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PRENATAL DEVELOPMENT OF THE LIVER IN THE RABBIT

(With 1 Table and 17 Figures)

By

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التطور الجنينى لكبد الأرنب

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أجرى هذا البحث على كبد ٤٠ من أجنة الأرانب البلدى التى تتراوح أطوالها من ١٥ - ١٣٠مم وذلك للتعرف على التغيرات المورفولوجية التى تحدث بها . وقد وجد أن الكبد يكون ضخم جدا فى المرحلة الجنينية الأولى ويشغل معظم التجويف البطنى وبتقدم العمر فإنه ينمو بمعدل أقل من الجسم ولذا فإنه يشغل الجزء الأمامى من التجويف البطنى فى نهاية فترة الحمل . فى الأجنة التى يبلغ طولها ١٥ - ٣٠ مم يتكون الكبد من فصين أيمن وأيسر حيث ينقسم الأخير الى فص أنسى وآخر وحشى . يظهر البروز المذيل والحلمى ويمكن تمييز أربطة الكبد عند طول ٤٠ - ٧٥ مم، بينما يمكن تمييز جميع فصوص الكبد وذلك بظهور الفص الرباعى عند طول ٩٠ -١١٠مم. ولقد أوضحت الدراسة أن الكبد فى المرحلة الجنينية الأولى يتكون من نسبة قليلة من خلايا الكبد التى تحتوى على فجوات صغيرة وعلى كمية كبيرة من الخلايا المسئولة عن تكوين الدم . وبتقدم العمر تزداد الخلايا الكبدية فى الحجم ويزداد حجم الفجوات الواقعة بداخلها والتى أثبتت الدراسة أنها ممتلئة بحبيبات دهنية ، وعلى العكس فأن الخلايا المسئولة عن تكوين الدم تقل بتقدم العمر . تظهر الجيوب الدموية فى المرحلة الأولى على هيئة فراغات صغيرة تزداد فى العدد والحجم فى إتجاه نهاية الحمل وتصبح ممتلئة بخلايا دموية ناضجة . ولقد تبين من البحث أن قطر أنوية الخلايا الكبدية يزداد بتقدم العمر . أوضحت الدراسة أيضا أن الحويصلة المرارية تظهر فى أجنة الأرانب التى يتراوح طولها ٤٠ - ٧٥مم .

SUMMARY

In the present work, the prenatal morphological changes of the liver in the balady rabbits were studied. In the rabbit fetuses of 15-30 mm CVRL, the liver consists of right and left lobes separated by umbilical

fissure. The left lobe is divided into medial and lateral lobes. In the rabbit fetuses of 40-75 mm CVRL, the caudate and papillary processes are appeared and the hepatic ligaments are identified. The quadrate lobe was observed in the fetuses of 90-110 mm CVRL. In the early embryonic stage, the outlines of the hepatic lobules can be distinguished. The hepatocytes have different shapes, their cytoplasm shows small vacuoles. During further development, the hepatocytes increase in size, their cytoplasm becomes greatly vacuolated. These vacuoles are filled with fat droplets. The hemopoietic tissue is predominant in the early embryonic stage. It contains blood cellular elements of various developmental stages. With the advancement of age, this tissue decreases in amount, the intravascular non-nucleated blood cells increase on the expense of the nucleated ones. The blood sinusoids appear in the early developmental stage as narrow blood spaces which increase in number and size towards the term of gestation. The nuclear diameter of the hepatic cells is increased with the advancement of the fetal age. The gall bladder is demonstrated in the rabbit fetuses of 40-75 mm CVRL.

Key words: *Prenatal development, liver, rabbit*

INTRODUCTION

The morphological studies indicated the importance of the mammalian liver as a main site for erythropoesis. The growth of the liver after birth differs distinctly from its embryonic growth (Mckellar, 1949). The development of the liver had been investigated by many authors (Deane, 1944; McCuskey, 1968; Patten, 1968; Severn, 1972; Snell, 1975; El-Morsy et al., 1979; Fouad et al., 1984; Anwar et al., 1989; Sadler, 1990; Godlewski et al., 1992 and Moustafa and Ahmed, 1995).

The available literatures on the fetal liver of rabbit especially those of the balady species are lacking. Therefore, the present work is carried out to give more informations on the morphological changes that occur in the rabbit liver during the prenatal development.

MATERIAL and METHODS

The present work was carried out on 40 normal rabbit fetuses of both sexes ranging from 15-130 mm CVRL. The fetuses were divided into four groups; the first group was ranged from 15-30 mm CVRL, the second from 40-75 mm CVRL, the third from 90-110 mm CVRL and the fourth group from 115-130 mm CVRL. Each group included ten specimens. The fetuses were obtained from pregnant rabbits (balady species), which were anesthetized with chloroform and were injected with fixative (10% neutral buffered formaline and Bouin's fluid) through the common carotid artery. After evisceration, the entire small fetuses (10-40 mm CVRL) and parts of the liver of the other fetuses were taken and immersed in fixative. Then the specimens were dehydrated, cleared and embedded in paraffin wax. Sections of 5-7 µm were taken and stained with Haematoxylin and Eosin, Periodic acid Schiff, Alcian blue and Weigert's elastic stain (Drury and Wallington, 1980).

For histochemical studies, fresh samples were taken and immersed in liquid nitrogen, then transfered to cryostat chamber. Serial sections of 10 µm were taken and stained with Suddan black B.

For semithin sectioning, small pieces of the fetal liver were fixed in formaldehyde-glutaraldehyde fixative (Karnovsky, 1965). Then the materials were osmicated in 1% osmium tetraoxide, washed in 0.1 M cacodylate buffer at pH 7.3, dehydrated in ethanol followed by propylene oxide and embedded in araldite. Semithin sections were taken and stained with Toluidine blue.

The nuclear diameter of the hepatic cells of the fetuses of each group was measured using Quantiment Q500 MC image processing and analysis system (Leica).

