after birth.

-There is an increase in the levels of cell activity during the development of the liver after birth.

Recommendations

- Using specific dyes to identify the development of fibers in the liver.

- Studying blood levels during the early postnatal period.

Acknowledgment

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Conflict of Interest

No conflict of interest

Funding statement

This research was special exertion

Ethical consideration

This study has been approved by the animal rights and ethical of Tikrit University

TABLE 1. Fetal measurements for days (1 - 3 - 5 - 7 - 10) after birth

day	Avareg E	Death	Activity %	Activity %
1	4200	0	100	0
3	4301	-2.40476	97.5952381	2.404761905
5	4436	-5.61905	94.38095238	5.619047619
7	4533	-7.92857	92.07142857	7.928571429
10	4878	-16.1429	83.85714286	16.14285714
dead			98	2

TABLE 2. Shows the readings for cell activity (MTT assay)

s	Day	Rat No.1		Rat No.2	
		Weight (g)	Length (cm)	Weight (g)	Length (cm)
1	First day after birth	5.5	6.7	5.5	6
2	Third day after birth	8	6	6.5	6
3	Fifth day after birth	11.7	8	11	7.5
4	Seventh day after birth	12.5	6	11	7
5	Tenth day after birth	13	8.5	12	8

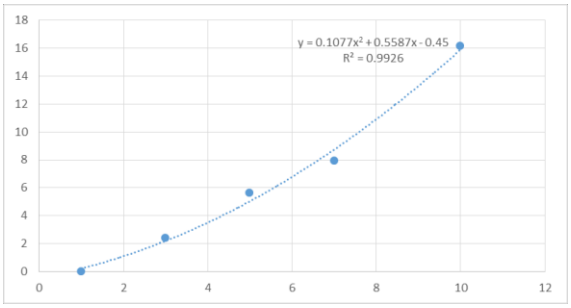

Fig. 1. A diagram showing the activity of hepatocytes with the MTT assay.

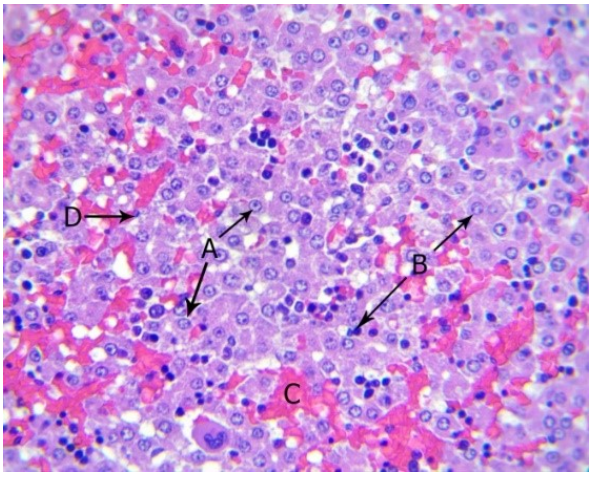

Fig. 2. depicts the liver tissue of a rat on the first day after birth. It illustrates: (A) Liver tissue - polygonal hepatic cells, (B) Hepatic cells with binucleation, (C) Blood-filled sinusoids, (D) Large-sized Kupffer cells. The magnification is (40x E & H).

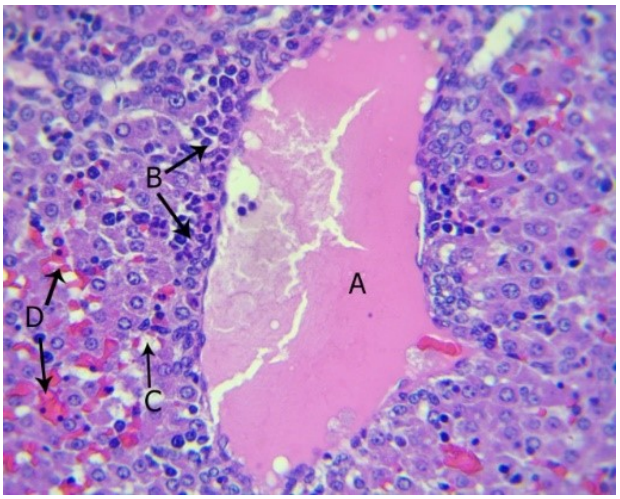

Fig. 3. illustrates the liver tissue of a rat on the first day after birth. It shows: (A) Central vein, (B) White blood cells, (C) Blood sinusoids with Kupffer cells, (D) Blood from the blood sinusoids. The magnification is (E & H x40).

