A LIGHT AND ELECTRON MICROSCOPIC STUDY ON THE LIVER OF EEL (ANGUILLA ANGUILLA L.)

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ABSTRACT

Liver specimens were obtained from six healthy adult male and female eels. The specimens were processed for light and electron (transmission) microscopic examination. The light microscopic observations revealed that, the liver of eel is covered by thin fibrous capsule formed mainly of reticular fibers. The hepatic parenchyma is not arranged in distinct lobules. The biliary channels and vascular elements did not exhabit the classical triads but seem to be randomly dispersed in biliary tracts, biliary-arteriolar tracts and venous-biliary-arteriolar tracts. Rather than the liver of most teleostis the liver of eel lacked pancreatic exocrine tissue (hepatopancreas). Electron microscopic observations revealed that cells of the liver differentiated to hepatocytes and non-hepatocytic cells. The hepatocytes are characterized by the presence of abundant rough endoplasmic reticulum, dense aggregates of glycogen and lipid droplets in their cytoplasm. The non-hepatocytic cells including firstly, sinusoidal cells, which are characterized by micropinocytotic activity. Secondly, perisinusoidal fat storing, Ito cells, which are the source of vit. A. Thirdly, interhepatocytic macrophages. There is no classical kupffer cells. The results were discussed on the light of available literature.

It was concluded that, the liver of eel have its own characteristic features, devoid of hepatic lobulation and hepatopancreas. Its cells are good source of glycogen, lipid and vitamin A.

INTRODUCTION

Marked attentions were focused on the liver of fish. This can be attributed to the fact that, the liver plays a major role in the process of vitellogenesis and in the energy production during spawning **(Wallace, 1978; Guebral, 1991 and Toru & Shozo, 1998)**.

Recently, the fish importance increased as a model to determine the environmental pollution. The liver as a target organ is used as biomarker for determining environmental quality **(Malins & Haimanot, 1991 and Gonzalez, et al., 1993).**

The histological study of the liver in fish was described by many authors **(Groman, 1982)**, striped bass **(Grizzle & Rogers, 1976)**, catfish **(Rocha et al., 1995 & 1997)** trout and **(Nelle et al., 1990)** Tilapia.

Eels live in rivers through out Europe and in North Africa, grow in fresh water, when reach sexual maturity make their way to river mouth and swim together to their spawning grounds in salt water sea **(Hisek, 1987)**. The available literatures have little information about the histological structure of the liver of eel.

So, the present study aimed to establish the normal histological structure of this organ in eel when present in Egyptian sea water.

MATERIAL AND METHODS

Liver specimens were obtained from six adult male & female healthy eels. Their body length ranges from 50-55 cm. The fishes were caught from Manzalla lake in March. Their liver were excised. For light microscopic examination, paraffin sections were prepared, stained with haematoxylin and eosin (H & E). Crossmon's trichrome and Gomori's reticulin stains (Bancroft and Stevens, 1990). For electron microscopic examination, very small pieces of liver were immediately fixed in 2.5% cold glutaraldehyde in phosphate buffer (pH 7.2) for 24 hours. Then post fixed in 1% cold osmium tetraoxide in phosphate buffer (pH 7.2) for 4 hours. The specimens then were dehydrated in graded alcohol and embedded in araldite resin (Hayat, 1989). Semi-thin section (1μm in thickness) were stained with 1% toluidine blue to select the field for ultrathin sections, mounted in grids, stained with uranyl acetate and lead citrate and examined by JEOL 100 C x transmission electron microscope.

RESULTS

I- Light microscopy:

The liver of eel is formed of reticulotubular gland which is covered with thin fibrous capsule formed mainly of reticular fibers. The hepatic parenchyma is not arranged in distinct lobules, that are appearing quite continuous. The radial pattern of cellular plates recognize the units of structure and assign imaginary boundaries to the lobule by relaying upon the regularly distributed central veins as a landmarks (Fig. 1).

Hepatocytes were observed as large, polyhedral cells with spherical single nucleus and prominent nucleolus, the cytoplasm is vacuolated (Fig. 2).

The biliary channels and vascular elements seem to be randomly dispersed throughout the parenchyma. The stromal components are three dimensionally arranged as venous - biliary - arteriolar tracts (VBAT) (Fig. 3) & biliary - arteriolar tracts (BAT) (Fig. 4) and biliary tract (BT) (Fig. 2). The hepatic tracts are surrounded by a moderate amount of connective tissue (Fig. 3). The medium- sized bile duct is lined by cuboidal epithelium (Fig. 2), while the large ones were lined by columnar epithelium with basally situated nuclei. The lamina epithelialis is followed by connective tissue layer and thick layer of smooth muscle fibers (Fig. 4).

Hepatic sinusoids were irregular in shape distributed in between hepatocytes forming an extensive network (Fig. 1). Gomori's sliver stain visualized a reticulum framework made up of argyrophilic inter-related fibers outlining the hepatic sinusoids (Fig. 5).

The liver of eel lacked pancreatic exocrine tissue (Hepatopancreas).

II-Electron microscopy:

A-Hepatocytes:

They were polygonal in shape. The nuclear information of hepatocytes showed large, spherical nuclei with few scattered euchromatin and aggregated peripheral heterochromatin (Figs. 6 & 7). They contained prominent nucleoli (Fig. 6). Rough endoplasmic reticulum was present as abundant cisternae and in parallel form, appeared all over most of cytoplasm (Figs. 6 & 8). Mitochondria exhibited large ovoid shape and were distributed haphazard in hepatocytic cytoplasm (Fig. 6). Golgi apparatus is made up of three to four closed parallel arrays. The ends of the cisternae are oftenly dilated (Fig. 7).

