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HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF LIVER IN FISHES

(With 20 Figures)

By

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دراسات هستولوجية وهستوكيمائية فى كبد الاسماك

منى عبد الفتاح على

اوضحت هذه الدراسات ان كبد سمك القرموط والبلطى النيلى محاط من الخارج بطبقة رفيعة من النسيج الضام يحتوى على بعض الالياف الشبكية والغروية. كان الجزء الخلوى من الكبد فى كلا النوعين غير منقسم الى فصيصات واضحة، ولم يلاحظ وجود ثلاثيات بوابية نموذجية، بل كانت تتكون من شريان كبدى او وريد بابى مع قناه مرارية. احتوت الخلايا الكبدية فى كلا النوعين على مواد ايجابية لمعامل شيف، حبيبات جليكوجين، دهون وحبيبات صبغية. فى سمك القرموط، كونت الالياف الشبكية شبكة تدعم الخلايا الكبدية، كما وجدت هذه الالياف فى جدار الاوعية الدموية وحول الحويصلات البنكرياسية: ولكنها فى سمك البلطى النيلى وجدت فى الغلاف الخارجى حيث امتدت على شكل الياف دقيقة بين الخلايا الكبدية الموجودة تحت الغلاف الكبدى، فى الاوعية الدموية وحول الحويصلات البنكرياسية. وقد وجدت خلايا دهنية وخلايا بالوعة للميلانين داخل الجزء الخلوى لكبد سمك القرموط. كانت انسجة البنكرياس ذات الافراز الخارجى موزعة بدرجة عالية فى الجزء الخلوى لكبد سمك البلطى تحيط تقريبا كل تفرعات الاوردة البابية الكبدية، ولكن فى سمك القرموط كانت مصاحبة للتفرعات الكبيرة فقط للوريد البابى.

SUMMARY

The liver of Cat fish and Nile telapia were surrounded by a thin fibroconnective tissue capsule contained reticular and collagen fibres, this connective tissue investment covered by a single layer of mesothelial cells. The capsule was thickened at hilus. The parenchyma of liver in both types was not divided into distinct lobules. Typical portal triads were not obvious. The portal area was composed either of hepatic artery or portal vein with bile duct. The hepatocytes of both types

contain PAS positive materials, glycogen granules, lipids and pigment granules. In Cat fish, the argyrophilic reticular fibres form a meshwork supporting the hepatocytes and were demonstrated in the wall of blood vessels and around acini of exocrine pancreas, but in Nile telapia it was restricted in the capsule, which sent fine fibres in-between hepatocytes underlying it, in the wall of blood vessels and around the pancreatic acini. Fat cells and melanomacrophage cells were also demonstrated within the parenchyma of Cat fish. Exocrine pancreatic tissues were highly distributed in the parenchyma of liver in Nile telapia surrounding nearly all branches of hepatic portal veins, but in Cat fish, these tissues were associated only with large branches of portal vein.

Key Words: *Histochemical-Cat fish-Telapia-Fibroconnective*

INTRODUCTION

The liver is essential for life, it is the largest internal organ of the body. The liver is interposed between two important vessels: (1) The large hepatic portal vein carrying nutrients from the stomach and intestine and (2) The smaller hepatic artery which provides the oxygen for the metabolically active liver cells. These cells are capable of many varied functions including synthesis and secretion of bile, storage of glucose, glycogen, fats, proteins and vitamins, detoxification of metabolic wastes, synthesis of blood-metabolic wastes and synthesis of blood-cloting and blood-thinning factors (Telford and Bridgman, 1990).

In addition, the liver of fish playes a major role in the process of vitellogenesis of oocytes (Wallace, 1978) and in the energy production during spawning (Mohallal and Al-Thani, 1990).

The histological structure of liver of many species of fish is essentially similar to mammals (Ferguson, 1989).

The present studies were carried out to illustrate the differences in the histological and histochemical structures in the liver of Nile telapia (*Telapia nilotica*) and Cat fish (*Clarias lazera*).

