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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE LIVER OF WEASEL (MASTELLA NIVALIS)

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ABSTRACT

Microscopical anatomy study of liver of weasel (Mustella nivalis) refers that, liver structure exhibit the normal lobular structure of mammalian liver, but the outline of each lobule is indistinct due to the presence of poor connective tissue partitions at the boundary. The parenchyma consists of anastomosing plates of hepatic cells, each plate being one cell thick. These plates are arranged radially around a central blood vessel. Wide sinusoids lie in the spaces enclosed between hepatic plates. Two types of cells lining the sinusoids, dilicate endothelium and numerous demarkated Von Kupffer cells. Hepatocytes are polyhedral in shape, with more eosinophilic cytoplasm and well demarkated membranes. Their nuclei are of the condensed type. Histochemical investigations referred to, that the carbohydrate content was found to be high in liver cells of weasel and that glycogen particles were densely accumulated in centrolobular areas. Hepatocytes were intensely stained with bromphenol blue stain exhibiting their high content in proteinic material. A high activity

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of acid phosphatase enzyme was restricted to kupffer cells and a granular staining (lysosomes) were seen in the cytoplasm of hepatocytes. Also, a high activity of alkaline phosphatase was found along the sinusoidal borders of hepatocytes in different areas.

INTRODUCTION

Anatomy, morphology and the fine structure of the parenchymal cells of the liver of several mammalian species and human have been described in details (Elias, 1952 ; Elias and Bengelsdorf, 1952 ; Deams, 1961 ; Rouiller and Jezequel, 1963 ; Wood, 1963 ; Bruni and Porter, 1965 ; Shnitka, 1966 ; Stein et al, 1966 and Flaks, 1971).

In the recent years the pig has been increasingly used as an experimental animal, especially in the field of liver transplantation (Hunt, 1967 and Hobbs et al, 1968) owing to its similarity to man in having no hepatic vein sphincters, in being omnivorous, and in having a similar blood biochemistry (Peacock and Terblanclie, 1967). Also, in nowadays there has been a growing interest in the histochemical demonstration of enzymes in mammalian tissues (Novikoff, 1959 ; Wachstein, 1959 ; Thorbecke et al 1961 ; Manns and Mortimer, 1969 and Sasse et al, 1975).

The present report gives an account of the morphological structure of weasel liver, together with several

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histochemical investigations considering that weasels are differ from the common experimental mammalian species, in being carnivorous animals.

MATERIAL AND METHODS

Liver samples were taken from the largest lobes of the livers of three healthy weasels (Mustella nivalis), as quickly as possible after killing them, some specimens were fixed in Bouin's fluid and stained with haematoxyline and eosin for the histological study. Other specimens were fixed in carnoy's fluid for histochemical demonestration of carbohydrates and protein materials using Periodic Acid Schiff's and mercuric bromphenol blue methods (Mazia et al, 1953). In addition other samples of liver tissue were freshly sectioned using the cryostat for the demonstration of acid and alkaline phosphatases activities according to Gomori (1952).

RESULTS

Histological results:

The liver of weasel exhibits the common normal lobular structure of mammalian liver, but the interdigitiation of the terminal branches of portal and venous systems gives

rise to a repeating pattern of structural units. The outlines of lobules in the liver of weasel are indistinct due to the presence of poor connective tissue partitions at their boundries.

Liver cells are found in the form of hepatic plates. Each plate is one cell thick. The hepatic plates are extended, anastomosed with each other and arranged in radiating manner around the central blood vessel (Fig.1).

A network of wide blood sinusoids which enclose inbetween hepatic plates is very clear. Two types of cells are distinguished in the sinusoidal lining, a dilicate endothelium, the cells which have small dark nuclei and their cytoplasm extending as thin film along the sinusoids; the other type of cells includes the phagocytic stellate cells of Von Kupffer which are numerous in number and distinctly larger than the former cells (Fig.2).