The cytoplasm of hepatocytes presents an extremely variable appearance. The principal source of variation is the content of stored material, glycogen and lipids. The glycogen appeared in the cytoplasm as a dense aggregates (Fig. 7), which is not uniformly distributed. Lipid appears in the form of osmiophilic (non membrane bounded) droplets of varying size, they are few small droplets (Fig. 7). Sometimes large droplets (Figs. 6 & 8). Spaces of Disse were located between blood sinusoids and perisinusoidal hepatocytes (Fig. 8).

B- Non-hepatocytic cells :

1- Sinusoidal cells (endothelium) :

The hepatic blood sinusoids is lined by one cell type only, the endothelial cell. It is formed of

an attenuated cytoplasmic sheet, which delimite space of Disse (Fig. 8), nuclei were generally bulging into the lumen. Numerous pinocytotic and micropinocytotic vesicles were observed indicating high endocytotic and/or exocytotic activity (Fig. 9). Reticulin fibers are attached to the endothelium by fine, fibrillar meshwork which filled the space of Disse (Fig. 8).

2-Perisinusoidal Ito cells (fat storing, perisinusoidal cells) :

Ito cells present immediately underneath the endothelial lining. The nuclei tend to be less elongated with deep indentation. Osmiophilic lipid droplets (non membrane bounded) were observed, rough endoplasmic reticulum and ribosomes also demonstrated. No junction complex between Ito cells and endothelial cells were observed. Cell membrane showed microvesicular exocytotic activity into blood sinusoids (Fig. 9).

3-Macrophages:

Interhepatocytic macrophages were located among hepatocytes. The nucleus is large lobulated with abundant euchromatin. The cytoplasm contained few mitochondria and rough endoplasmic reticulum. Electron dense bodies of different diameter, phagosomes and lysosomes were demonstrated (Fig. 10).

DISCUSSION

The obtained results revealed that, the fibrous stroma of the liver was formed mainly of reticular fibers. Similar findings were reported by **Speilberg et al. (1994)** in Atlantic salmon.

In the present study, the hepatic parenchyma is not arranged in distinct lobules. The same result was reported in several fish species **(Grizzle and Rogers, 1976)** in Cat fish, **(Groman, 1982)** in striped bass, **(Speilberg et al., 1994)** in Atlantic salmon, **(Nell et al., 1990)** in Tilapia. In contrast, **Timashova (1981)** reported that liver of placia has well-developed connective tissue fibers, clearly demarkate the parenchyma into distinct lobules.

Regarding the arrangement of hepatocytes, they are radially organized in plates around central vein. Different opinions were reported, the same observation was reported by **Anderson & Mitchum (1974)** in trout, **Salama (1995)** in carp. However, hepatocytic arrangement were recorded as circular profiles in rainbow trout **(Hampton et al., 1989)**, branching and anastomosing cellular tubules in Atlantic salmon **(Speilberg et al., 1994)**.

The present investigation revealed that, the hepatocytes in general are large polyhedral cells with spherical nucleus having prominent nucleolus. The cytoplasm is vacuolated. **Grizzle & Rogers (1976)** suggest that these vacuoles in liver of catfish results from removal of glycogen and fat in routine paraffin preparation.

Despite of the absence of hepatic lobulation in this study, the biliary channels and vascular elements seem to be randomly dispersed throughout the parenchyma as (VBAT), (BAT) and (BT). Rather than hepatic triads which were reported in several fresh and salt water fishes **(Timashova, 1981; Leatherland & Sonstegard, 1988 and Howse et al., 1992)**, the term BAT was first introduced for fish liver histology by **Hampton et al., (1988) and Rocha et al. (1994)** in trout. It has been reported that, large diameter veins are occasionally associated with small bile channels and arteriole (VBAT) **(Schar et al., 1985; Hampton et al., 1989 and Rocha et al., 1995)** in trout.

The present study revealed that medium and large sized bile ducts were lined by cuboidal to columnar epithelium in parallel to results of **Hampton et al. (1988)** in trout.

The present investigation revealed that, the liver of eel lacked pancreatic exocrine tissue (hepatopancreas). This result is in agreement with the results of **Eastman and Devries (1997)** in Antarctic notothenids. On the other hand, hepatopancreas is a specific character of the liver of most fresh and salt water fish species **(Hinton et al., 1972)** in large mouth bass, **(El-Habbak, 1995)** in Tilapia.

The electron microscopic observation of the present study revealed that, the functional units of the liver formed of numerous cell types, which are differentiated to hepatocytes and non-hepatocytic cells (sinusoidal, perisinusoidal cells and macrophages). Hepatocytes exhibited abundant cisternae of rough endoplasmic reticulum, dispersed allover the most of cytoplasm and associated with free ribosomes, this result in close parallel to results of **Takahashi et al. (1977)** in salmon. However, little amount cisternae were reported in Zebra fish **(Peute et al., 1978)**.

Lipid droplets and glycogen were demonstrated with variable degrees in the cytoplasm of hepatocytes. The stored material reflects to some extent the functional state of the cells **(Santos, 1995)** in marine fish. The present study revealed large glycogen deposits similar to that observed in Atlantic croaker, rainbow trout **(Eurell & Haensly, 1982 and Hampton et al., 1989)**. The present study demonstrates lipid as osmiophilic droplet of varying size. The cytological demonstration of the liver of fish species revealed few droplets of lipid in hepatocytes of salmon **(Leatherland & Sonstegard, 1988)**. However, lipid form large mass in Antarctic fish **(Eastman & Devries, 1981)**.