RESULTS

In the rabbit fetuses of 15-30 mm CVRL, the developing liver is very large in size occupying most of the abdominal cavity extending from the diaphragm cranially to the pelvic inlet caudally (Fig.1). At this stage the liver consists of right and left lobes, each occupies the corresponding half of the abdominal cavity. The left lobe is divided into left medial and left lateral lobes. The right and left lobes are

separated by longitudinal umbilical fissuure which corresponds to the median plane.

The parietal surface of the liver is convex and related to the ventral as well as to the lateral abdominal walls, and is separated cranially from the large-sized heart by the Septum transversum. The visceral surface is concave and is related to the developing stomach and Kidneys (Fig.1).

During this developmental period, the light microscopical examination shows that the liver is composed of hepatic parenchyma and hemopoietic tissue. The outlines of the hepatic lobules can be distinguished (Fig.2). The hepatocytes are variable in size, they are distributed in irregular manner. These cells show different shapes; elongated, polyhedral, triangular and irregular with basophilic cytoplasm containing few small vacuoles. Their nuclei are large, vesicular, rounded or oval in shape (Fig.3,4). They measure 4.61 µm in diameter (Table 1 & Fig.17). The liver parenchyma is surrounded by a single layer of flattened cells. PAS positive material was observed within the cytoplasm of the hepatic cells at this stage.

The hemopoietic tissue is relatively abundant, it is intermingled with the hepatic cells. Most of the hemopoietic cells appear to be arranged in nests (Fig.2). They are rounded in shape with large deeply stained and centrally located nuclei (Fig.4). Different developmental stages of immature and mature blood cellular elements are also present. The extravascular nucleated erthroid cells are predominant, while the non-nucleated red blood cells are infrequently demonstrated. Megakaryocytes can be demonstrated at this stage.

The developing central veins (Fig.2,3) are represented by rounded or elongated wide blood spaces lined by thin layer of the flattened cells; the primitive endothelium. They are free from blood cellular elements. In addition, small narrow elongated blood spaces; primitive blood sinusoids, are demonstrated at this stage. Some of these spaces are continuous with the developing central veins.

In the rabbit fetuses of 40-75 mm CVRL, the rate of the development of the liver is relatively slower than that of the body compared with the previous stage. Therefore, in the fetuses of 40 mm CVRL the liver occupies large part of the abdominal cavity (Fig. 5). Then its caudal termination moves forwards to end about 0.7 cm behind the umbilicus. The right and left lobes are separated by deep umbilical fissure which lodges part of the intestine The caudate

During this stage of the intrauterine life, the microscopical observations indicate that the hepatocytes become larger with large rounded vesicular nuclei which measure 5.52 μm in diameter. Their cytoplasm is more vacuolated than those observed in the previous stages (Fig.10). Frozen sections stained with Suddan black show considerable amount of fat droplets located within the cytoplasm of the hepatic cells (Fig.11,12).

The hepatic sinusoids become larger and some of them are engorged with non-nucleated blood cells (Fig.10). Intravascular nucleated blood cells are disappeared. The liver of the rabbit contains small amount of the hemopoietic tissue, so some extravascular nucleated blood cells are demonstrated at this period (Fig.10). The hemopoietic cells have relatively small rounded nuclei.

In the rabbit fetuses of 115-130 mm CVRL (last stage of gestation), the rate of the development of the liver is markedly slower than that of the body as a whole compared with the previous stages. Therefore, it occupies the cranial portion of the abdominal cavity. The liver situates in the intrathoracic part of the abdominal cavity, where its strongly convex parietal surface lies against the diaphragm and almost covers it. It extends caudally to terminate about 0.6 cm cranial to the umbilicus. All the hepatic lobes except the right lobe and the caudate process are located to the left of the median plane.

In the full term fetuses, the liver of the rabbit acquires the shape and relations of the adult animal (Fig.13,14). The hepatic ligaments are clearly demonstrated but still have the membranous character. The gall bladder increases relatively in size and has the form of small elongated sac.

At this developmental stage, semithin sections stained with Toluidine blue show that the cytoplasm of the hepatic cells contains large vacuoles of different sizes (Fig.15). The nuclei of these cells are rounded and vesicular. They have different locations and larger diameter compared with the previous stages (Table 1 & Fig.17). The hepatic cells are still arranged in irregular manner and do not form hepatic cords.

The fat deposition in the cytoplasm of the hepatic cells reaches its highest level than those observed in the preceding stages.

During this period of the intrauterine life, the rabbit liver becomes highly vascularized, thus the liver parenchyma are invested with numerous large blood sinusoids. Most of these sinusoids are

process is represented by small quadrilateral outgrowth develops on the dorsolateral part of the right lobe. The papillary process appears as small structure on the visceral surface of the left lobe. The visceral surface of the liver is related to the stomach, intestine, right and left kidneys (Fig.6).

During this developmental stage, the falciform, coronary, triangular and hepatorenal ligaments as well as the lesser omentum can be distinguished as a thin membranes.

At this stage of intrauterine life, the fetal rabbit liver is more organized, so the outlines of the hepatic lobules are more distinguished. The hepatocytes are increased in number and size than those observed in the preceding stage. Their nuclei contain large distinct nucleolei, and their cytoplasm becomes less basophilic and more vacuolated (Fig.7,8).

The hemopoietic tissue is relatively decreased in amount than that of the previous stage. Extravascular non-nucleated blood cells are demonstrated at this stage. Most of the blood cellular elements within the blood sinusoids are non-nucleated (Fig.7,8). Megakaryocytes are frequently demonstrated outside the blood sinusoids and near the hemopoietic nests. They have multilobed or polymorphous nuclei (Fig.8).

The blood spaces increase in number and size. The lining epithelium of the blood sinusoids is well demonstrated and is represented by flattened endothelial cells with distinct flattened nuclei and lightly stained cytoplasm (Fig.8). The central veins increase in number and decrease in size. During this period of intrauterine life, the gall bladder is demonstrated (Fig.9).