MATERIALS and METHODS

Ten randomly selected Cat fish and others of Nile telapia of both sex were freshly obtained from Kafr El-Sheikh governorate. The livers were removed, and small pecies were taken, fixed either in Bouin's fluid

for paraffin sections or in formal calcium for frozen sections. After proper fixation, the specimens were dehydrated, cleared and embedded in paraffin. Sections were obtained at 5-7 µm and stained with haematoxylin and Eosin (Harris, 1898), Crossman's trichrome stain (Crossman, 1937). van Gieson technique (van Gieson, 1899). Periodic acid Schiff's technique (McManus and Mowry, 1960), Gomori's method for reticular fibres (Gomori, 1937), Verhoeff's method for elastic fibers (Verhoeff, 1908) and Best's carmine method for detection of glycogen granules (Best, 1906). Fresh or fixed frozen sections were obtained at 10-15 µm and stained with Sudan black (Lison and Dagnelie, 1935) for demonstration of neutral lipid.

RESULTS

The liver of both types of fish was surrounded by a thin fibro-connective tissue capsule contained reticular and collagen fibres this connective tissue investment covered by a single layer of mesothelial cells. (Fig. 1a). At hilus, the capsule was thickened (Fig. 1b). The capsule was sent fine threads of reticular fibres inbetween underlaying hepatocytes.

The parenchyma of liver in both types was not divided into distinct lobules. In Nile telapia it composed of branching two thick rows of hepatocytes separated by blood sinusoids and radiated outward from the central vein (Fig. 2), but in Cat fish it composed of irregular interconnecting plates of hepatocytes separated by more dilated blood sinusoids (Fig. 3).

The hepatocytes in both types were polyhedral in shape contain highly vacoulated cytoplasm with one or two rounded peripherally located nuclei (Fig. 4).

Their cytoplasm contain PAS positive materials (Fig. 5). These materials were more abundant in the hepatocytes laying near the central vein than those laying away from it.

In addition the cytoplasm contains pigments, glycogens and lipids (Fig. 6, 7a, b, 8). The glycogen granules were concentrated at the peripheral sides of the cells facing blood sinusoids (Fig. 9).

Typical portal triads were not obvious, the portal area was composed either of hepatic artery with bile ducts or portal vein with bile ducts (Fig. 10a, b).

The bile ductules of different sizes were randomly distributed throughout the liver parenchyma singly or in groups. They were lined with columner cells with basely located nucleu. Their apices gave a strong reaction with both PAS and acid fuchsin stains (Fig. 11). In Nile telapia, the cells of bile ductules demonstrated cillia at their apical surfaces (Fig. 12).

The bile ductules were surrounded by a thin layer of connective tissue followed by a relatively circularlly arranged smooth muscle fibres and covered from outside with connective tissue layer.

No elastic fibres were demonstrated within the parenchyma of liver in both types.

The argyrophilic reticular fibres form a meshwork supporting the hepatocytes of Cat fish, and were demonstrated in the wall of blood vessels and around the acini of exocrine pancreas (Fig. 13). But in Nile telapia, the reticular fibres were restricted in the capsule which sent fine fibres between hepatocytes underlying it, in the wall of blood vessels and around the pancreatic acini (Fig. 14).

Fat cells and melanomacrophage cells were demonstrated within the parenchyma of Cat fish (Fig. 15, 16). The macrophages were always related to blood vessels.

Exocrine pancreatic tissues were observed intrahepatically in both types, highly distributed surrounding nearly all the branches of hepatic portal veins and central veins in Nile telapia, but in Cat fish, their were associated only with large branches of portal vein. (Fig. 17a, b and 18).

The exocrine pancreas was formed of tubulealveolar secretory end-pieces. The glandular cells were pyramidal or columner in shape and contained large rounded basally situated nuclei, basophilic cytoplasm was located at the basal portion of the cell. The secretory granules filled large proportion of the pancreatic cells (Fig. 19).

Oval or rounded cells with peripherally situated deeply stained nuclei and granular acideophilic cytoplasm were demonstrated between pancreatic acini of Nile telapia, these cells always were related to blood vessels, macrophages were also demonstrated between pancreatic acini (Fig. 20a, b).

DISCUSSION

The present study revealed that the liver of fish surrounded by a thin connective tissue capsule contained reticular and collagen fibres. This capsule functions as tough, outer "stocking" to give support and shape to the liver (Telford and Bridgman, 1990).

In the present investigation, the parenchyma of Nile telapia and Cat fish were not divided into distinct lobules, Kendall and Howkins (1975), Groman (1982), Ferguson, (1989) and Elmohdy (1993) appreciated the same observation.