Hepatocytes appear polyhedral in shape, large in size and limited by distinct membranes. The cytoplasm of hepatic cells is prominently eosinophilic and is more granulated. Each hepatocyte has a centerally placed nucleus. The nuclei are densely stained, spherical in shape with well demarkated boundaries. Most nuclei are of the condensed type where chromatin particles are lying mainly free in the nucleoplasm

with large particles lining the nuclear membrane (Fig. 2). Small spherical and dense centric nucleoli are seen in most nuclei of hepatic cells (Fig. 3).

Liver of weasel is invested by the outer tunica serosa which is derived from the peritoneum and encloses a delicate connective tissue capsule (Fig. 4).

Histochemical results:

i. General proteins:

Hepatic cells exhibit a considerable amount of proteinic material. Cytoplasm of hepatocytes is densely and homogenously stained with bromphenol blue stain. Each hepatocyte is limited by intensively stained plasma membrane. The nuclei appear condensely stained, each of which contains positively stained chromatin particles and weakly stained nucleolus. (Fig. 5)

Some hepatocytes contain a number of clear unstained vacular areas. Kupffer cells as well as the endothelial lining of blood sinusoids are moderately stained with bromphenol blue (Fig. 5).

ii. Carbohydrates:

Glycogen content was found to be high in the liver

of weasel. The centrolobular area exhibit high accumulation of glycogen material than other regions (Fig 6).

Glycogen particles are more scattered in the cytoplasm of hepatic cells in the form of small to large sized dense granules and mases. The perinuclear position shows more concentration of glycogen material. The nuclei of hepatocytes are negatively stained. The walls of blood vessels as well as surrounding tissues are weakly stained (Fig. 7).

iii. Enzymes:

- Acid phosphatase:

High activity of acid phosphatase enzyme was similar throughout the different areas. The walls of blood vessels were positively stained (Fig. 8).

Marked activity was restricted to kupffer cells lining the blood sinusoids. Also, dilicate endothelial cells were positively stained. Enzyme activity was seen in the cytoplasm of hepatocytes as dense fine particles (lysosmes) exhibiting granular staining. Sites of enzyme reaction were seen as intensely stained particles scattered in the ground cytoplasm of hepatocytes (Fig.9).

- Alkaline phosphatase:

Intensive activity of alkaline phosphatase was observed

along the sinusoidal borders of hepatocytes in the different areas, mainly centrolobular areas; and sometimes within sinusoids, often related to kupffer cells. The walls of blood vessels and the endothelium of sinusoids are weakly stained while the endothelium of bile duct is moderately stained (Figs.10 , 11).

DISCUSSION

In the present study, the general resemblance of the morphological structure of the adult weasel hepatocytes to that of other mammalian hepatocytes has been noted. Since the fine structure of the mammalian liver has been extensively reviewed by (Elias, 1952 ; Elias and Bengelsdorf, 1952 ; Bruni and Porter, 1965 and Flaks, 1971), discussion will be confined mainly to those features which appear to be characteristic of the weasel.

Liver parenchyma of weasel exhibit the lobular arrangment which is characteristic to the mammalian liver structure, but the outline of the lobules is indistinct due to the poor connective tissue partitions at the boundary.

Hepatic plates are one cell thick and this result accords very well with the accounts of Elias (1952) who reported that, the parenchyma of mammalian liver is made up of anastomosing sheets of hepatic cells, and that these

sheets are one cell thick. He also showed that the mammalian structure of sheets of a single cell thickness provides a better constructional stability over the structure of the avian liver from a mechanical view point, and also provides for greater physiological efficiency by an increase of blood/cell contact surface and biliary outlet surface.

Flaks (1971) found that the fibrous interlobular septa of the porcine liver are thicker than those of most other species. In addition, he noticed that the appearance of the sinusoid of the pig liver is unusual characteristically, much of the lumen is occupied by cytoplasmic processes of the kupffer cells, which contain distinctive phagosomes. In other species, the hepatic sinusoids generally possess only a thin lining of kupffer cell cytoplasm.

