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THE POST-HATCHING DEVELOPMENT OF THE LIVER IN QUAIL

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ABSTRACT

Forty Japanese quails of both sexes ranging from 1- 45 days old were used to elucidate the gross and structural changes in the liver during the post-hatching period. The liver of quails develops at a slower rate than the body cavity. In one day old birds, it occupies most of the cranial part of the body cavity and extends caudal to the sternal crest, but by advancement of age it moves cranially to become completely hidden by the sternal crest in mature birds. The liver of newly hatched quails consists of right and left lobes that are equal in length. The relative development of the left lobe is less active, so that in mature birds, it is shorter and smaller than the right one. Two intermediate lobes are also recognized at one day old, but the left one develops at a slower rate, so it becomes smaller than the right one at 30-45 days old. Structurally, the liver of newly hatched quails is markedly immature. There are few portal triads and central veins, which occupy 8.17% of hepatic tissue. The hepatocytes are not yet arranged in cords. By advancement of age, the portal triads and central veins increase in number and occupy 11.2% of hepatic tissue, the hepatocytes become arranged in cords with intervening sinusoidal network in premature and mature quails. The nucleus/cell percentage of the hepatocytes decreases during the post-hatching period that may reflect an increase in function. The hepatocytes of newly hatched quails contain large quantities of fat, which decrease to a minimal amount at 15 days old, then reincrease in premature and mature birds. However traces of glycogen are demonstrated in the hepatocytes of newly hatched birds, which increase toward the mature stage. In conclusion, the liver of quails surpasses obvious post-hatching changes not only grossly but also structurally indicating structural and functional hepatic maturation during this period.

INTRODUCTION

The quail has become an important bird not only as a source of meat and egg production but also as a standard laboratory bird. The liver plays a very important role in the assimilation of feed. In most birds the liver consists of a large right and a small left lobes. Although the avian liver is similar in basic structure to that of mammals, the two differ in numerous points of structural detail. The lobule as a traditional unit of liver structure is not evident in the liver of fowl ***(Romanoff, 1960)***. The postnatal development of the liver has been studied in some animals. In marsupials, the liver appears markedly immature at birth, lobular arrangement becomes evident and small bile ductules are observed at the 20th postnatal day ***(Krouse et al., 1975)***. In rabbit, the liver at one-day old consists of differentiating hepatic lobules together with portal areas. Hepatic cords become well-established at the beginning of 6th week up to one year old ***(El-Keshawy et al., 1985)***. Although the development of the avian liver was studied extensively during embryonic stage ***(Elias, 1955; Herrmann and Barry,1955;Kingsburg et al., 1956)***, information about the post-hatching development of the liver are meager. This work was outlined to throw light on the gross and structural changes in the liver of the quail during the post-hatching period.

MATERIALS AND METHODS

The current work was carried out on 40 apparently healthy Japanese quails (Coturnix coturnix japonica) of both sexes ranging from 1-45 days old. The birds were divided into 5 groups namely;1,7, 15, 30 and 45 days old. Five birds from each group were used for gross morphology. After weighing and euthanasia of the birds,their body cavities were opened and the gross morphological features and topographical relations were described. The livers were weighed and the liver/body weight percentages were estimated in all ages. In addition some morphometric aspects, concerning the length of the liver and its lobes as well as the termination of the liver in relation to the sternal crest, were measured. For light microscopy, three birds from each group were used. Small pieces from the liver were sampled and used for cryostat sectioning and stained with Sudan black. Also,

another samples were fixed in neutral buffered formalin and Carnoy's fixatives ***(Bancroft and Cook, 1994)***. These samples were then washed in 0.1M phosphate buffer (pH 7.4), dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Sections of 5-7µm thick were stained with H & E, PAS and Best's carmine stains. Some stereological aspects of the liver tissue were made using an image analysis system (Leica Q500). Volume percentage of portal triads and central veins in the total hepatic tissue as well as the nucleus/cell percentage and nuclear diameter of hepatocytes were measured in all studied ages.

RESULTS

Macroscopic observations:

The liver of one day old quails is relatively large in size occupying the ventral part of the cranial half of the body cavity (Fig.1). At this age the liver constitutes 4.44% of the total body weight. This percentage decreases gradually until the mature stage (45 days old) where it represents 2.85% (Table 1 and Histogram 1).

The caudal termination of the liver in the newly hatched birds lies 3.80mm behind the caudal end of the sternal crest. By advancement of age, the liver develops at a slower rate than the body cavity. As a result, the caudal termination of the liver becomes cranially displaced where it lies at the level of the caudal end of the sternal crest at 7 days old. In 15, 30 and 45 days old quails the caudal termination of the liver lies 3.00, 9.28 and 15.22mm cranial to the sternal crest respectively.