The present work revealed that the parenchyma of Nile telapia liver composed of branching two thick rows of hepatocytes seperated by blood sinusoids, Kendall and Howkins (1975) found this arrangements in channel Cat fish where in our study the parenchyma of liver in Cat fish was formed of irregular interconnecting plates of hepatocytes. Elmohdy (1993) revealed that the hepatocytes of Cat fish were arranged in branched curvilinear or circular tubules around the central veins. Tanuma et al. (1982) and Hampton et al. (1985) and (1989) have confirmed the tubular arrangement in the liver of teleost species, while Simon et al.(1967) could not found the tubular nature of the rainbow trout liver.

The present study revealed that the hepatocytes contained highly vacoulated cytoplasm. Kendall and Howkins, (1975) reported that this vacoulation results from the removal of glycogen and fats during routine preparation. The present investigation revealed that the hepatocytes of Nile telapia and Cat fish were rich in glucogen. Ferguson (1989) mentioned that fish such as trout poorly, utilize diatary carbohydrates and an excess can accumulate in hepatocytes as glycogen. It is generally accepted that the hepatocytes of fish contain glycogen, but its amount varies in different species of fish. Kramar et al. (1974), Hinton and Pool (1976), Hacking et al. (1978) and Sakono and Fujita (1982) noticed that hepatocytes of numerous teleost were rich in glycogen. While some other teleosts contain scanty amount of glycogen, (Welsch and Storch 1973, Vonnahme 1981 and Taverny et al. 1984). These variations in the glycogen contents of hepatocytes in different teleosts can be correlated with age, Barni et al. (1985), sex, Braunbeck et al. (1989), nutritional condition, Affandi and Biagiani (1987) and gonadal maturation, Tveranger (1985).

The present study showed that the hepatocytes, of liver of both types were rich in fat content. Kendall and Howkins (1975) and

Ferguson (1989) reported the same observation. Moreover, the hepatocytes of Nile telapia contain a yellowish-brown pigments, reacted positively with PAS. This pigment might be hemosiderin pigments. Telford and Bridgman (1990) mentioned that this pigment appear as yellowish brown glycoprotein derived from the degrading of hemoglobin of senile red blood cells.

In the present investigation, typical portal triads were not obvious. The portal area composed of branches of hepatic artery or portal vein with bile duct. While, Hampton et al. (1985) reported that the portal area in the liver of Rainbow trout was composed of branches of hepatic artery and bile duct and El-Mohdy (1993) reported that portal triads of Cat fish were similar to those of mammalian liver.

The present study revealed that the bile ductules of different sizes were randomly distributed throughout the liver parenchyma singly or in groups. This observation was in agreement with El-Mohdy (1993), while Hampton et al. (1989) revealed that small bile ductules were found in the periadventitia of large diameter portal veins.

The present study showed that the blood sinusoids between hepatocytes of Cat fish were more dilated and more demonstrated than that in Nile telapia. The exchange of nutrients between hepatocytes and the blood flowing through the sinusoids (Telford and Bridgmen 1992) where the mixed arterial and venous blood percolates the sinusoids to empty into the central vein.

The present study demonstrated that argyrophilic reticular fibres form a meshwork supporting the hepatocytes of Cat fish, also, it was located in the wall of blood vessels and around the acini of exocrine pancreas. This results were in-agreement with that demonstrated by Kendall and Hawkins (1975) in liver of channel Cat fish. The present study showed that, the reticular fibres were demonstrated only in capsule, between hepatocytes underlying capsule and around pancreatic acini in liver of Nile Telapia.

In fish the liver is hepatopancreatic organ (Kendall and Howkins, 1995; Ferguson, 1989 and El-Mohdy, 1993). The pattern of distribution of exocrine pancreatic acini was different in Nile Telapia than that in the cat fish. In Nile Telapia they were located around all branches of the hepatic portal vein (Kendall and Howkins, 1975 and El-Mohdy, 1993). They were only associated with the major portal vessels of liver in cat fish (Ferguson, 1989).

Oval or rounded cells with peripherally situated deeply stained nuclei and granular acidophilic cytoplasm were demonstrated between

pancreatic acini of Nile telapia, these cells always were related to blood vessels. The number of zymogen granules in the pancreatic acinar cells is variable and depends on the digestive phase, reach its maximum in fasted animal. These cells are pancreatic acinar cells in storage stage.