The present results refer that, kupffer cells lining the sinusoids are simillar to those noticed by Flaks (1971) in which these cells are large in size and numerous in number.

Protein in liver is labile, at least for the most part, and many of the enzyme proteins in the liver undergo changes in amount with changes in the protein content of the diet. Tarver (1951) stated that the liver is capable of meeting a large variety of metabolic situation because

the pabulum offered varies with changes in the dietary regime.

Liver cells of weasel exhibit more content in proteinic inclusions. Their cytoplasm is homogeneously diffused and showed granular staining with bromphenol blue stain. This findings agree with those of Banhawy and Riad (1972) who reported similar observation in the liver of guinea pig.

The amount of histochemical demonstrable glycogen shows marked dependence on food intake (Minjer 1957). Cardell et al (1973) observed significant quantities of glycogen in hepatocytes located around the portal tract than in cells near central veins.

The liver exert metabolic adaptation to withstand any condition. Such adaptive changes could facilitate the uptake of glucose by hepatic cells, its release into the blood stream, its conversion into glycogen and synthesis of fat or protein from glucose or vice versa as the situation demands. Deane (1944) observed that glucogen in the mouse liver cells exhibited variations in relation to the feeding cycle. Also, the effect of feeding on the liver glycogen content and blood glucose in the hepatic and hepatic portal veins were investigated by Laghans et al (1982) and Abo Shafey (1985).

The present observations prove that, liver cells of weasel show high content of carbohydrate material and that the centrolobular areas exhibit more concentration of glycogen material than other regions.

In the liver, the roles played by both acid and alkaline phosphatases can only be judged from the site of their activity.

Acid phosphatase is considered to be a lysosomal enzyme and hydrolytic in nature; therefore, the presence of this enzyme in the kupffer cells is understandable. Manns and Mortimer (1969) found that a marked activity of acid phosphatase was restricted to kupffer cells in the liver of sheeps, calvs and rats. Also, Ratzlaff and Tyler (1973) found similar observations in the liver of birds. As Wachstein (1963) opined, the degree of acid phosphatase in kupffer cells reflected the functional state of reticuloendothelial cells in the liver.

The liver of weasel exhibits high acid phosphatase activity restricted to kupffer cells and in cytoplasm of hepatocytes as fine granules and such findings are basically similar with those mentioned by Manns and Mortimer (1969). Also, Shah et al (1973) stated that both vulture and kite liver showed high acid phosphatase resp-

onse in the kupffer cells.

Liver of weasel shows comparativily high alkaline phosphatase activity in peribiliary regions; however, in other parts the enzyme reactivity was similar. The areas surrounding the central collecting veins (centrolobular regions of the liver lobule) showed practically least enzyme reactivity.

Manns and Mortimer (1969) noticed that, alkaline phosphatase staining was generally located on the sinusoidal borders of hepatocytes in the regions of the terminal hepatic venules in sheep, calf and rat. However, in the cells of rat liver, increased staining occurred in the areas of the terminal portal venules.

The quantitative variations in activity of certain enzymes, as displayed by the density of the reaction product from portal to central zones of the lobules is well recognized. Novikoff (1959) emphasized the importance of this phenomenon in terms of variation in metabolic function within the lobule. Sasse et al (1975) reported that, hepatocytes from one and the same physiological state have a different outfit of active enzymes and of cell constituents and thus probably different metabolic functions.

On the basis of these facts and from the present observations, it could be stated that the distribution and concentration of alkaline and acid phosphatases in the liver of weasel comparable with other mammalian species such as sheeps, calves, rats and pigs have some relationship with the type of food they ingest.

Fig.1 : Liver section of weasel showing the lobular structure. Hepatic plates (H.P.) arranged radially around central vein (C.V.) . X 100 . HX.