The present investigation demonstrates non-hepatocytic cells; firstly, the sinusoidal endothelial cells, which showed micropinocytotic vesicles. This is indicating the high endocytosis and/or exocytotic activity. It has been demarkated by **McCuskey et al. (1986)**. Secondly, Ito perisinusoidal cells, these cells were clearly distinct from the adjacent cells by their situation,

absence of microvilli, nuclear deep indentation and presence of lipid droplets. Ito cells have no junction between them and endothelial cells. **Rocha et al. (1997)** in brown trout described Ito cells as fat storing cells and source of vit. A. **Eng and Youson (1992)** added that Ito cells implicated in collagen production, responsible for fibrosis associated with natural degeneration of biliary tree in brook lamprey. Thirdly, the interhepatocytic macrophages, which are situated in between hepatocytes. No classical kupffer cells were apparent. Also kupffer cells were not seen in some fish species, rainbow trout **(Hampton, et al., 1989)** and Atlantic salmon **(Speilberg, et al., 1994)**. However, kupffer cells have been encountered in cat fish **(Hinton and Pool, 1979)**, cod **(Fujita et al., 1986)**. The authors agreed that the kupffer cells were occasionally observed between the endothelial cells blocking the sinusoidal lumen.

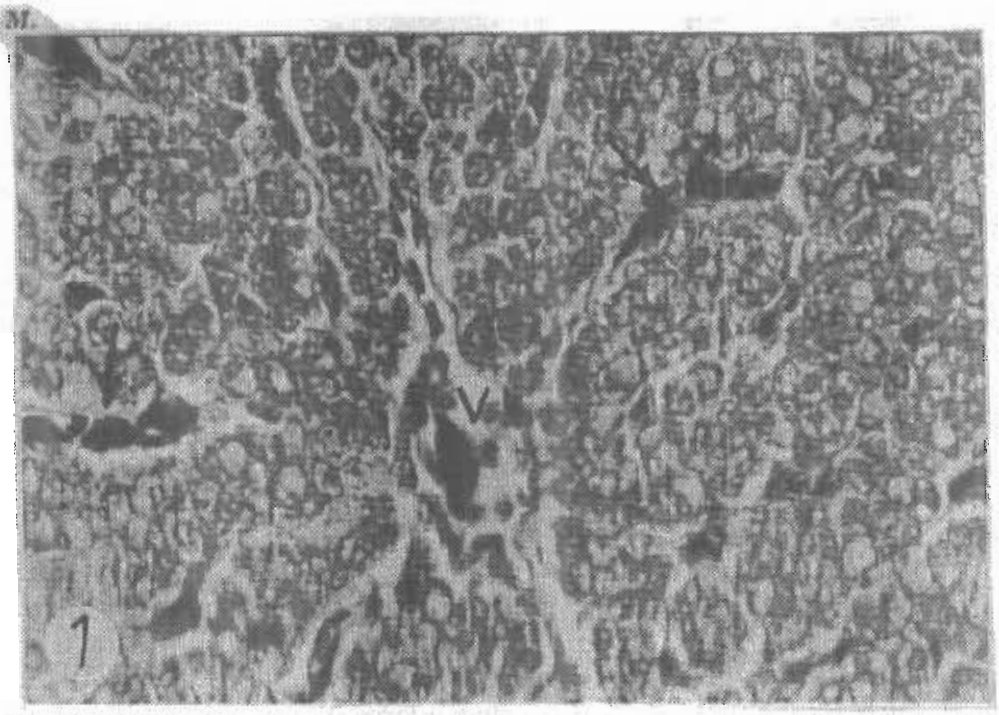

Fig. (1) : A light micrograph of the eel liver showing, the arrangement of hepatic parenchyma into cellular plates radiating from the central vein (v), notice irregular shaped, distributed hepatic sinusoids (arrows). (Crossmon's, trichrome st., Oc. X 10 & Obj. x 10).

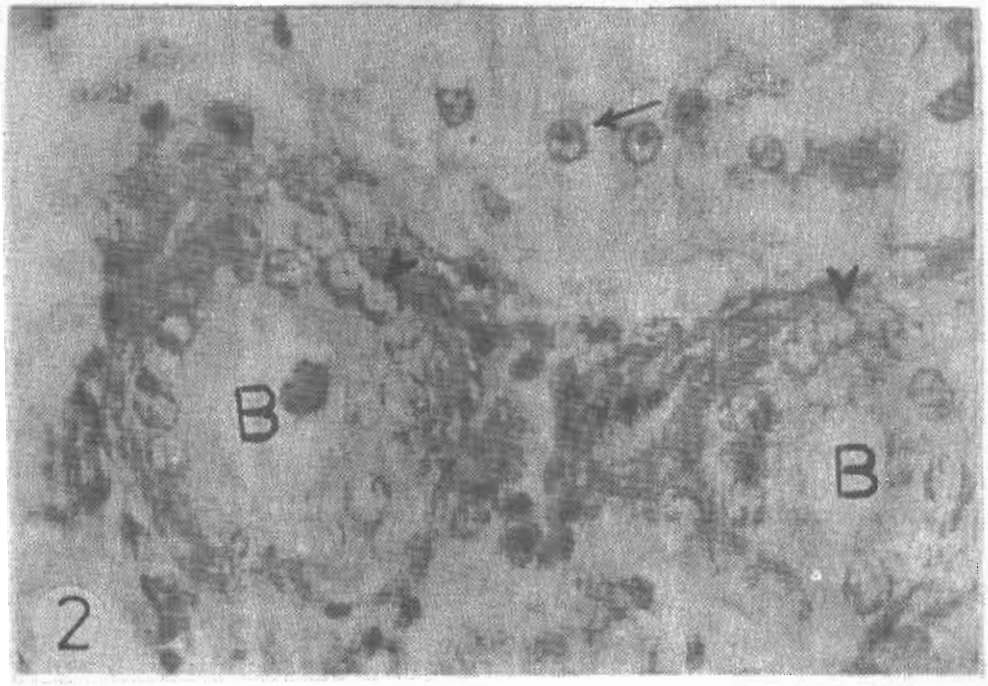

Fig. (2) : A light micrograph of the eel liver showing, large polyhedral hepatocytes (arrow) with spherical nucleus, prominent nucleolus and vacuolated cytoplasm. A large biliary tract holding two medium sized biliary ducts (B). The latters are lined by cuboidal cells (arrow heads). (H & E, st., Oc. X 10 & Obj. x 40).