In conclusion: The liver of fish was hepatopancreatic organ. The parenchyma was not divided into distinct lobules. The hepatocytes were filled with Glycogen and fats. Typical portal triads were not obvious. The differences in the histological structure of liver of Nile telapia and Cat fish were summarised in the arrangement of hepatocytes, arrangement of exocrine pancreatic tissue, and distribution of reticular fibres.

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FIGURES

Fig.1a,b :Paraffin section in liver of Cat fish showing connective tissue capsule; reticular fibres (arrows), collagen fibres (1) (Gomori's tech. (a) x 100 x 12.5 and (b) x 40 x 12.5).

Fig.2 :Paraffin section in liver of Nile telapia showing the arrangement of hepatocytes (1), central vein (2) and blood sinusoids (arrows). (Best's carmine stain, x 40 x 12.5).

Fig. 3 :Paraffin section in liver of Cat fish showing the arrangement of hepatocytes (1) and blood sinusoids (arrows). (Van Gisson's stain, x 40 x 12.5).

Fig. 4 : Paraffin section in liver of Nile telapia showing hepatocytes (1), pigments (2) and Nucleus (arrows) (H & E stain, x 100 x 12.5).

Fig. 5 : Paraffin section in liver of Nile telapia showing PAS + ve materials inside the hepatocytes (arrows). (PAS. haematoxylin tech. x 100 x 12.5).

Fig. 6 : Paraffin section in liver of Cat fish showing hepatocytes contain pigments granules (arrows) (H & E stain x 100x 12.5).

Fig. 7a,b : Frozen section in liver of Nile telapia showing hepatocytes contain lipid droplets (arrows) (sudan black stain (a) x 10 x 12.5 (b) x 40 x 12.5.

Fig. 8 : Frozen section in liver of Cat fish showing hepatocytes contain lipid droplets (arrows) (sudan black stain x 100x 12.5).

Fig. 9 : Paraffin section in liver of Nile telapia showing the arrangement of glycogen inside the hepatocytes (arrows) (Best's carmine stain x 100 x 12.5).

Fig. 10a, b : Paraffin section in liver of Cat fish showing hepatic artery (1), portal vein (2) and bile ducts (3) (H & E stain (a) x 40 x 12.5 and (b) x 100 x 12.5.

Fig. 11 : Paraffin section in liver of Cat fish showing the structure of bile ductules. Epithelium (1), smooth muscle (2) and connective tissue (3) (Crossmon's trichrome stain, x 40 x 12.5).

Fig. 12 : Paraffin section in liver of Nile telapia showing the structure of bile ductules, notice cilia (arrow) (PAS-Haematoxylin tech. x 100 x 12.5).

Fig. 13 : Paraffin section in liver of Cat fish showing the distribution of reticular fibres (arrows) (Gomri's tech. x 40 x 12.5).

Fig. 14 : Paraffin section in liver of Nile telapia showing reticular fibres around pancreatic acini (arrows). (Gomori's tech. x 40 x 12.5).

Fig. 15 : Paraffin section in liver of Cat fish showing adipose tissue between hepatocytes (arrow) (H & E stain x 40 x 12.5).

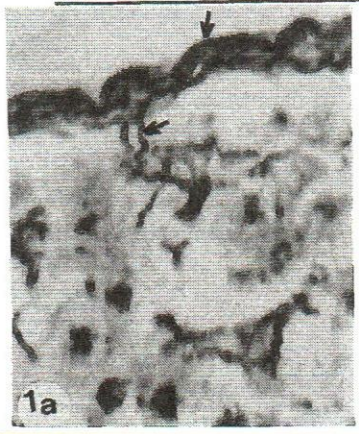
Fig. 16 : Paraffin section in liver of Cat fish showing melanomacrophage cells (arrow) and blood vessels (1). (H & E stain x 100 x 12.5).

Fig. 17a, b : Paraffin section in liver of Nile telapia showing the distribution of exocrine pancreas in the parenchyma of liver (arrows) around the central vein (1). (H & E stain, (a) x 4 x 12.5 (b) x 40 x 12.5).

Fig. 18 : Paraffin section in liver of Cat fish showing the pancreatic acini (arrows) (H & E stain, x 10 x 12.5).