The liver is generally composed of right and left lobes (Figs.2, 3). At one day old, the two lobes are loosely attached cranially by weak connective tissue bridge, which soon at 7 days old becomes invaded by hepatic tissue. The length, cranial and caudal extensions of hepatic lobes differ in various studied ages (Table 1). At 1-7 days old, the right and left lobes are equal in length, they begin and terminate at the same level. At 15 days old, the right lobe becomes longer due to its cranial extension when compared with the left lobe. In premature and mature birds (30-45 days old), the right lobe extends cranial and caudal to the level of the left lobe.

The ventrolaterally directed parietal surface of the liver is smooth and convex except in its cranioventral part, which presents a cardiac impression (Fig. 2). At 1-15 days old, the apex of the heart is not seen from the parietal surface, but by advancement of age and due to enlargement of the cardiac impression, the apex of the heart becomes easily demonstrated on the parietal surface of the liver in 30-45 days old quails. The relations of the parietal surface of the liver vary in different studied ages. In one day old birds, it is related to the sternum and body wall ventrally and ribs laterally. By advancement of age and due to differential growth of the liver and body cavity, the former becomes completely hidden by the sternum and ribs in 30-45 days old quails.

The dorsomedially directed visceral surface of the liver shows impressions of the related viscera (Fig.3). At 1-15 days old, the dorsal parts of the right and left lobes are related to the terminal part of the esophagus and proventriculus while their ventral parts are related to the duodenum and pancreas on the right side and the gizzard and spleen on the left side. At 30-45 days old, the visceral surface of the liver appears irregular since it becomes closely moulded to the above-mentioned viscera. In addition, at this developmental period, the visceral surface is also related to the abdominal air sacs and lungs on both sides.

The visceral surface of the liver of one-day old quails also demonstrates two small intermediate lobes projecting from the right and left lobes. These lobes develop and extend caudomedially toward each other by advancement of age, but the left one develops at a slower rate so that it becomes smaller than the right one in 30-45 days old birds (Fig.3).

The left hepatic lobe is divided caudally by deep longitudinal intralobar fissure into a small caudodorsal and a large caudoventral parts (Fig.4). The cranial extension of this fissure varies in different ages. It extends along the caudal third of the left lobe at one-day old, caudal half at 7 days old, caudal three fourths at 15 days old and almost the whole length at 30-45 days old. The caudal border of the right lobe demonstrates a small notch at 30 days old. This notch becomes deeper at 45 days old, it lodges the ascending limb of the duodenum.

The gall bladder of 1-7 days old quails is represented by a small elongated sac lying on the caudal part of visceral surface of the right lobe, its apex terminates just cranial to its caudal border. In older quails, it attains a cylindrical shape, its apex protrudes from the border of the liver in full state only (Fig.3). The gall bladder demonstrates an increment in length by advancement of age (Table 1).

Microscopic observations:

In newly hatched quails (Figs. 5, 6), the liver is composed of variably sized clusters, rarely cords of hepatocytes with narrow sinusoids inbetween. The hepatic lobules are illdistinct due to poor interlobular connective tissue. Few portal triads containing small branches of blood vessels and bile ductules are demonstrated. In addition, few small central veins are encountered among the clusters of hepatocytes. Both portal triads and central veins constitute 8.17% of the hepatic tissue. At this age, the hepatocytes are large polyhedral in shape with unclear cell boundaries. The nuclei are large and vesicular with one or two distinct nucleoli. The cytoplasm shows numerous vacuoles of different size and shape. The nucleus measures 5.30µm in diameter, while the nucleus/cell percentage accounts for 23.65% (Table 2 and Histogram 2). Blood cells, mostly erythrocytes, are seen intravascularly.

By advancement of age and in 7-15 days old quails, the vascular component of the liver becomes well recognized. The portal triads and central veins occupy more volume percentage in the hepatic tissue than in one day old birds (Table 2 and Histogram 2). The branches of the blood vessels and bile ductules are clearly seen in the portal triads. The sinusoidal network is also better developed between the hepatic cell cords. The hepatocytes are arranged mostly in anastomosing cords, which radiate from the central veins representing the putative hepatic lobules. The hepatocytes are polyhedral or irregular, their cell boundaries are better seen than the previous age (Figs.7, 8). The nuclei are slightly smaller, the cytoplasmic mass expands and contains fewer and smaller vacuoles than those demonstrated in one day old quails. The nucleus/cell percentage decreases when compared with the previous age (Table 2 and Histogram 2).