In the rabbit fetuses of 90-110 mm CVRL, the liver terminates caudally directly behined the umbilicus. All the hepatic lobes can be easily identified (Fig.13). The quadrate lobe appears on the medial side of the right lobe, it lies between the gall bladder on the right side and the umbilical fissure on the left side. The gall bladder has the form of small structure embedded on the visceral surface of the liver. The left lateral lobe is larger than the medial one.

The parietal surface of the liver faces cranioventrally and its relations similar to those of the preceding stages. The visceral surface faces caudodorsally and is related to the stomach, intestine and right kidney. The hepatic ligaments are easily identified at this stage.

completely filled with non-nucleated blood cells (Fig.15). The central veins are greatly increased in number but decreased in size. The megakaryocytes become more prominent (Fig.16). The fetal rabbit liver is characterized at this developmental period by reduction in the number of the hemopoietic cells (Fig.15).

Table (1): Mean values of the nuclear diameter (μm) of hepatic cells.

Item	15 - 30 mm CVRL	40 - 75 mm CVRL	90 - 110 mm CVRL	115 - 130 mm CVRL
Nuclear diameter of Hepatic cells (μm)	4.61	5.15	5.52	5.91

DISCUSSION

The present study shows that, at the early embryonic stage the fetal rabbit liver is very large in size. Thus it occupies most of the abdominal cavity. With the advancement of age, the liver develops in a slower rate than the body as a whole, so at the last stage of gestation it occupies the cranial portion of the abdominal cavity. Similar to that reported by Ibrahim et al. (1991) in camel as well as Langman (1984) and Sadler (1990) in human. These results support the fact that there is a reverse correlation between the rate of development of the fetal liver and the body as a whole.

The present work indicates that, in the rabbit fetuses of 15-30 mm CVRL the liver consists of right and left lobes. The left lobe is divided into left medial and left lateral lobes. The right and left hepatic lobes are separated by the umbilical fissure which corresponds to the median plane. In this connection, Hamilton and Mossman (1972) stated that, in human the two lobes are inseparable in the midline. The subdivision of the liver into its characteristic lobes is largely the result of unequal growth, while its final shape is a passive response to adjacent pressure (Arey, 1965).

The caudate process of the liver appears, in the examined fetuses of 40-75 mm CVRL, as a small quadrilateral outgrowth develops on the dorsolateral part of the right lobe. This result is confirmed by Arey (1965) as well as Hamilton and Mossman (1972), who indicated that both caudate and quadrate lobes of the human liver appear as

subdivision of the right lobe. The present work reveals that the caudate process of the fetal rabbit liver lies to the right of the median plane. On the contrary, this process lies mainly on the left side of the midline as reported in the adult rabbit by Mclaughin and Chiasson (1990).

The gall bladder is demonstrated in the rabbit fetuses of 40-75 mm CVRL. At the last stage of gestation, it increases relatively in size and attains the form of small elongated sac embedded in the visceral surface of the liver. A similar result was mentioned in the adult rabbit by Thakur and Puranik (1984).

The microscopical investigations show that, in the rabbit fetuses of 15-30 mm CVRL the outlines of the hepatic lobules can be distinguished. This finding simulates that of Arey (1965), who stated that the human embryo of 7.5 mm CVRL has two primary lobules. On the other hand, the lobulation of the fetal camel liver begins at 72 cm CVRL (Abou-Easa, 1987), or at 94 cm CVRL (Fouad et al., 1984), and that of the fetal buffalo liver begins at 55 cm CVRL (Osman et al., 1984). The present work reveals that, during the last period of intrauterine life, the hepatic cells of the rabbit are still arranged in irregular manner and do not form hepatic cords. This result is confirmed by the statement of El-Keshawy et al. (1985), that the hepatic cords become well established beginning from rabbits of six weeks of age up to over one year.

In the examined fetuses, the cytoplasm of the hepatic cells demonstrates vacuoles which are increased in size with the advancement of age. So at the last stage of gestation the cytoplasm of the hepatic cells appear to be completely vacuolated. The frozen sections stained with Suddan black indicate that these vacuoles are filled with fat droplets. This result is agreed with that of Deane (1944), who mentioned that the fat fills the liver cells of the mouse at the time of birth. In contrast to the present work, Herzberg and Orlic (1981) observed the fat droplets later on in the rabbit fetuses at the day twenty fourth. The presence of the vacuolation in the cytoplasm of the hepatic cells was also observed in the stillborn human fetus by Sarrut and Nezeloof (1959), but they attributed this to the pathological changes. On the other hand, Du Bios (1963) pointed out that the fat content of the hepatic cells forms only a part of lipid reserve, contained within the liver.

The present study indicates that the PAS positive material (mostly glycogen) is observed in the fetal rabbit liver at the early embryonic stage. This result is in confirmity with the finding of Deane (1944) in mouse, that the ability of the liver cells to store glycogen originates early in the fetal liver.

The results of the present work reveal that, in the rabbit fetuses of 15-30 mm CVRL, the hemopoietic tissue is relatively predominant. It contains various stages of developing blood cells. With the advancement of the fetal age, the hemopoietic tissue decreases in amount till it reaches its lowest value at the end of the gestation period. This finding resembles that of Fouad et al. (1984) in camel, as well as Sadler (1990) in human. The reduction of the hemopoietic activity of the fetal liver may be componsated by the bone marrow which is considered the main hemopoietic site.

In accordance with the statement of Osman et al. (1984) in buffalo; Abou-Easa (1987) in camel and that observed in the present study in rabbit, the hemopoietic process occurs extra-and intravascular. On the other hand, Mohamed et al. (1986) mentioned that the hemopioesis is only extravascular in the fetal camel liver.