Fig. 19 : Paraffin section in liver of Nile telapia showing the structure of pancreatic acini (1) notice the zymogen granules (arrows) (H & E stain, x 100 x 12.5).

Fig. 20a, b : Paraffin section in liver of Nile telapia showing acidophlic cells between pancreatic acini (arrows) and tissue macrophage cells (arrows heads), pancreatic acini (1), central vein (2) (H & E stain x 100 x 12.5).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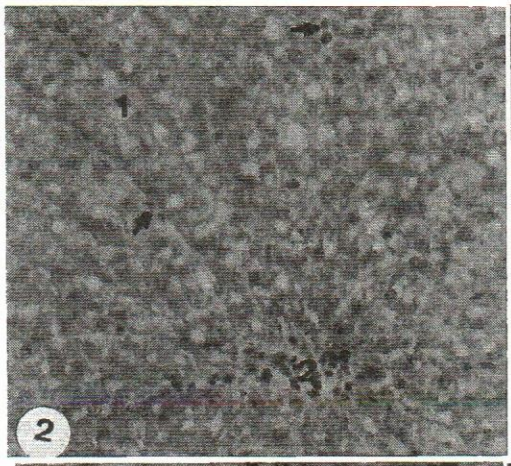
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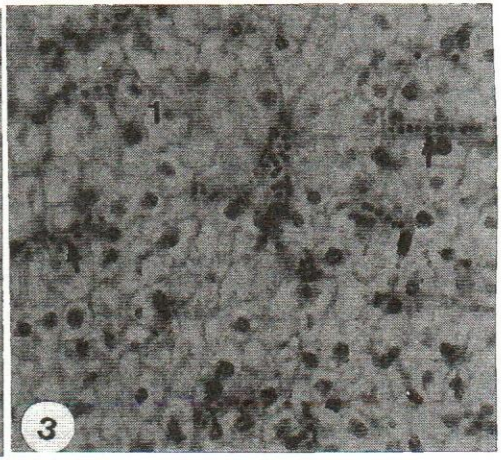
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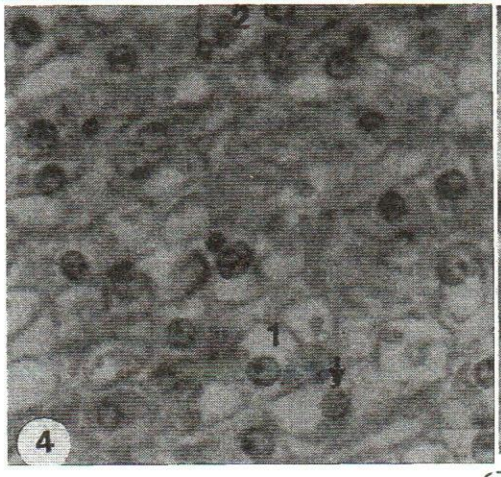