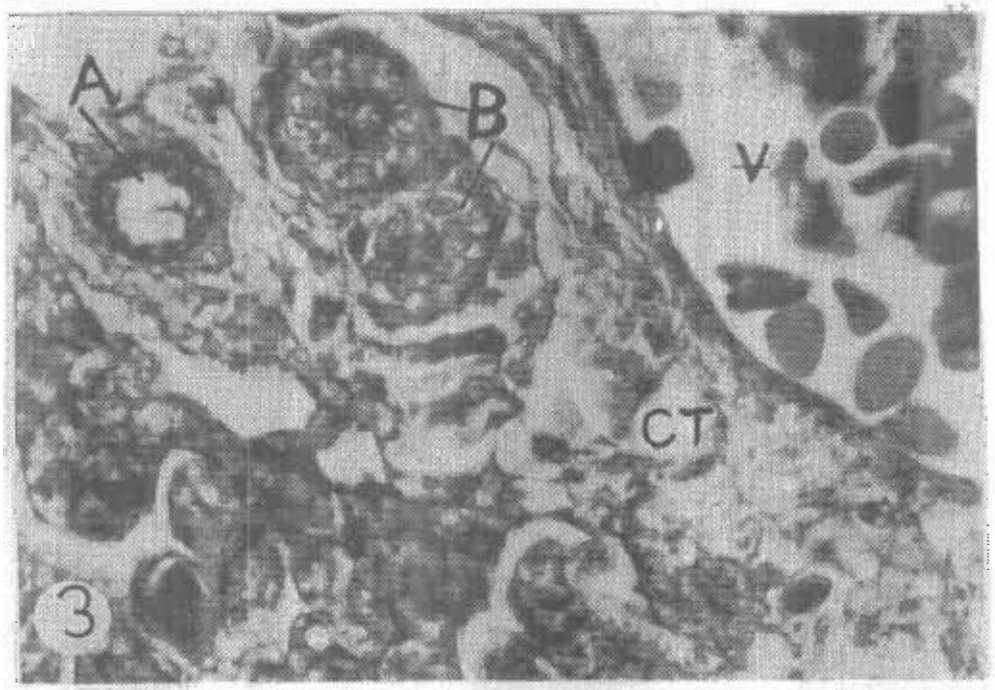

Fig. (3) : A light micrograph of the eel liver showing, a large (VBAT) displaying one vein (v), small biliary ducts (B) and arteriole (A). The ducts and vessels surrounded by a moderate amount of connective tissue (C.T) (Crossmon's trichrome st., Oc. X 10 & Obj. x 100).

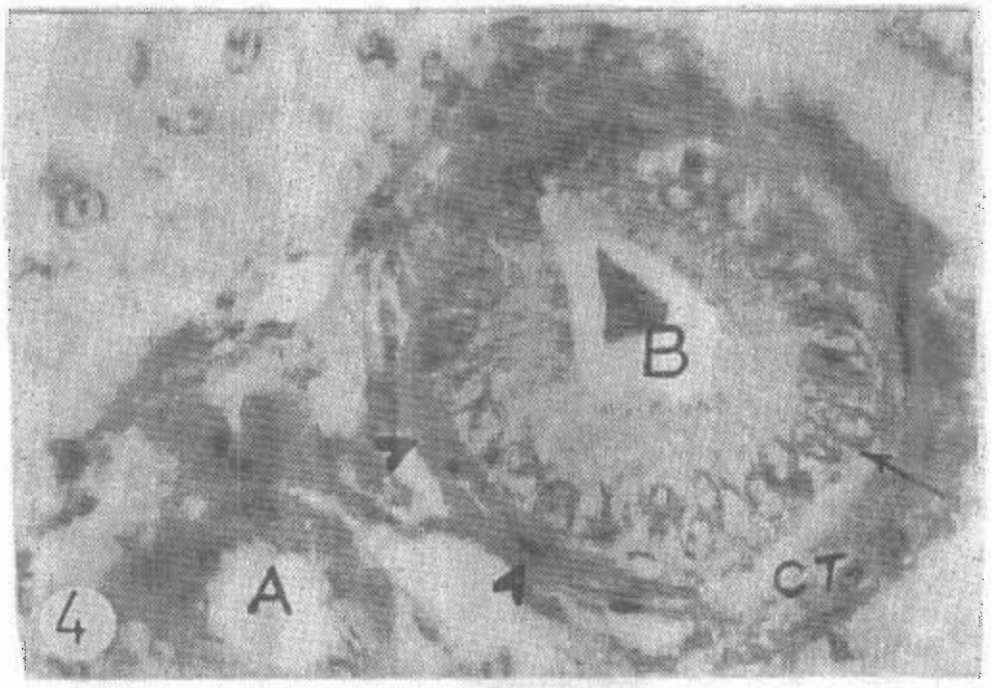

Fig. (4) : A light micrograph of the eel liver showing, aspect of bigger (BAT) holding large biliary duct (B) and arteriole (A). The bile duct is lined by high columnar epithelium with basally situated nucleus (arrow) followed by connective tissue sheath (C.T) and smooth muscle fibers (arrow heads) (H & E st., Oc. X 10 & Obj. x 100).