In agreement with Harrison et al. (1974) in mouse and Osman et al. (1984) in buffalo, the hemopoietic nests are described as hemopoietic progenitor cells. However, they are considered to be erythroblast progenitor cells as reported in guinea pig by Rosse and Beaufait (1978).

The present work shows that, the megakaryocytes as a member of the hemopoietic tissue can be demonstrated at the early stage of development (15-30 mm CVRL). Then they become prominent during the further developmental periods. The megakaryopoiesis begins also early in camel fetuses of 3.8 cm CVRL as recorded by Abou-Easa (1987), but it begins later on in the same animal at the fetuses of 40 cm CVRL as reported by Mohamed et al. (1986). In the same situation, Paone et al. (1975) pointed out that the megakaryocytopoiesis in the developing liver of the opossum is carried out within the blood sinusoids, they added that the number of megakaryocytes decreases after 9th day.

The central veins appear, at the early embryonic stage, as few wide blood spaces. During further development, they are greatly increased in number but decreased in size. The same observation was also given in camel by Abou-Easa (1987). On the other hand, Osman

et al. (1984) mentioned that the central veins appear in the buffalo fetuses of 35 cm CVRL as narrow space piercing a mass of differentiating hepatocytes.

The present work shows that, the blood sinusoids are demonstrated as narrow spaces in the rabbit fetuses of 15-30 mm CVRL. Then they become larger in size and some of them are engorged with non-nucleated blood cellular elements in the fetuses of 40-75 mm CVRL. At the last stage of gestation, numerous blood sinusoids invest the hepatic parenchyma, most of them are filled with non-nucleated blood cells.

The morphometric study shows that the nuclear diameter of the hepatic cells increases with the advancement of fetal age. This may reflect the increment of the size of the hepatic cells towards the end of the gestation period.

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LEGENDS

Fig. (1): Sagittal section in rabbit fetus of 15 mm CVRL showing: liver (V), stomach (S), kidney (K), lung (L) and Septum transversum (ST). Notice: the liver occupies most of the abdominal cavity. (H & E., X 31.5).

Fig. (2): Paraffin section in rabbit fetus liver of 15 mm CVRL showing: central vein (C), blood spaces (arrow). Notice: hemopoietic cells are arranged in nests. (H & E., X 125).

Fig. (3): Paraffin section in rabbit fetus liver of 15 mm CVRL showing: central vein (C) lined by flattened cells, hepatocyte (H) and hemopoietic cell (M). (H & E., X 500).

Fig. (4): Paraffin section in rabbit fetus liver of 15 mm CVRL showing: hepatocyte (H), hemopoietic cell (M), immature blood cells (T), (H & E., X 1250).

Fig. (5): Fotograph of the rabbit fetus of 40 mm CVRL after removal of the ventral abdominal wall. Notice: the right lobe (1), left medial lobe (2), left lateral lobe (3) and umbilical fissure (4) of the liver.

Fig. (6): Sagittal section in rabbit fetus of 50 mm CVRL showing: liver (V), stomach (S) and intestine (I). Notice: position and relation of the liver. (H & E., X 16).

Fig. (7): Semithin section in rabbit fetus liver of 60 mm CVRL showing: hepatocyte (H), hemopoietic cell (M), blood sinusoid (D). (Toluidine blue., X 500).

Fig. (8): Semithin section in rabbit fetus liver of 60 mm CVRL showing: hepatocyte (H), hemopoietic cell (M), megakaryocyte (K), blood sinusoid (D) lined by endothelial cell (arrow). (Toluidine blue., X 630).

Fig. (9): Paraffin section in rabbit fetus liver of 50 mm CVRL showing: developing gall bladder (G) and central vein (C). (H & E., X 31.5).

Fig. (10): Semithin section in rabbit fetus liver of 90 mm CVRL showing: distinct nucleolus within the nucleus (N) of hepatocyte, vacuoles within the cytoplasm (U), hemopoietic cell (M), blood sinusoid (arrow) contain non-nucleated blood cells (Toluidine blue., X 1000)..

Fig. (11,12): Frozen section in rabbit fetus liver of 100 mm CVRL showing: fat droplets stored within the cytoplasm of hepatocytes. (Suddan black B. 11: X 128, 12: X 400).

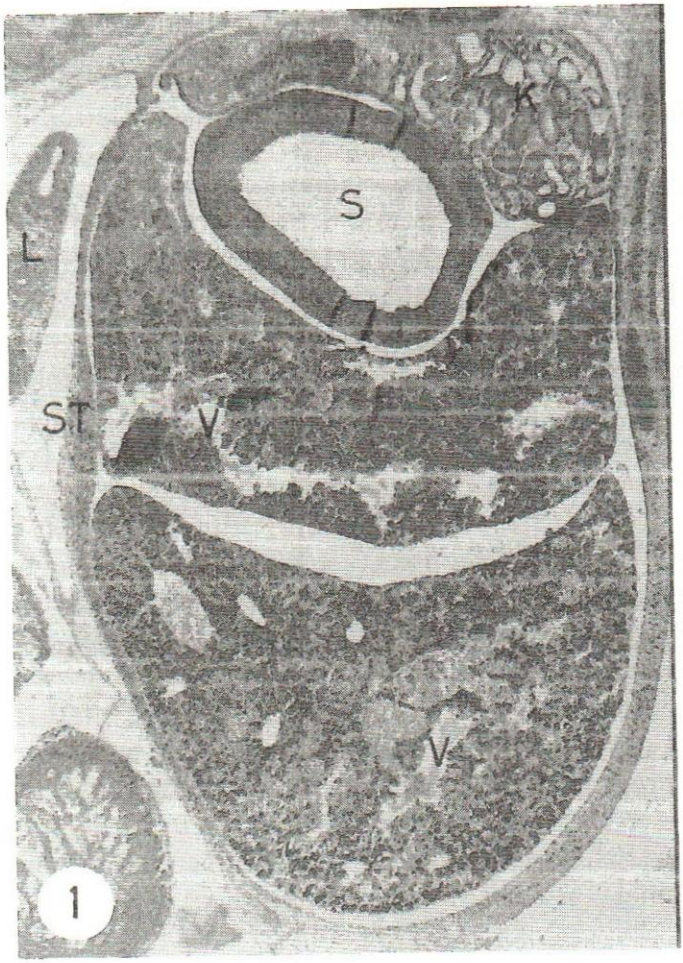
Fig. (13): Fotograph showing developmental changes in the rabbit fetus liver of: 90 mm CVRL (A) and 130 mm CVRL (B). Notice: right lobe (1), left medial lobe (2), left lateral lobe (3) and caudate process (4).