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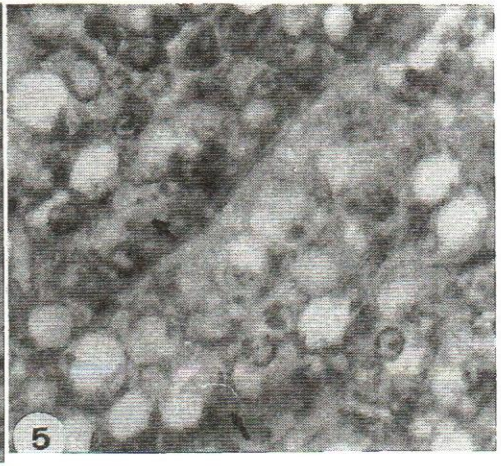
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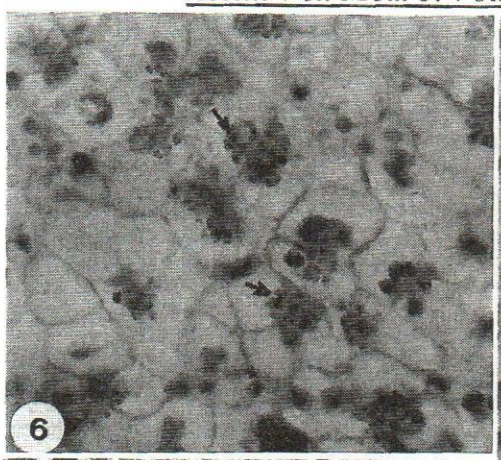
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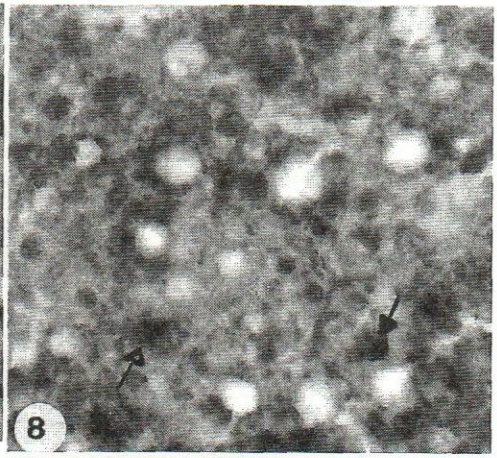
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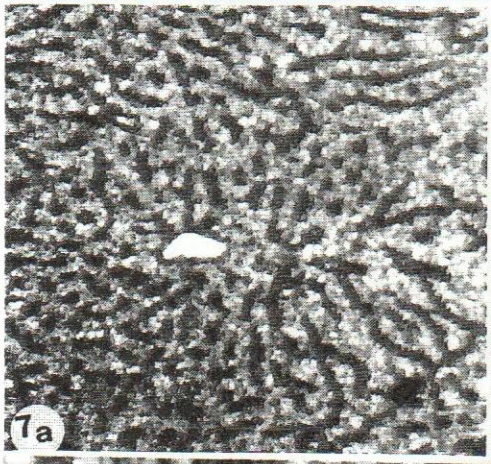
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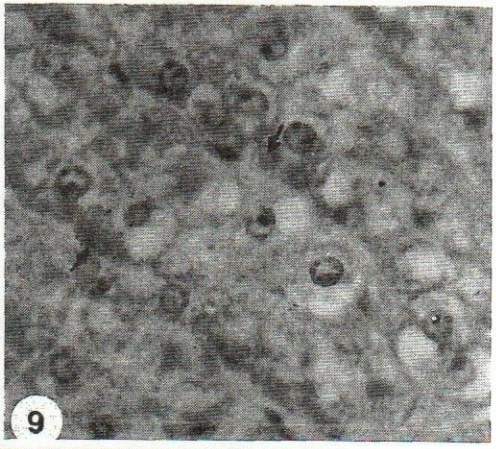
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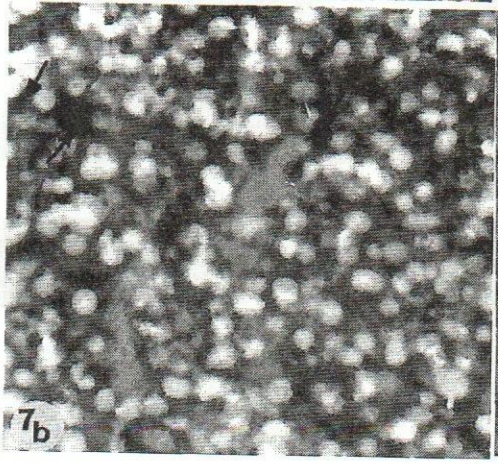
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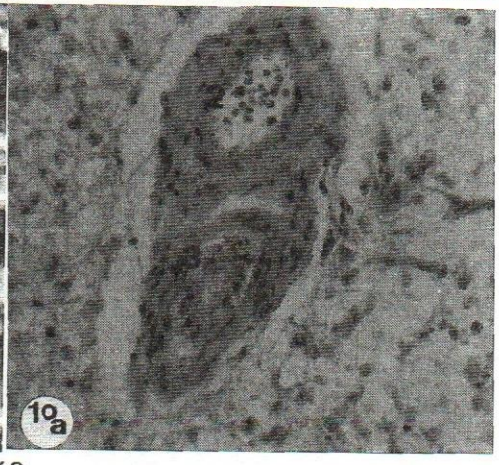
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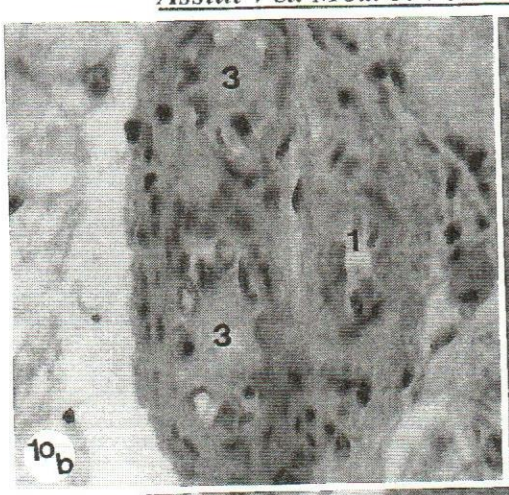