In 30-45 days old quails (Figs. 9, 10), the liver structure attains the picture of the mature stage. The volume percentage of the portal triads and central veins in the hepatic tissue increases as compared to the previous age. The nucleus/cell percentage of the hepatocytes records a slight decrease as compared to the case at 7-15 days old (Table 2 and Histogram 2). Hepatic cell cords and the sinusoidal network are clearly demonstrated. The cytoplasm shows reincrement in the frequency of the vacuoles compared to the previous age.

Table 1: Some gross morphometric aspects of the liver during post-hatching period.

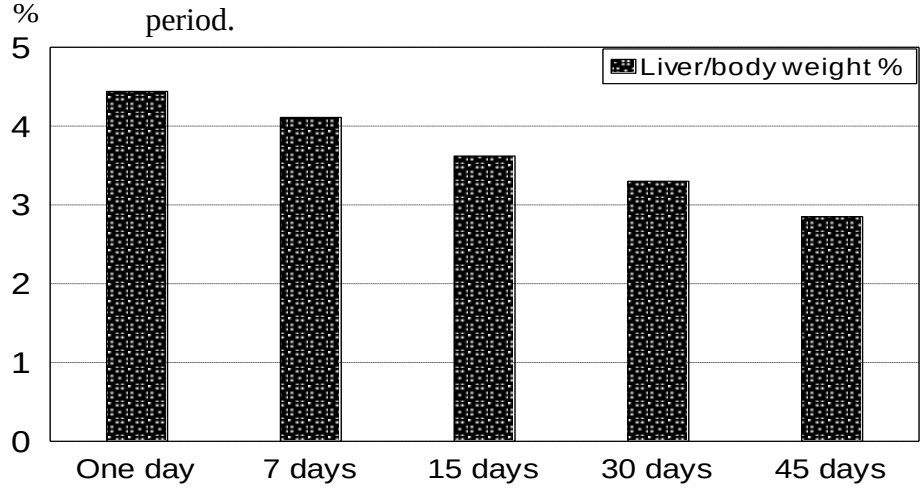
Item	**1 day old**	**7 days old**	**15 days old**	**30 days old**	**45 days old**
Total body weight (g)	7.20 ±0.4	17.50 ±1.2	32.80 ±1.5	90.90 ±5.4	182.40 ±8.3
Liver weight (g)	0.32 ±0.02	0.72 ±0.09	1.19 ±0.1	3.00 ±0.2	5.2 ±0.4
Liver/ body weight %	4.44±0.2	4.11 ±0.3	3.62 ±0.3	3.30 ±0.3	2.85 ±0.2
Length of liver (mm)	10.47 ±0.8	13.11 ±1.1	15.41 ±1.0	20.57 ±1.3	29.34 ±1.9
Length of right lobe (mm)	10.47 ±0.8	13.11 ±1.1	15.41 ±1.0	20.57 ±1.3	29.34 ±1.9
Length of left lobe (mm)	10.47 ±0.8	13.11 ±1.1	14.78 ±0.9	18.42 ±1.2	25.08 ±1.7
Distance between liver termination and sternal crest (mm)	3.80 ±0.1 caudal to it	0.00 ±0.0	3.00 ±0.2 cranial to it	9.28 ±0.8 cranial to it	15.22 ±1.2 cranial to it
Length of gall bladder (mm)	3.60 ±0.1	5.20 ±0.4	6.50 ±0.5	10.00 ±0.8	14.70 ±1.0

Table 2: Some stereological aspects of the liver tissues during post-hatching period.

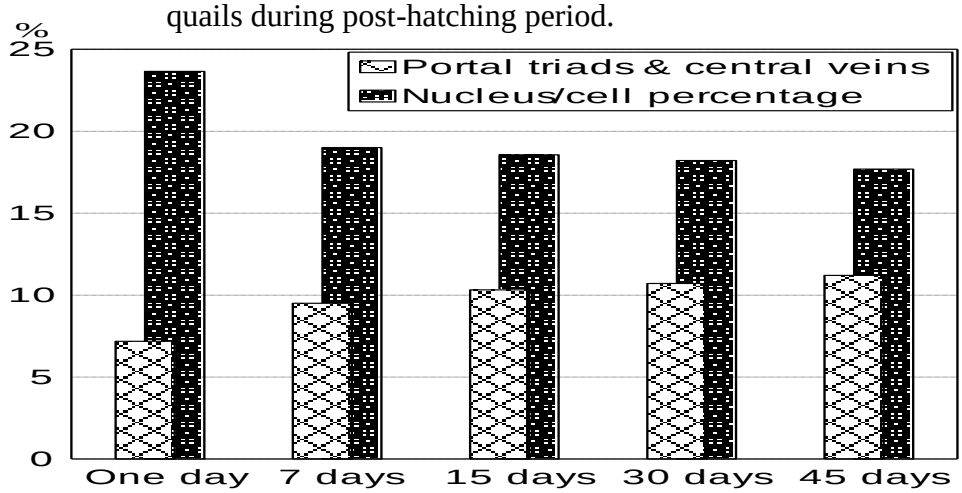
Item	1 day old	7 days old	15 days old	30 days old	45 days old
Volume percentage of portal triads & central veins / total hepatic tissue	8. 17 ±0.6	9.50 ±0.6	10.32 ±0.9	10.71 ±0.8	11.2 ±1.0
Nucleus / cell percentage of hepatocytes	23.65 ±1.2	19.00 ±1.4	18.56 ±1.3	18.21 ±1.2	17.69 ±1.1
Nuclear diameter of hepatocytes in µm	5.30 ±0.3	5.10 ±0.4	4.50 ±0.4	4.30 ±0.2	4.10 ±0.1