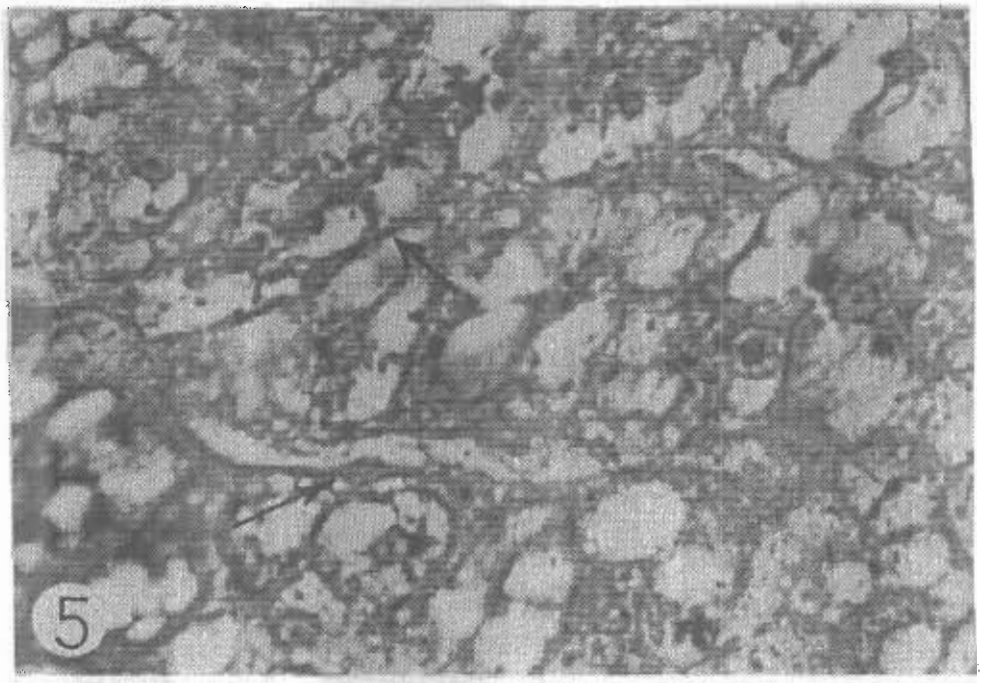

Fig. (5) : A light micrograph of the eel liver showing, arygrophilic reticular fibers (arrows) outline the hepatic sinusoids (Gomori's Silver st., Oc. X 10 & Obj. x 100).

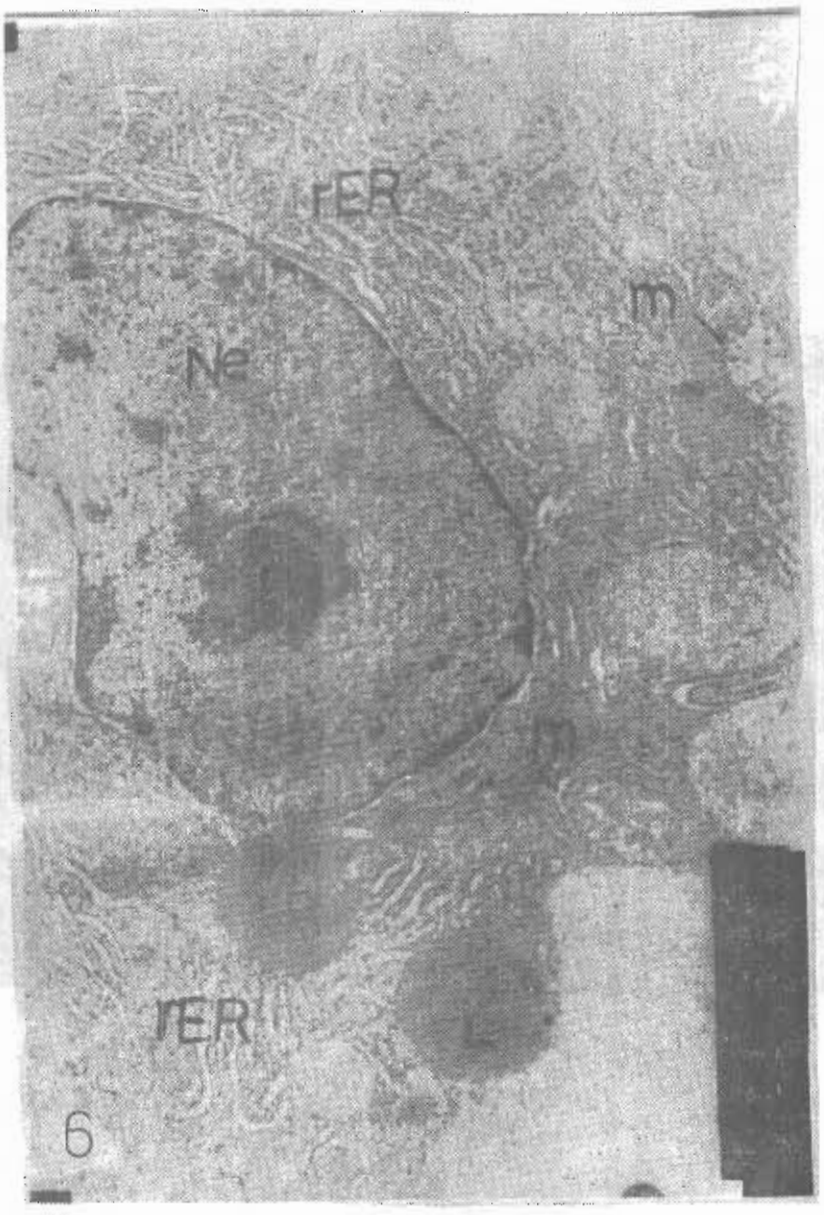

Fig. (6) : An electron micrograph of the eel liver showing, a hepatocyte having an euchromatic nucleus (Ne) with prominent nucleolus (n), proliferative rough endoplasmic reticulum (rER), mitochondria (m) and lipid droplets (L). (Uranyl acetate-lead citrate, X 12,000).