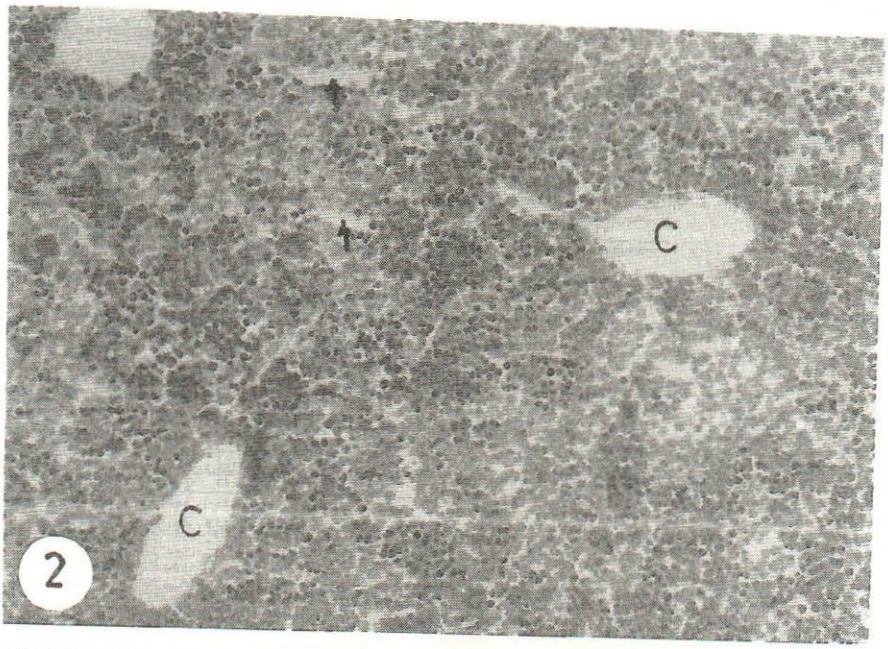
Fig. (14): Fotograph of the visceral surface of the rabbit liver of 130 mm CVRL. Notice: right lobe (1), left medial lobe (2), left lateral lobe (3), quadrate lobe (4), caudate process (5) and papillary process (6).

Fig. (15): Semithin section in rabbit fetus liver of 130 mm CVRL showing: large hepatocyte (H) with large vacuoles (U) within its cytoplasm, blood sinusoid (arrow) filled with non-nucleated blood cells. (Toluidine blue., X 1000).

Fig. (16): Paraffin section in rabbit fetus liver of 120 mm CVRL showing: non-nucleated blood cells (O), hemopoietic cell (M), and megakaryocyte (K). (H & E., X 252).

Fig. (17): Histogram showing the nuclear diameter of the hepatic cells at different stages.

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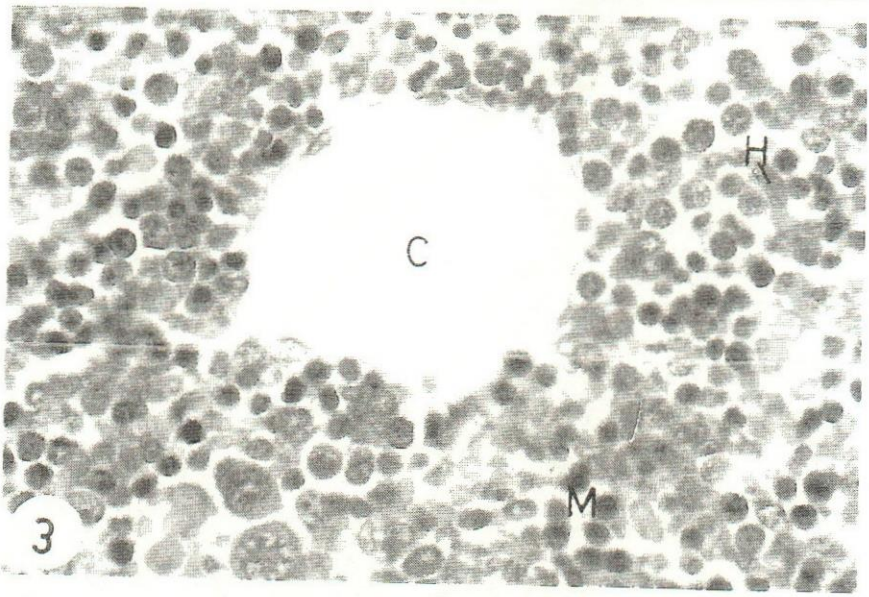
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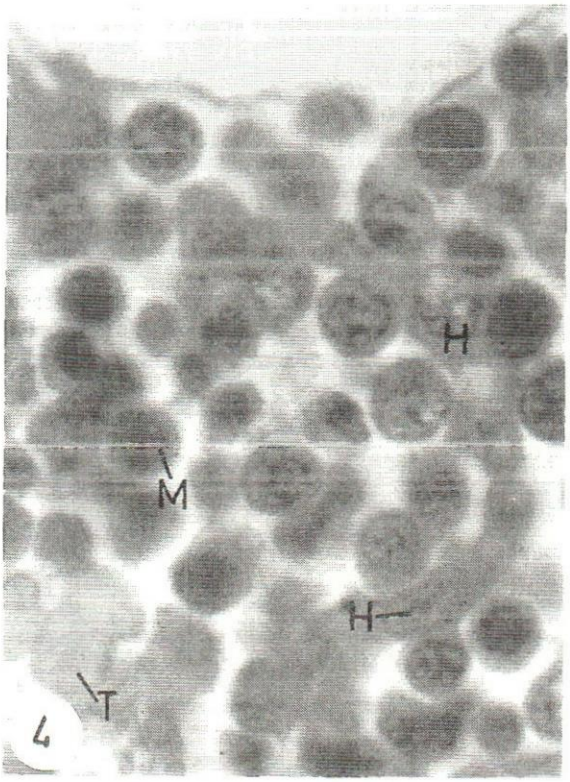