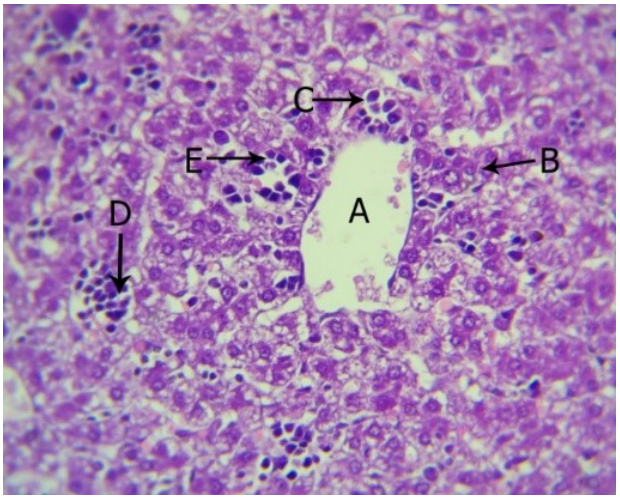

Fig. 4. depicts the liver tissue of a rat at three days after birth (Day 3 post). It illustrates: (A) The central vein, (B) White blood cells, (C) Blood sinusoids with Kupffer cells, (D) Blood from the blood sinusoids. The magnification is (E & H x40).

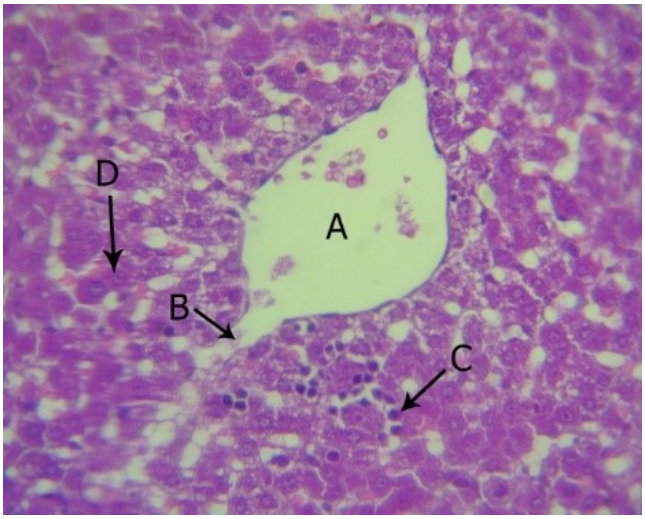

Fig. 5. Liver tissue of a rat at five days after birth (Day 5 post) illustrates: (A) Liver tissue - central vein, (B) Blood sinusoids, (C) Kupffer cells, (D) Hepatocytes. The magnification is 40x (E & H x40).

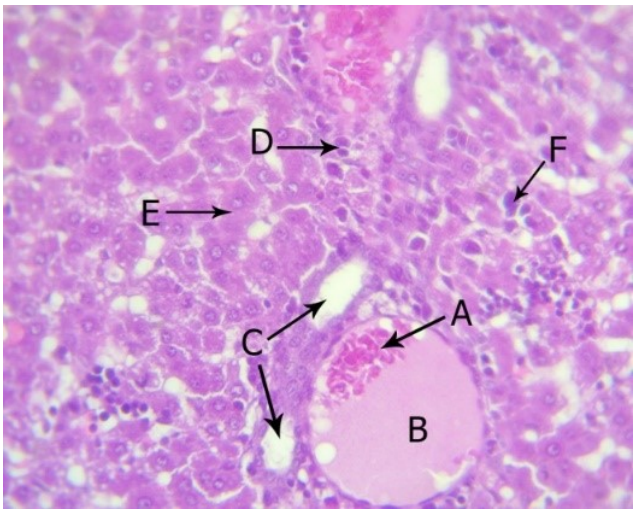

Fig. 6. Liver tissue of a rat at five days after birth (Day 5 post) illustrates: (A) Portal area of the liver - portal vein, (B) Degenerated blood, (C) Branches of bile ducts, (D) Kupffer cells, (E) Clusters of hepatocytes, (F) Enlarged Kupffer cells. The magnification is (E & H x40).

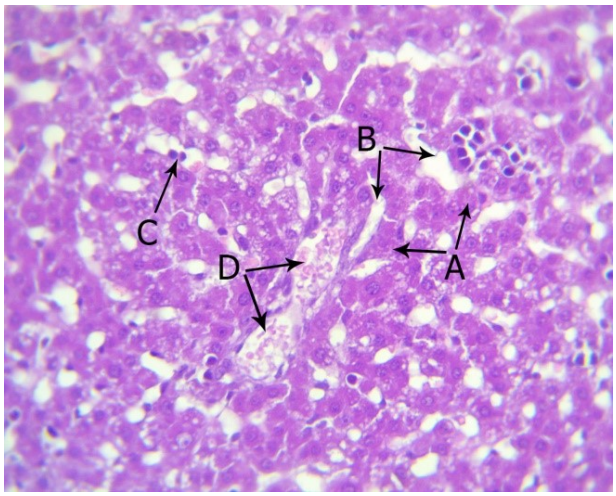

Fig. 7. Liver tissue of a rat at seven days after birth (Day 7 post) illustrates: (A) Rows of hepatocytes, (B) Network of vascular channels for the hepatic sinusoids, (C) Kupffer cells, (D) Central vein. The magnification is (E & H x40).