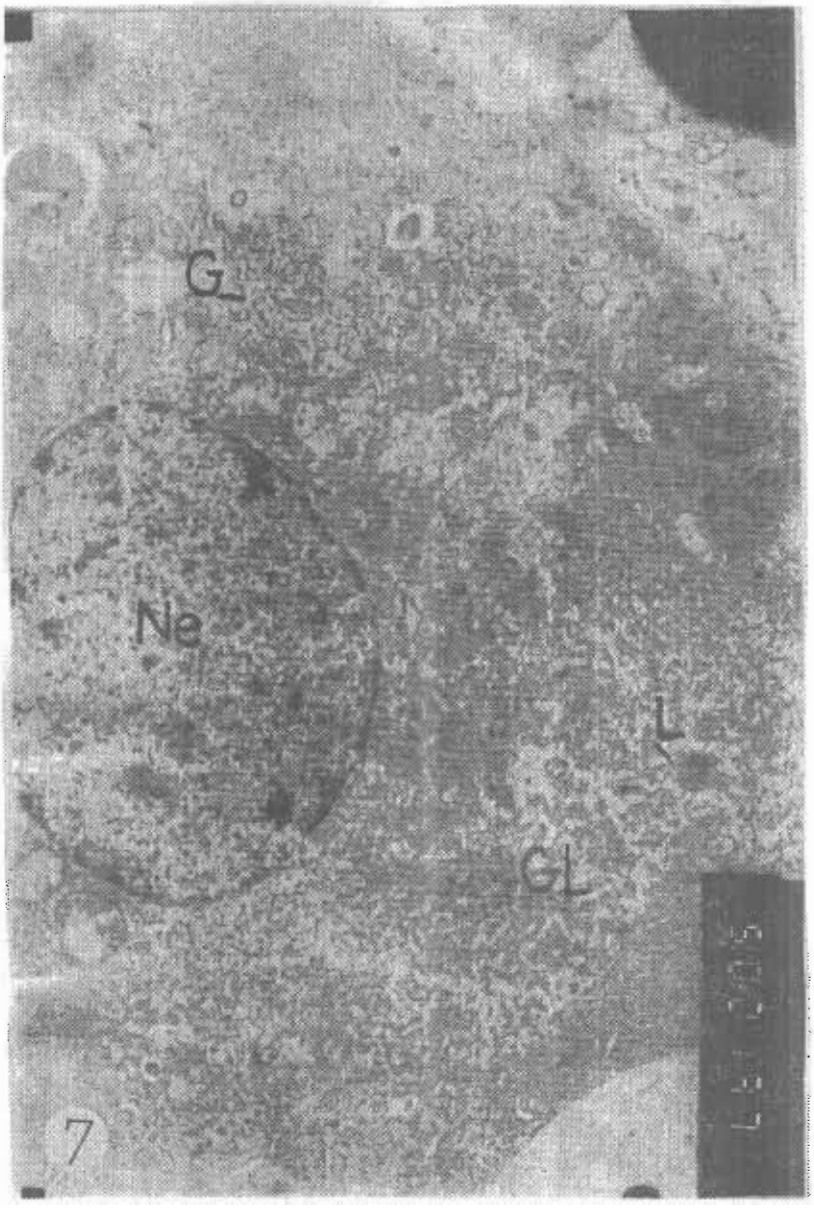

Fig. (7) : An electron micrograph of the eel liver showing, a hepatocyte with a large spherical euchromatic nucleus (Ne), profiles of Golgi apparatus (G), glycogen depots (GL) and few small lipid droplets (L). (Uranyl acetate-Lead citrate X 10,000).