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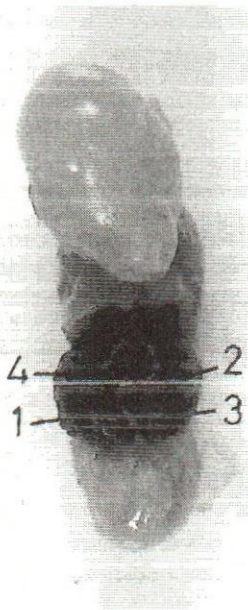

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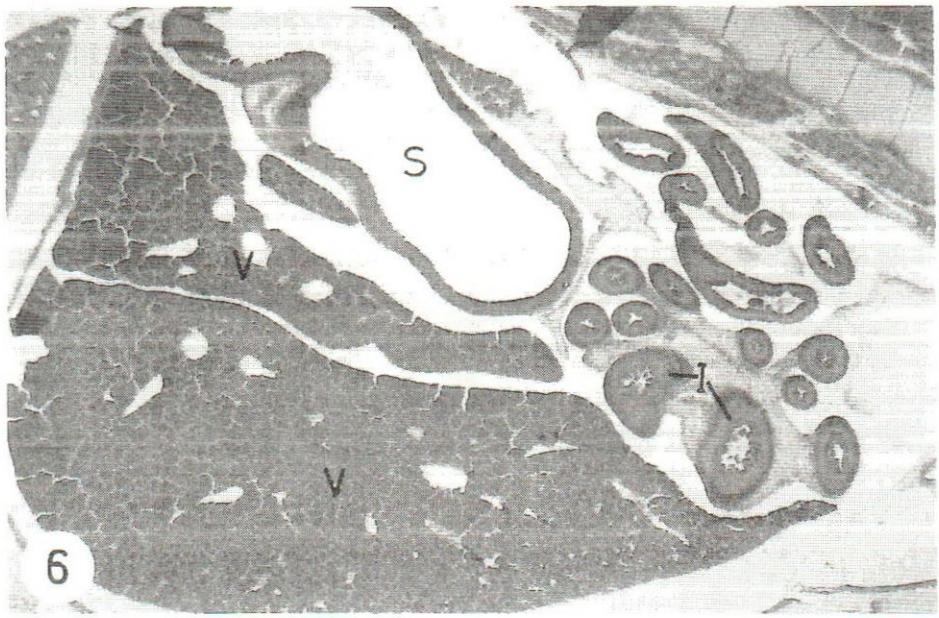