Histochemically,the hepatocytes of newly hatched quails are proved to contain many variably sized lipid droplets, (stain positive with Sudan black), which decrease to a minimal amount at 15 days old. These lipid contents reincrease again in 30-45 days old quails (Figs.11-13). However traces of glycogen are demonstrated in the hepatocytes of newly hatched birds, which increase toward the mature stage (Figs. 14-17). The distribution of lipid contents in all ages is irregular and patchy while the glycogen particles are always seen in peripheral locations.

Histogram 1: The liver/body weight percentage of quails during post-hatching period.



Histogram 2: The volume percentage of portal triads and central veins in the hepatic tissue and the nucleus/cell percentage of hepatocytes of quails during post-hatching period.



DISCUSSION

The present study reveals that the liver of the quails develops during the post-hatching period at a slower rate than the body cavity. In one day old birds, it occupies most of the cranial part of the body cavity and extends caudal to the sternal crest, but by advancement of age it moves cranially to become completely hidden by the sternal crest in mature birds. In this respect, ***Langman (1984) and Sadler (1990) in human, Ibrahim et al. (1991) in camel and Abdalla (1997)*** in rabbit mentioned that the liver development proceeds at a slower rate than the body cavity during the late prenatal stages. ***Likely, William and Warwick (1980)*** reported that the relative development of the liver is less active in late prenatal stages, but until birth the liver remains relatively larger than in the adult.

The shape of the liver in the newly hatched quails is essentially that in adults, but its weight at this time is greater in proportion to the total body weight, than the case in adult. It constitutes 4.44% of the total body weight in newly hatched quails, which decreases to 2.85% in mature birds. The weight percentage of the liver in adult quails is nearly similar to that recorded in the same bird by Ibrahim et al. (1992). However this weight percentage is relatively high when compared with many other birds like dove and sparrow (Hassouna and Zayed, 2001). This finding may indicate a high feed conversion in quails.

The liver of newly hatched quails consists of right and left lobes that are equal in length. By advancement of age, the relative development of the left lobe is less active, so that in mature birds, the left lobe is shorter in length and smaller in size when compared with the right one. The relatively slow development of the left lobe during the post-hatching period may be due to the repression exerted by the gizzard, which lies in close contact with it. A less active development of the left lobe in comparison to the right one has been reported to occur in the late prenatal life in the human liver and explained by a partial degeneration in the left lobe during this stage (Williams and Warwick, 1980).

Structurally, the liver of newly hatched quails is markedly immature. The lobular organization as well as the final distribution of the portal triads and central veins are not seen until the premature and mature ages. In this respect, Arey (1965) mentioned that toward the end of the fetal period, but mostly after birth, the primary hepatic lobules subdivide into smaller secondary units and this is accompanied by branching and division of the portal and central veins to keep the vascular relationship unchanged.

In newly hatched quails, the hepatocytes are not yet arranged in cords.By advancement of age,these hepatic cords start to appear irregularly and later on, they become well constructed with intervening sinusoidal network in premature and mature quails. Similar observations were reported in marsupials ***(Krouse et al, 1975)*** and rabbit ***(El-Keshawy et al., 1985)***. These changes point to an amplification of the hepatic cell function during the post-hatching development.

The nuclear diameter of the hepatocytes is slightly larger in newly hatched quails, but it decreases by advancement of age. The large nuclei in young birds may be attributed to the active dividing state of the hepatocytes in this period. The nucleus/cell percentage of the hepatocytes decreases during the post-hatching period indicating an increase in the cytoplasmic mass that may reflect an increase in function.The differences in function between young and adult have been broadly ascribed to relative differences between the immature and mature. Such a view fails to recognize those instances in which a delay in the maturation of a specific system may be essential to normal function at a specific developmental period ***(Timiras, 1972)***.