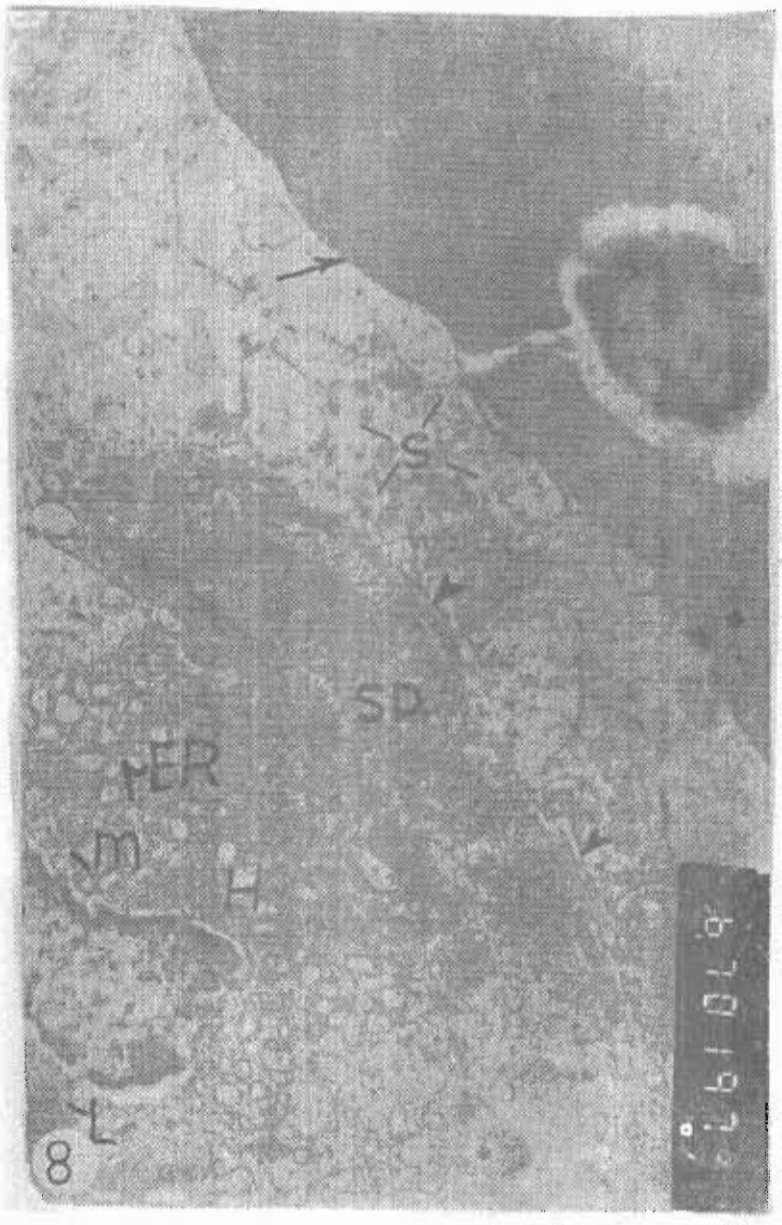

Fig. (8) : An electron micrograph of the eel liver showing, blood sinusoid(s) contained red blood cell (arrow) and lined by flattened extenstons of endothelial cells (arrow heads) which delimite the space of Disse (SD) filled with reticulin fibers meshwork attached to the endotheliu... Notice perisinusoidal hepatocyte (H). Its cytoplasm is filled with rought endoplasmic reticulum (rER), few mitochondria (m) and lipid droplets (L) (Uranyl acetate-lead citrate, X 12000).

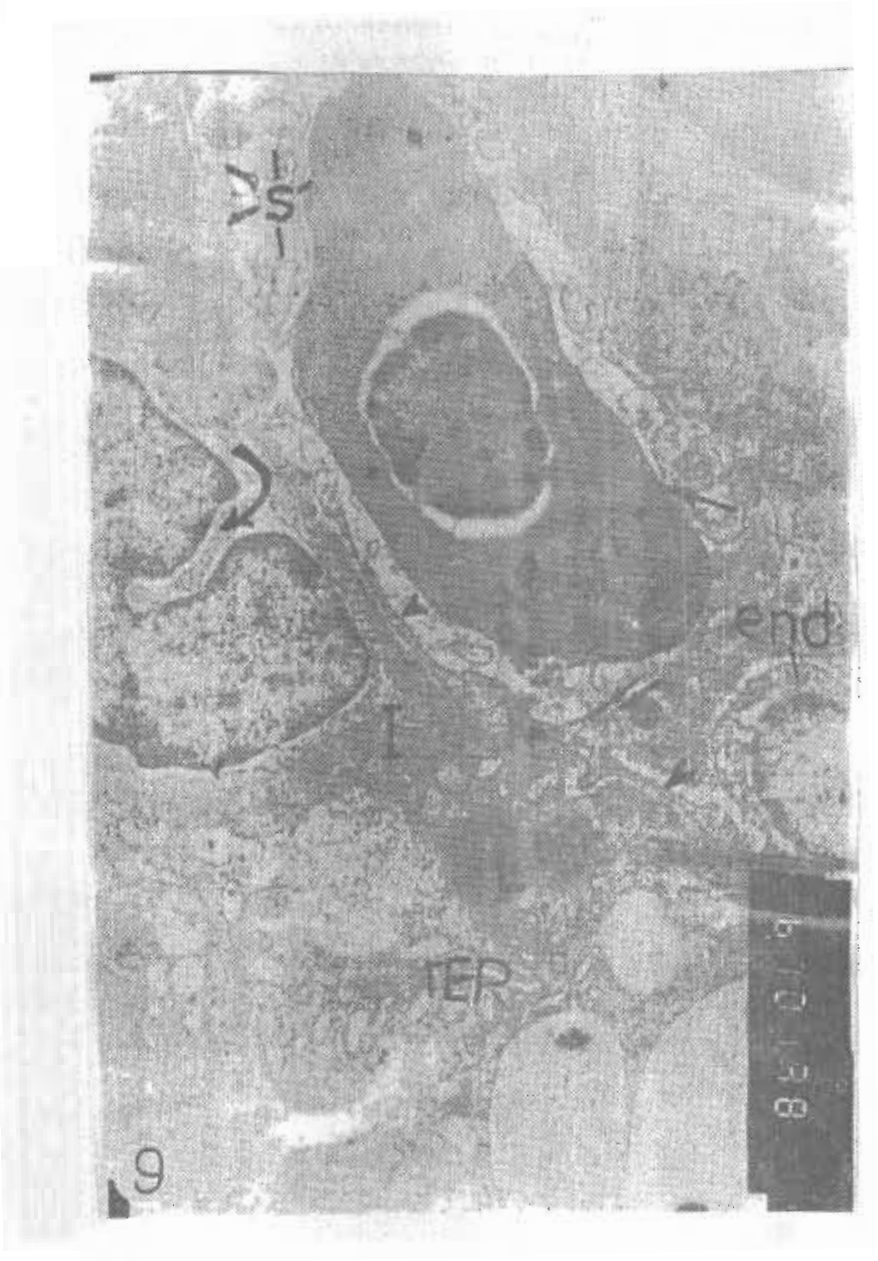

Fig. (9) : An electron micrograph of the eel liver showing, blood sinusoids (S) containing red blood cell (arrow). An endothelial cell (end) with attenuated cytoplasmic sheet (arrow heads). Notice perisinusoidal Ito cell (I). Its cytoplasm contains lipid droplets (L), cisternae of rough endoplasmic reticulum rER. The nucleus has a deep indentation (curved arrow). Membranous micropinocytotic vesicles were observed (double arrow). (Uranyl acetate-Lead citrate X 12,000).