Fig.2 :High magnification of liver section showing blood sinusoids (S), kupffer cells (K), endothelial cells (E) and hepatocytes. X 400.HX.

Fig. 3 : Liver section showing the hepatic cell nuclei of condensed type with centric nucleolei (arrows)

Fig. 4: Liver section showing blood vessel (B.V.). Outer : tunica serosa which forming the liver capsule (L.Cp.). X160. HX.

Fig. 5: Liver section stained with bromphenol blue to show the protein material throught the hepatic tissue component. X 400.

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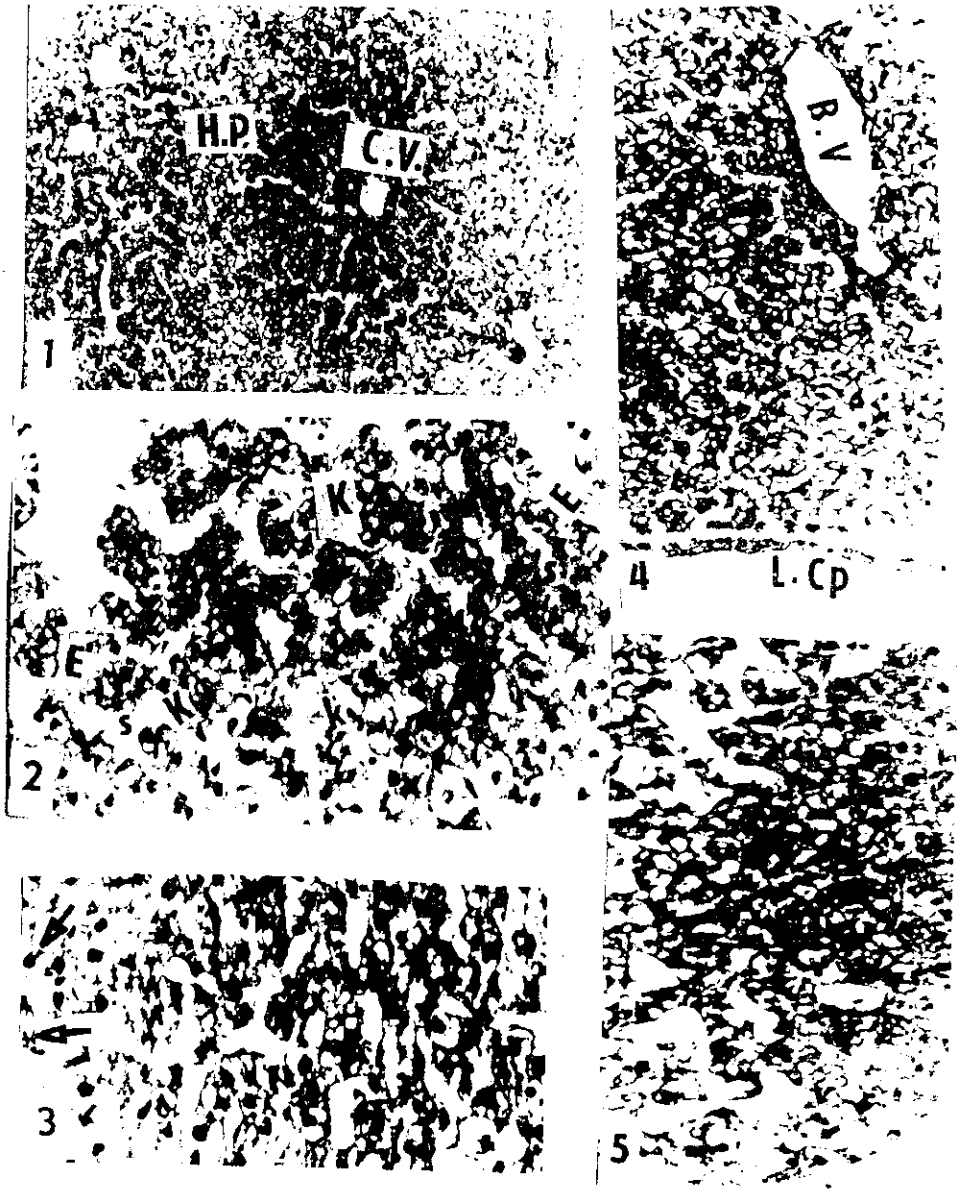