Lipid droplets are numerous and relatively large in size in newly hatched quails. These droplets decrease in both number and size to reach its minimum at 15 days old then reincrease again until the mature stage. Abundant lipid contents of the liver were reported in newly born rabbits ***(Traina, 1904)*** and ***rats (Smith, 1931; Rice and Jackson, 1934)***. Large amount of fat contents in the liver of newly born rats were attributed to the fat contents of the suckling milk ***(Rice and Jackson, 1934)***. In the current work the high fat contents in the liver of newly hatched quails may be stored from the absorbed yolk during embryogenesis,that decrease by advancement of age as a result of its utilization in the growing period. ***Breazile (1971)*** reported that fats are important source of stored energy and the half-life of the lipid depots is about 8-days. The reincrease of fat droplets in premature and mature quails may be due to decrease in catabolism during this stage.

The hepatocytes of newly hatched quails are also proved to contain traces of glycogen, which increases by advancement of age until being abundant in premature and mature birds. Unlikely, ***Stieve and Kaps (1937)*** reported that the ability of liver cells of the rabbit to store glycogen originates early in the fetal liver and is already maximal at the time of birth and decreases to a minimal amount before the third postnatal week. This controversy may be related to the species difference since quail and rabbits are quietly distant species. The ability of the liver to store glycogen is important in the systematic and efficient utilization of metabolic energy over a short period, it may constitute as much as 8% of the liver ***(Breazile 1971)***. The presence of glycogen in peripheral locations in the liver of quail may point to a less active blood circulation in these areas.

LEGENDS

Fig. 1: Photograph showing the position of the liver in relation to the sternal end in one, 15 and 45 days old quails arranged from left to right. Note that the liver (arrow) lies behind the sternal end (arrowhead) at one day old and completely hidden by it at 45 days old.

Fig. 2: Photograph showing the parietal surface of the liver in one, 15 and 45 days old quails arranged from left to right. The right (R) and left (L) lobes are nearly equal in length in one day old, but the left one becomes shorter at 45 days old. Note the cardiac impression (arrow).

Fig. 3: Photograph showing the visceral surface of the liver in one, 15 and 45 days old quails arranged from left to right. Note the right and left intermediate lobes (arrows) attached to the right (R) and left (L) lobes. The left one becomes smaller at 45 days old. The gall bladder (arrowhead) increases in length by advancement of age.

Fig. 4: Photograph showing left lateral view of the liver in one, 15 and 45 days old quails arranged from left to right. The left lobe (L) is divided caudally by a intralobar fissure (arrow), which increases in depth by advancement of age.

Fig. 5: Paraffin section showing overview of the liver of quail at one day old. The portal triads (PT) are few in number. H & E, X100.

Fig. 6: Paraffin section showing a hepatic lobule around a central vein (CV) at one day old. The hepatocytes are not yet arranged in cords. They contain numerous variably sized vacuoles. H & E stain, X400.

Fig. 7: Paraffin section showing overview of the liver of quail at 15 days old. The portal triads (PT) are relatively increased in number compared to the previous age. H & E, X100.

Fig. 8: Paraffin section showing a hepatic lobule around a central vein (CV) at 15 days old. The hepatocytes are arranged in irregular cords. They contain few variably sized vacuoles. H & E stain, X400.