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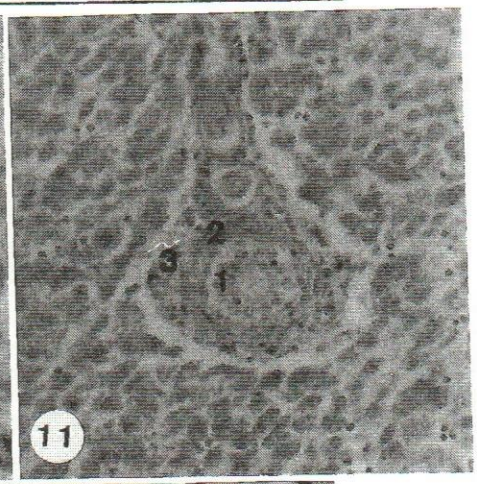

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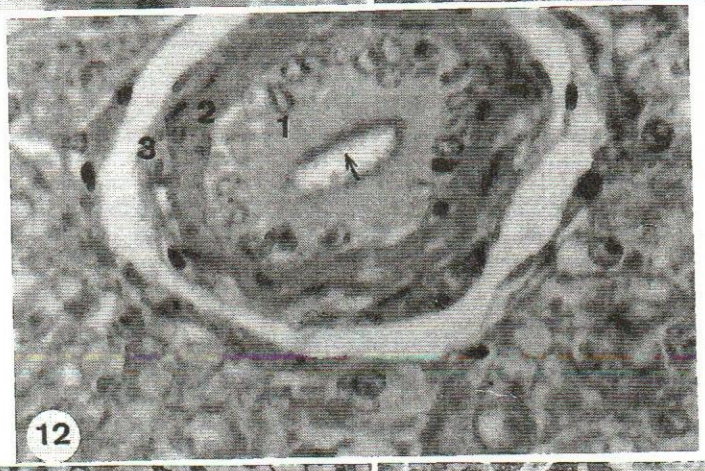

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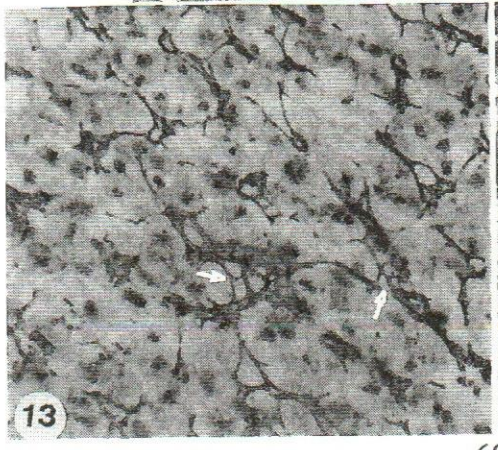
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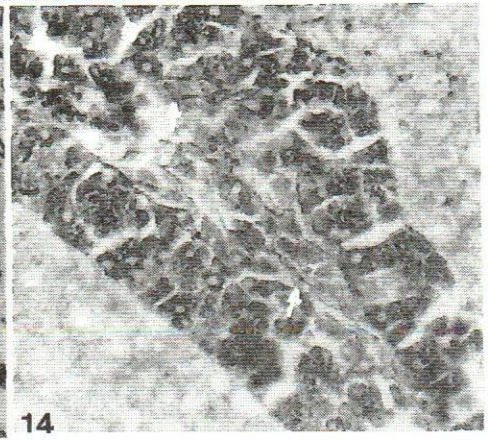
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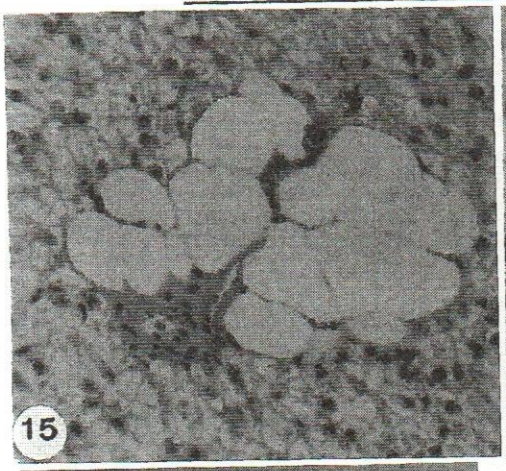
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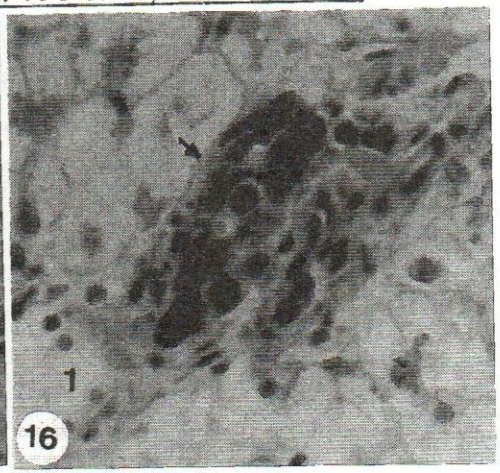