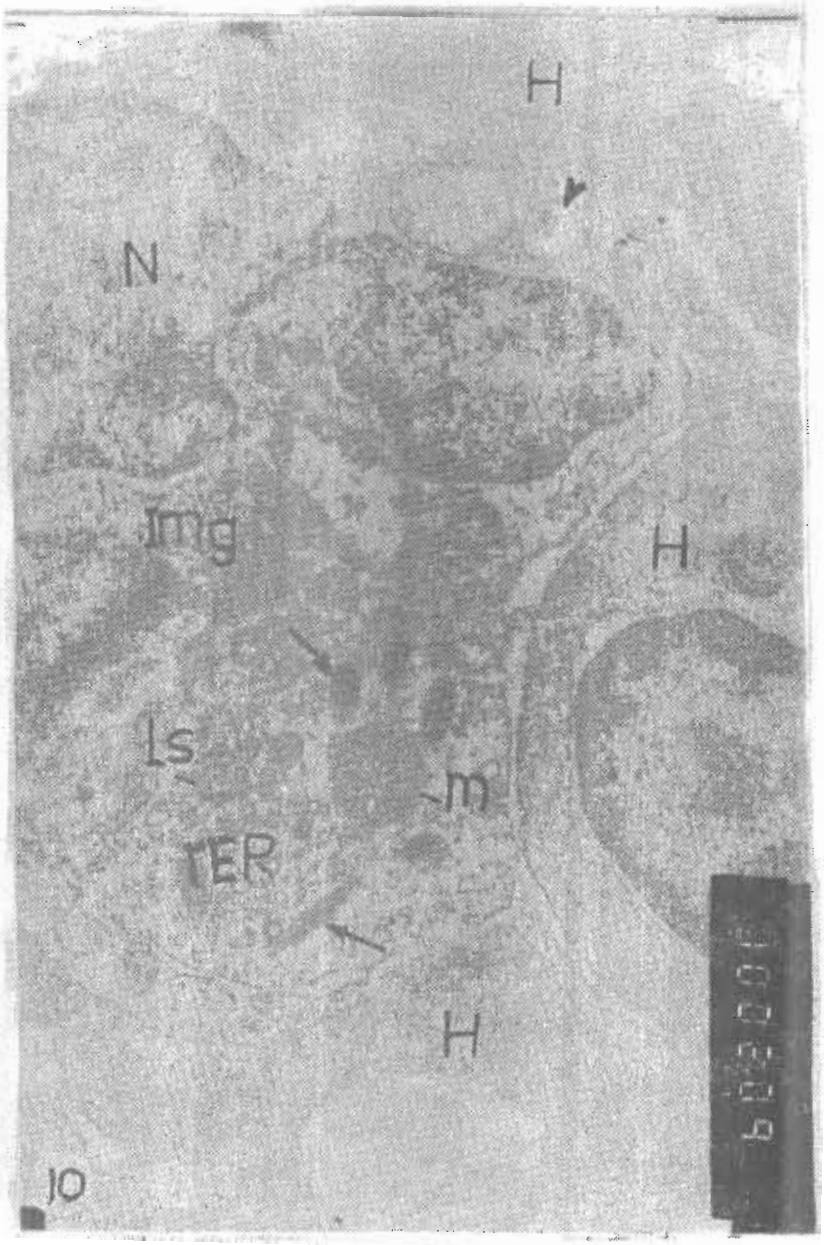

Fig. (10) : An electron micrograph of the eel liver showing, interparenchymal macrophages (I mg) in between hepatocytes (H). The macrophages have a large lobulated nucleus (N). The cytoplasm contained large mitochondria (m), rough endoplasmic reticulum (rER) lysosomes (Ls), electron dense bodies with different diameters (arrows) and phagosome (arrow head). (Uranyl acetate-lead citrate, X 20,000).

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الملخص العربى

دراسة بالميكروسكوب الضوئى والإلكترونى على الكبد فى سمك الثعابين

سوسن محمود غطاس

قسم التشريح والهستولوجيا - كلية الطب البيطرى - جامعة طنطا فرع كفر الشيخ

لقد أستخدم فى هذا البحث عدد ستة من إناث وذكور أسماك الثعابين البالغة، وقد جهزت العينات للفحص بالميكروسكوب الضوئى والميكروسكوب الإلكترونى (النفاذى).

وقد أوضحت النتائج بالميكروسكوب الضوئى أن الكبد فى أسماك الثعابين كانت محاطة بحافظة رقيقة من الألياف الشبكية، وكانت البرانشيما الكبدية غير مقسمة إلى فصيصات، ولم تسلك القنوات المرارية والوعائية نظام الثلاثيات المعتاد، ولكنها وزعت بطريقة عشوائية إما فى مدد مرارى شريانى أو مدد مرارى أو مدد شريانى وريدى مرارى.

وعلى خلاف الكبد فى غالبية الأسماك العظمية لم يحتوِ الكبد فى أسماك الثعابين على أنسجة حويصلات بنكرياسية ذات الإفراز الخارجى (بنكرياس كبدى).

كما أظهرت النتائج بالميكروسكوب الإلكترونى أن الخلايا فى كبد أسماك الثعابين قد تنوعت إلى :

(أ) الخلايا الكبدية : والتى تميزت بوجود شبكة أندوبلازمية خشنة وغزيرة فى السيتوبلازم، كذلك إختزنت الجليكوجين والدهون فى تجمعات كثيفة.

(ب) الخلايا الغير كبدية فقد تضمنت :

١- خلايا جدارية وعائية أظهرت نشاطاً إفرازياً حويصلياً دقيقاً.

٢- خلايا جار وعائية وهى خلايا ايتو المخزنة للدهون وهى مصدر لڤيتامين "أ".

٣- خلايا بالوعة بينية.

ولم تظهر النتائج وجود خلايا kupffer المعتادة.

وقد نوقشت النتائج على ضوء الأبحاث المتاحة.

والخلاصة :

أن الكبد فى سمك الثعابين له خواص ذاتية، وهى الخلو من التفصيص الكبدى، والبنكرياس الكبدى وخلاياه مصدر جيد للجليكوجين والدهون وفيتامين "أ".