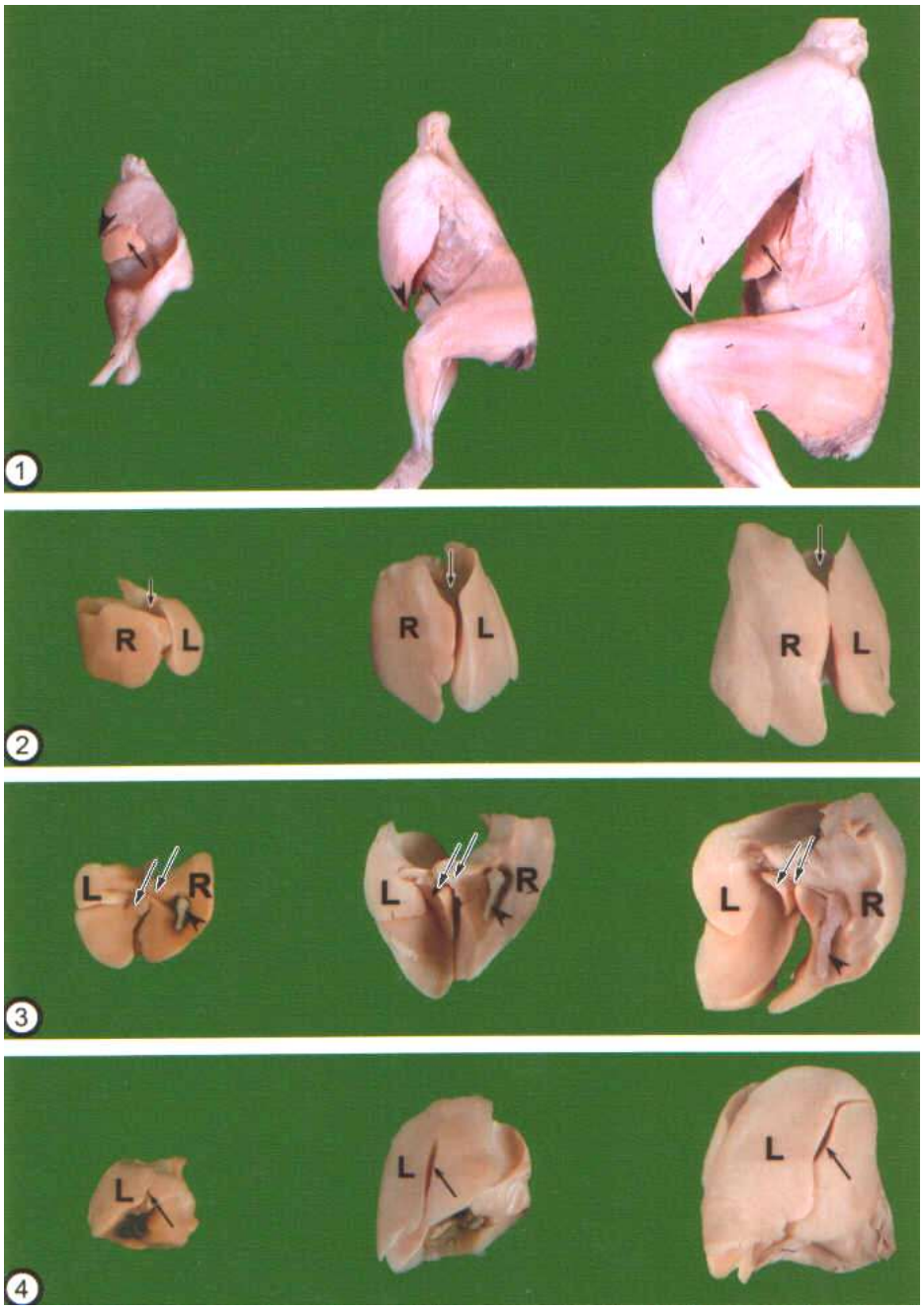
Fig. 9: Paraffin section showing overview of the liver of quail at 45 days old. The portal triads (PT) relatively increase in number compared to the previous age. H & E, X100.

Fig. 10: Paraffin section showing a hepatic lobule around a central vein (CV) at 45 days old. The hepatocytes are arranged in cords with branched sinusoidal network in between. The hepatocytes contain relatively numerous variably sized vacuoles. H & E stain, X400.

Figs 11-13: Fresh cryostat sections stained with Sudan black at one day (Fig. 11), 15 days (Fig.12) and 45 days old (Fig. 13). The hepatocytes demonstrate variably sized lipid droplets (arrow), which are numerous at one day old, decrease to a minimum amount at 15 days old then increase again at 45 days old. X400.

Figs 14-16: Paraffin sections of the liver of quail at one day (Fig. 14), 15 days (Fig.15) and 45 days old (Fig. 16). At one day old, the liver contains traces of PAS positive granules (arrow), which increase in number and size toward the mature birds (45 days old). Note the central vein (CV) at one day old. PAS stain, X1000.