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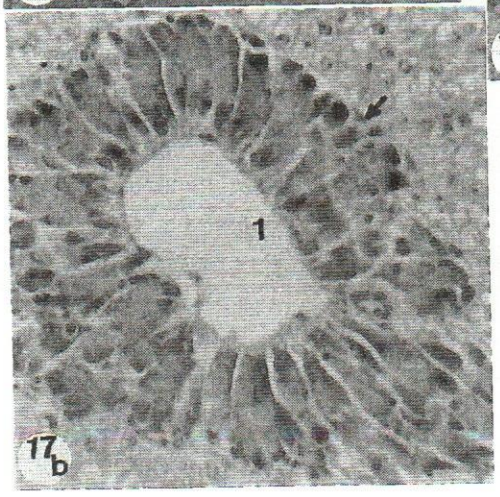

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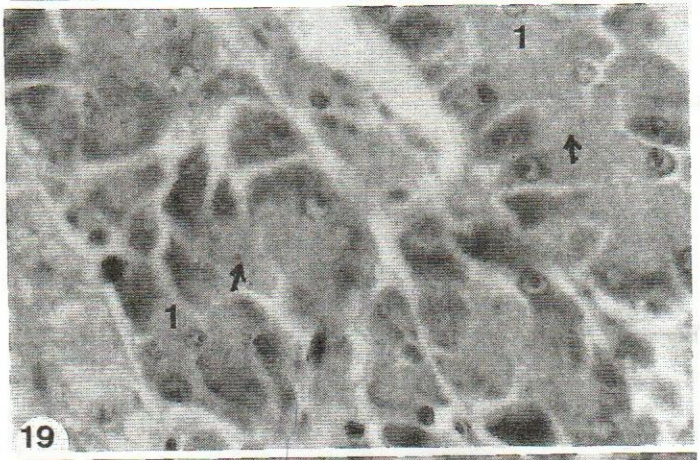
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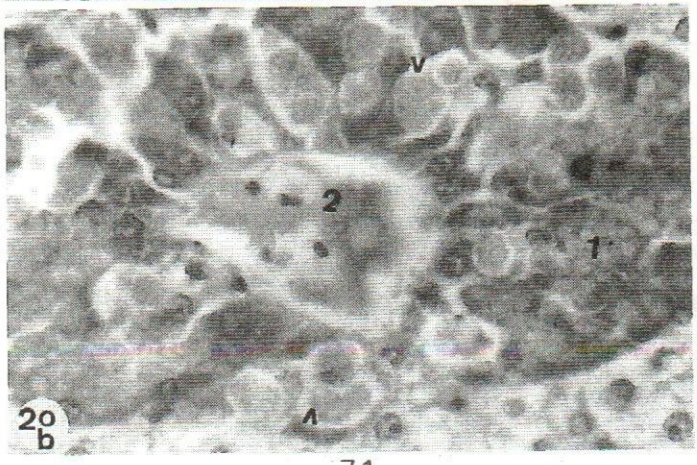
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