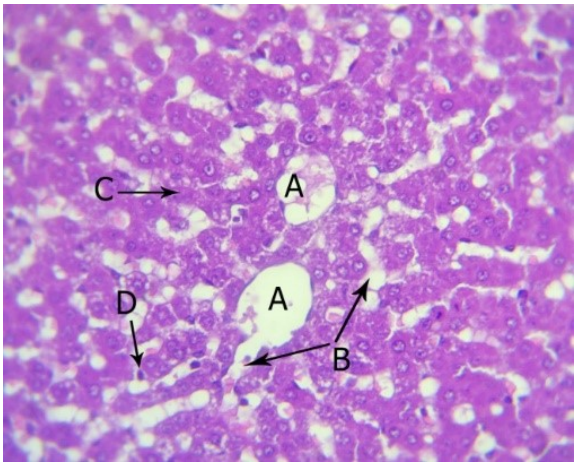

Fig. 8. Liver tissue of a rat at seven days after birth (Day 7 post) illustrates: (A) Central vein, (B) Hepatic sinusoids, (C) Rows of hepatocytes. The magnification is (E & H x40).

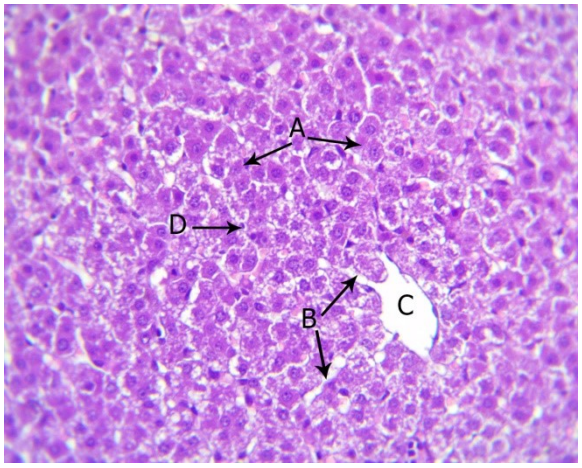

Fig. 9. Liver tissue of a rat at ten days after birth (Day 10 post) illustrates: (A) Polygonal hepatocytes, (B) Hepatic sinusoidal network, (C) Central vein, (D) Kupffer cells. The magnification is (E & H x40).

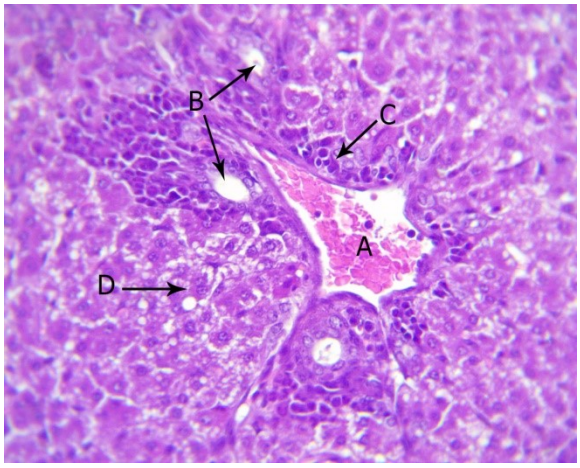

Fig. 10. Liver tissue of a rat at ten days after birth (Day 10 post) illustrates: (A) Portal area of the liver - portal vein, (B) Branches of bile ducts, (C) Infiltration of white blood cells, (D) Stacking of liver cells. The magnification is (E & H x40).

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التطورات النسيجية الطبيعية لكبد الجرذان حديثي الولادة للأيام (1، 3، 5، 7، و 10)

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الكبد بشكل عام عضو مهم في الجسم وهو مسؤول عن مجموعة من الوظائف التي تساعد في دعم عملية التمثيل الغذائي والمناعة والهضم وإزالة السموم وتخزين الفيتامينات من بين الوظائف الأخرى لذا هدفت هذه الدراسة الى معرفة اهم التغيرات النسيجية الحاصة في الخلايا الكبدية لأكباد الجرذان البيض المختبرية بعد الولادة للأيام (1،3،5،7،10) بالإضافة الى ذلك معرفة نشاط الخلايا الكبدية في نفس الفترة باستخدام اختبار (MTT assay) حيث اجريت هذه الدراسة في البيت الحيواني لكلية الطب البيطري لجامعة تكريت في العراق وقد استخدمت في هذا التجربة جرذان وكان العدد الكلي ثمانية (8) حيوانات مقسومة على خمسة (5) اناث للاستفادة من ولاداتها وثلاثة (3) ذكور لأغراض التزاوج بعد ذلك تم عمل المقاطع النسيجية للأعضاء المنتخبة (اجزاء الكبد) باستخدام صبغة الهيماتوكسلين وصبغة الايوسين حيث اظهرت نتائج تلك المقاطع ان هناك تطورات وتغيرات نسيجية واضحة لتلك الخلايا بالإضافة الى نشاطها العالي اثناء عملية التطور مما يتلاءم مع حجم التطور الحاصل في تلك الفترة.

الكلمات الدالة : الفئران، الكبد، التغيرات والتطورات النسيجية عند الأطفال حديثي الولادة