Fig. 17: Paraffin section in the liver of 45 days old quail stained with Best's carmine showing numerous variably sized glycogen particles (arrow). X1000.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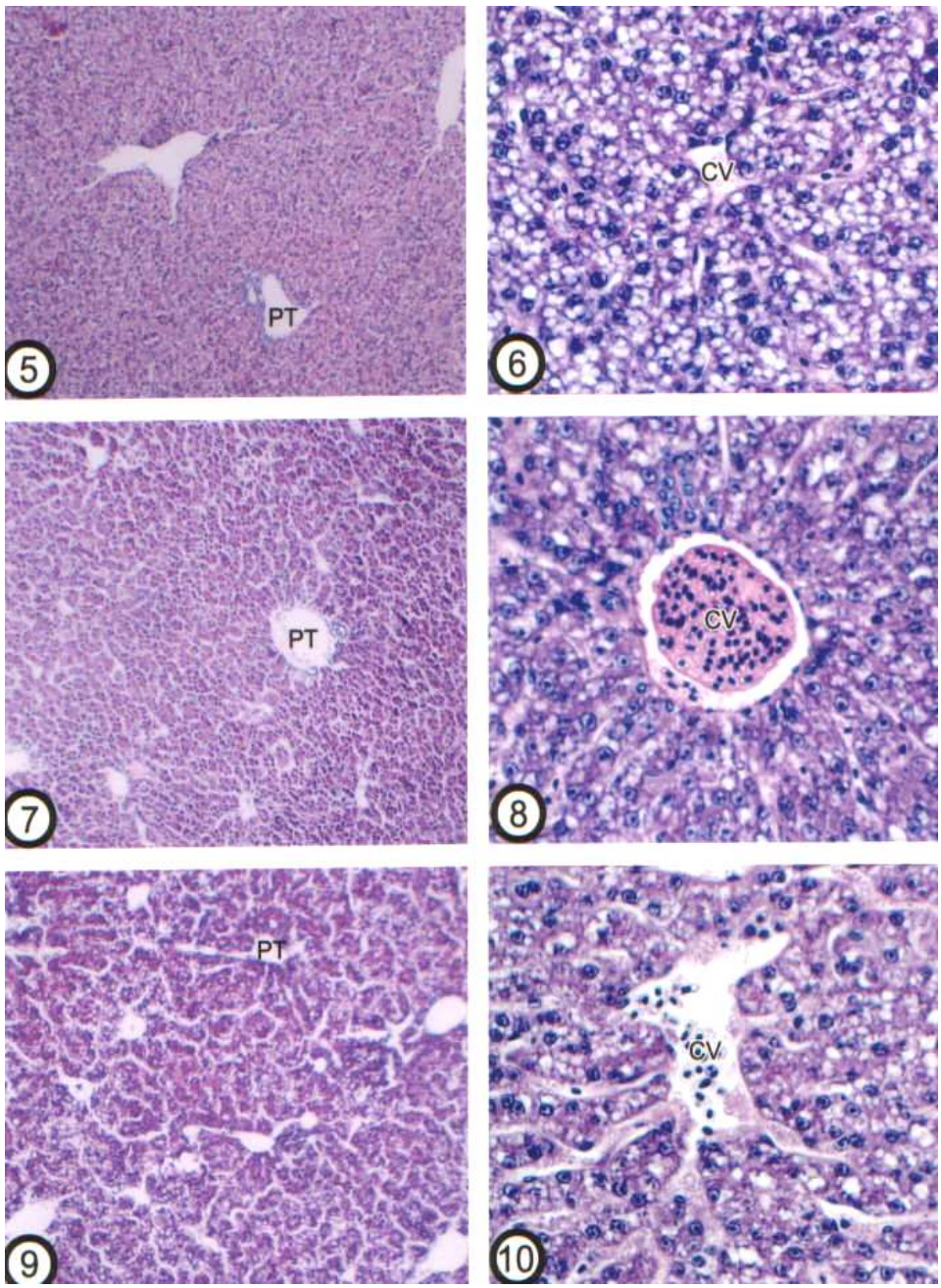
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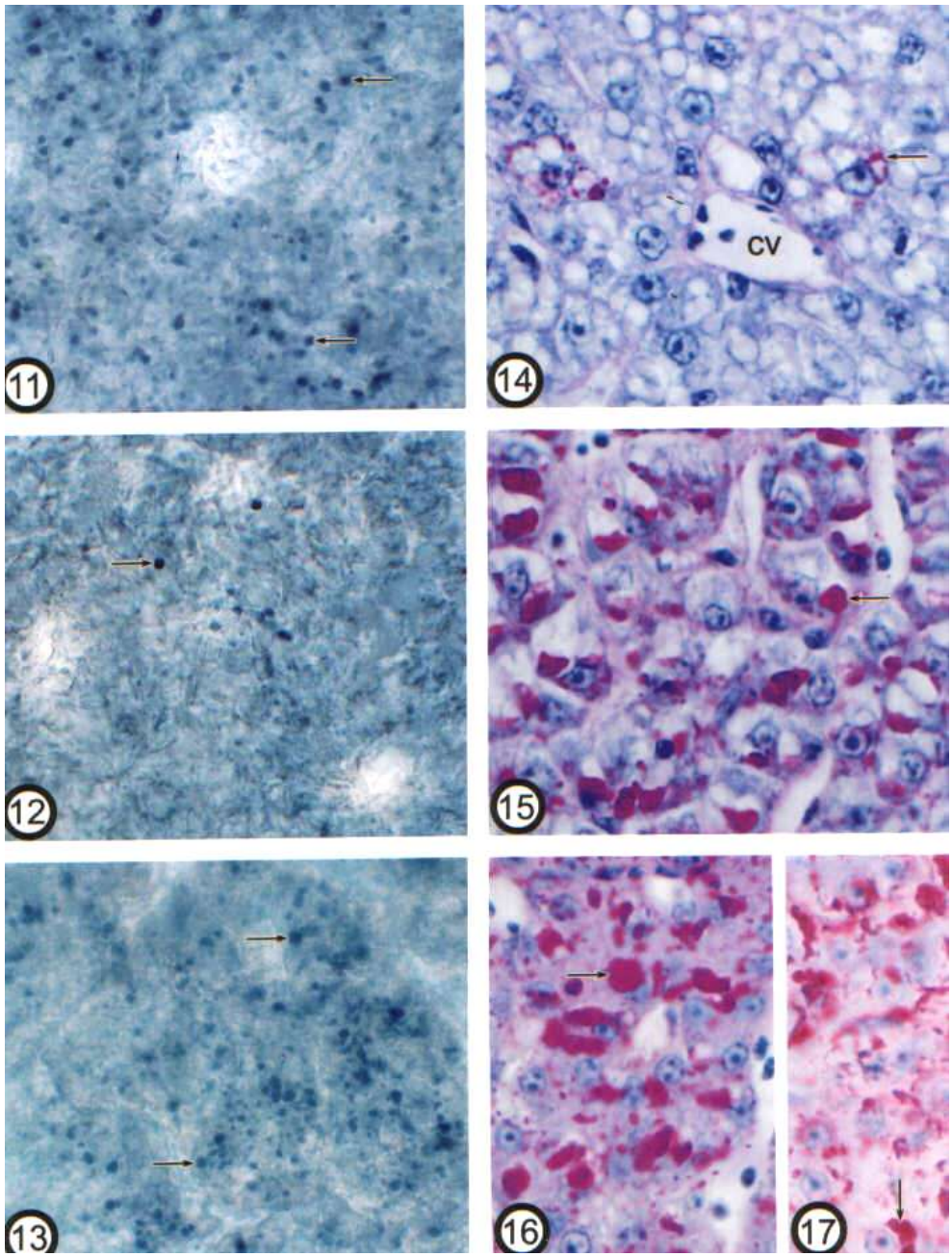
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تطور كبد السمان فى فترة ما بعد الفقس