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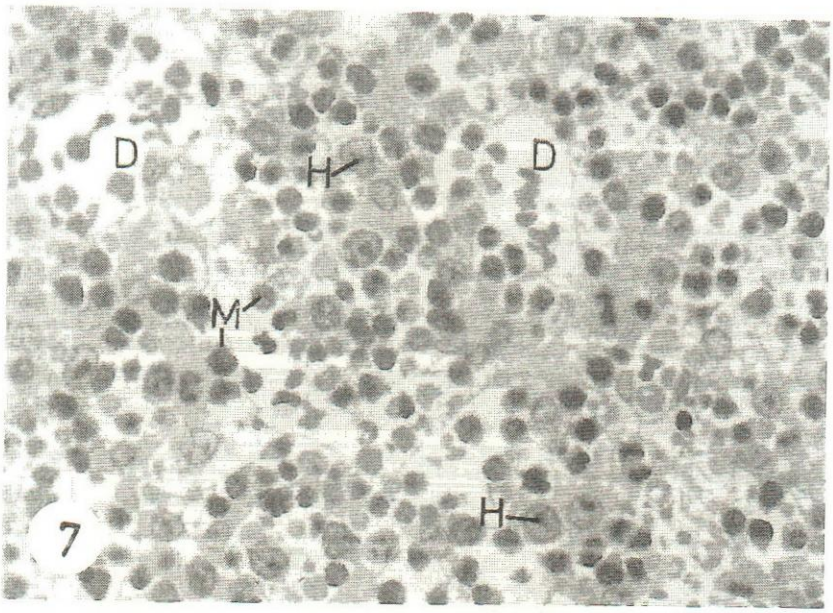
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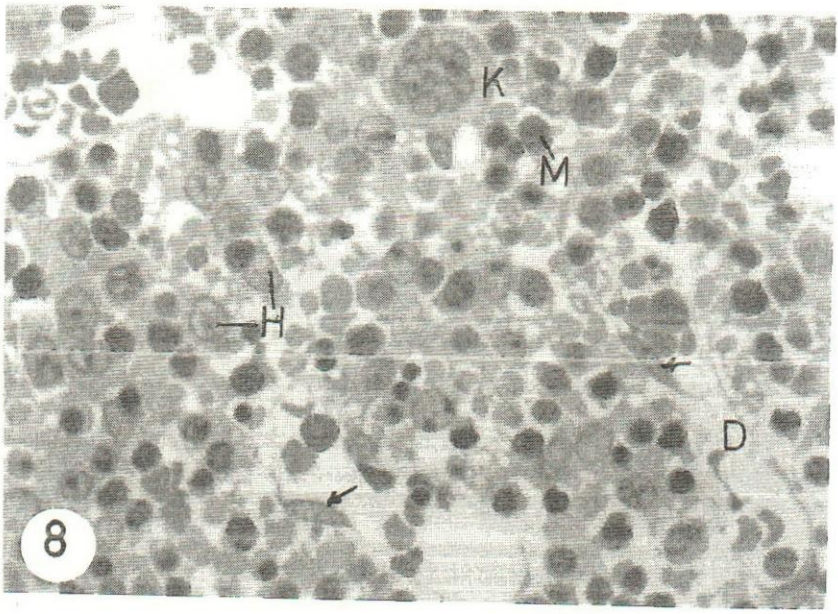
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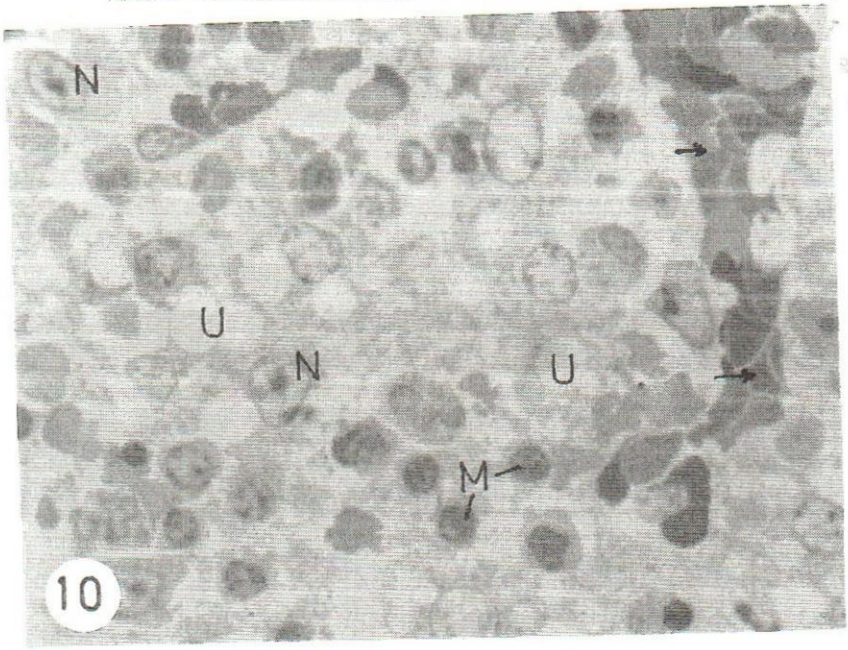
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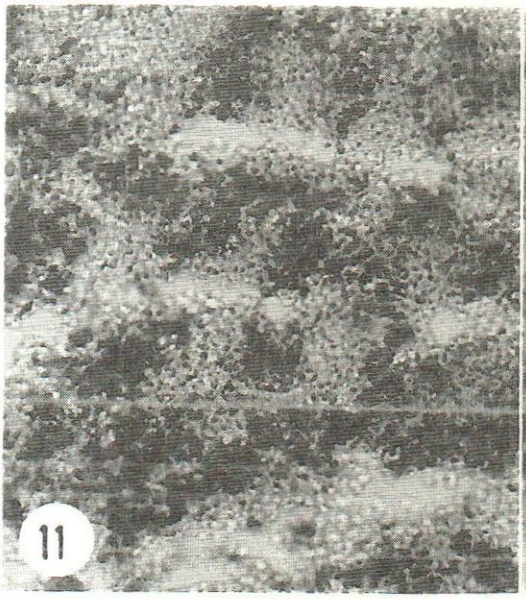

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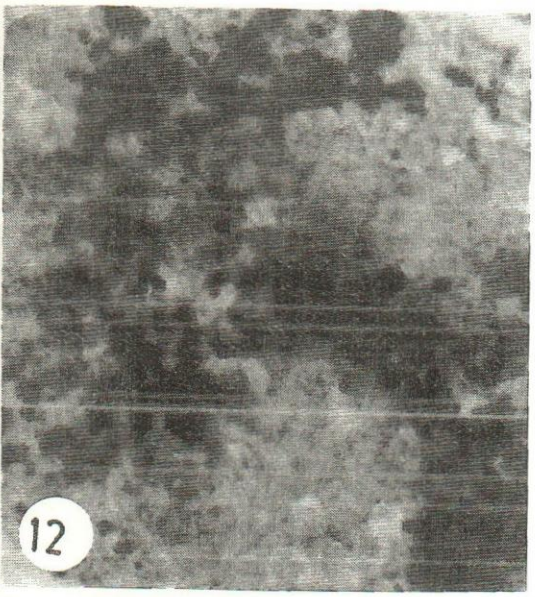

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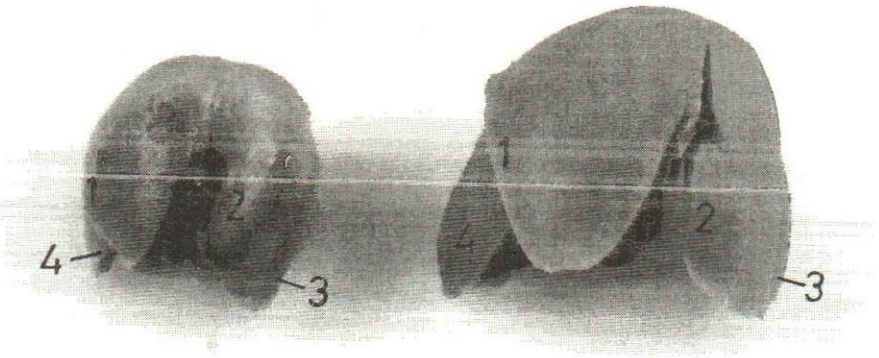
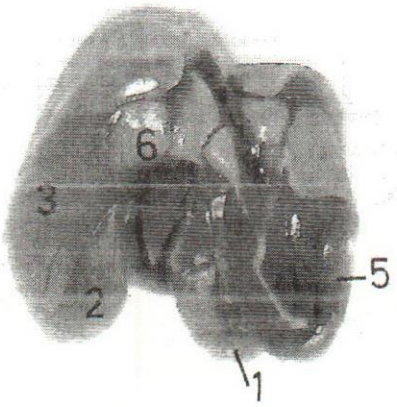