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استخدم فى هذه الدراسة عدد أربعين سمان من كلا الجنسين، تتراوح أعمارهم من 1–45 يوما لاستيضاح التغيرات العينية والتركيبية للكبد فى هذه المرحلة. ينمو كبد السمان بمعدل أقل من تجويف الجسم، ولذلك فإن الكبد يشغل معظم الجزء الأمامى من تجويف الجسم ويمتد قليلا وراء عظمة القص فى عمر يوم واحد، بينما يتحرك للأمام بتقدم العمر ليصبح مغطى بالكامل بعظمة القص فى الطيور البالغة. وقد لوحظ أن فصى الكبد الأيمن والأيسر لهما نفس الطول فى عمر يوم بينما يصبح الفص الأيسر أقصر وأصغر حجما فى الطيور البالغة. إضافة إلى ذلك فالفصان الوسطيان واللذان يتصلان بالفصين الأيمن والأيسر ينموان بمعدل مختلف مما ينتج عنه صغر حجم الفص الأوسط الأيسر فى الطيور البالغة. أما تركيبيا فإن كبد الطيور حديثة الفقس يبدو بشكل غير ناضج، فعدد المثلثات البابية والأوردة المركزية قليل حيث تمثل17، 8% من النسيج الكبدى، كذلك فإن الخلايا الكبدية تكون غير مرتبة فى أشكال حبلية ولكن بتقدم العمر تزداد المثلثات البابية والأوردة المركزية لتمثل 2 ،11% من النسيج الكبدى، أما الخلايا الكبدية فتصبح مرتبة فى أشكال حبلية تتخللها شبكة من الجويبات الدموية فى الطيور شبه البالغة والبالغة. أما فيما يخص النسبة المئوية للنواه فى الخلايا الكبدية فإنها تتناقص تدريجيا بتقدم العمر مما يعكس تزايد الأداء الوظيفى للكبد. كذلك أوضحت الدراسة أن الخلايا الكبدية تحتوى على كميات كبيرة من الدهون فى الطيور حديثة الفقس و التى تتناقص إلى أقل مستوى عند عمر خمس عشر يوما ثم تزداد فى الطيور شبه البالغة والبالغة. على أنه لوحظ وجود أثار من الجليكوجين فى الطيور حديثة الفقس والذى يزداد فى الكمية باتجاه مرحلة النضوج. يستخلص من هذه الدراسة أن كبد السمان يمر بتغيرات واضحة فى فترة ما بعد الفقس ليس شكليا فقط ولكن نسيجيا مما يدل على نضوج نسيجى ووظيفى فى هذه الفترة.