13 A B



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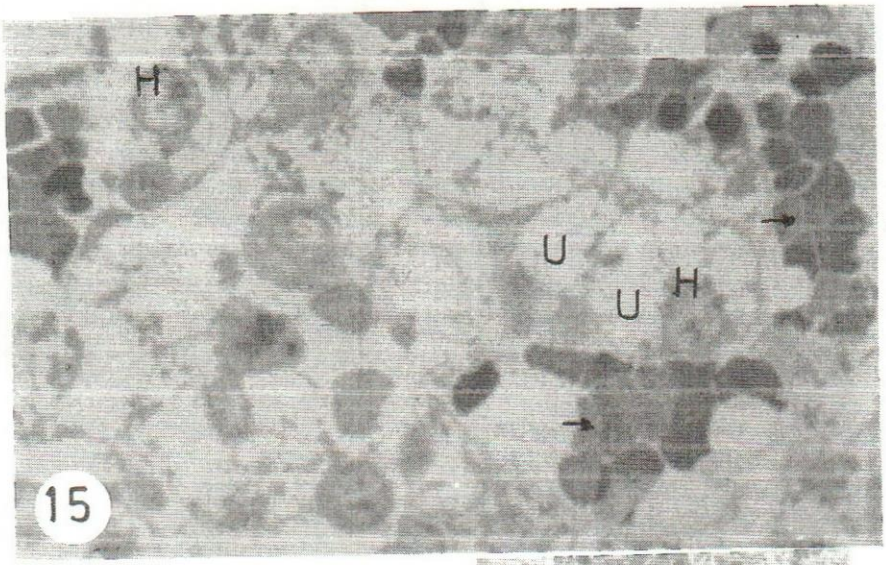

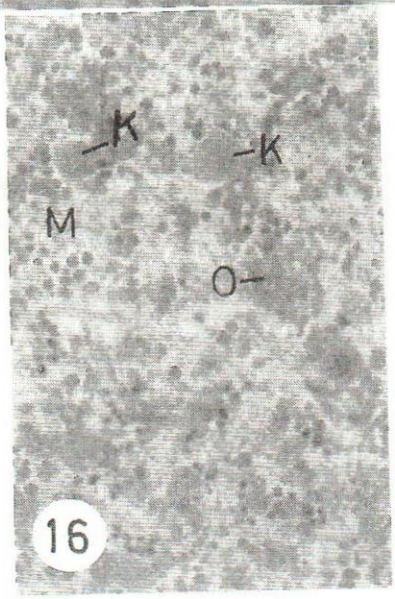

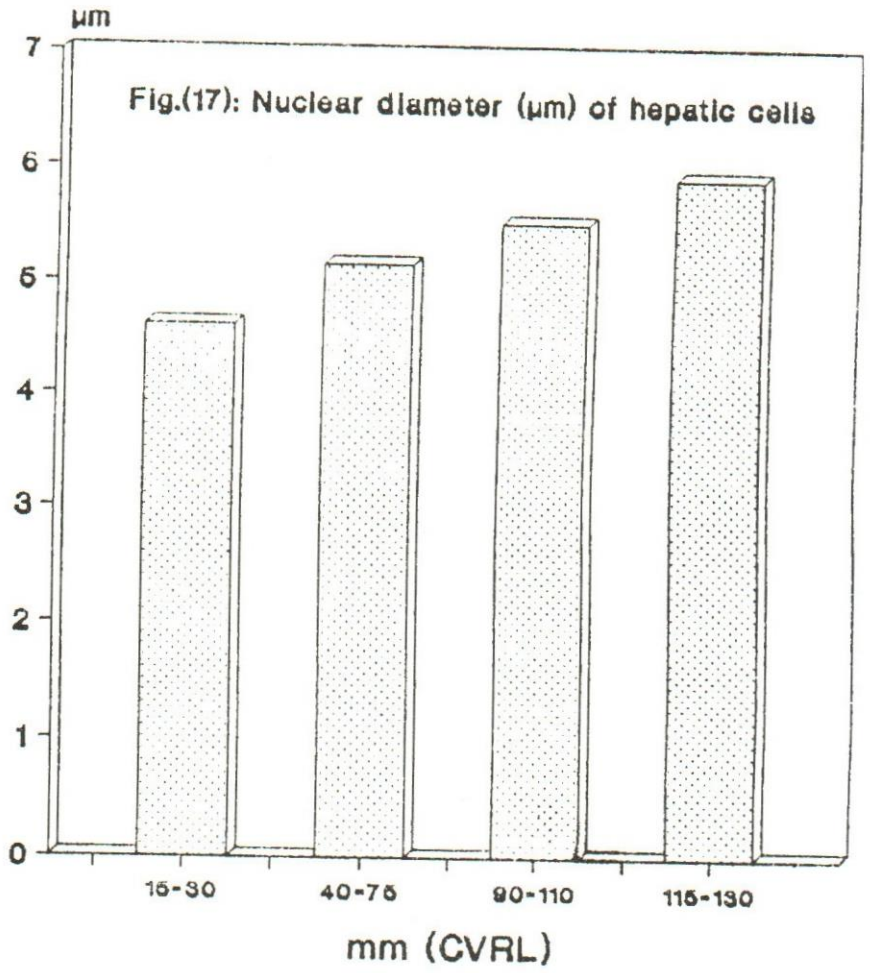

Fig.(17): Nuclear diameter (µm) of hepatic cells