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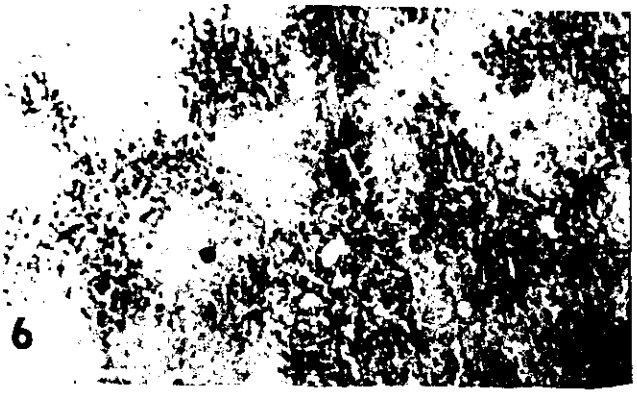
Fig. 6: Liver section stained by PAS reaction exhibiting carbohydrate content, Centrolobular areas showing more accumulation of glycogen particles. X 65.

Fig. 7: High magnification of the same above section. X 250.

Fig. 8: Liver section showing high activity of acid phosphatase throughput all hepatic areas. X 65.



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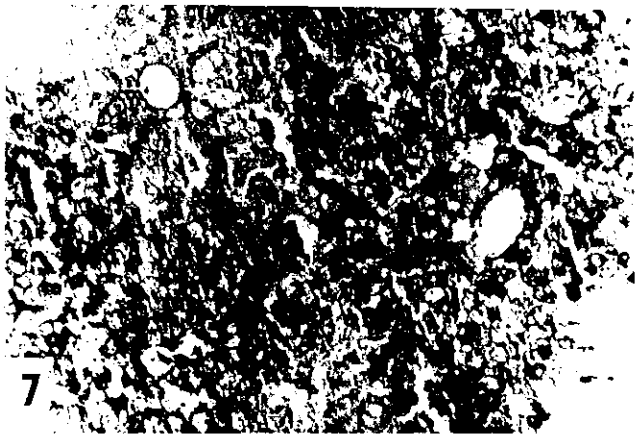

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Fig. 9: High magnification of the above section . Kupffer cells are strongly stained. Cytoplasm of hepatocytes exhibit granular staining. X 250.

Fig. 10: Section of liver exhibiting high activity of alkaline phosphatase. More activity appeared arround blood vessel. Endothelium of bile duct are positively stained (B.D.). X 65.

Fig. 11: High magnification of the same above figure. X 160.

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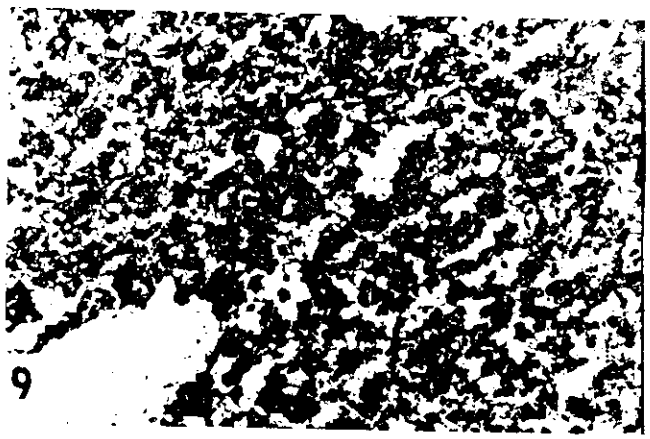

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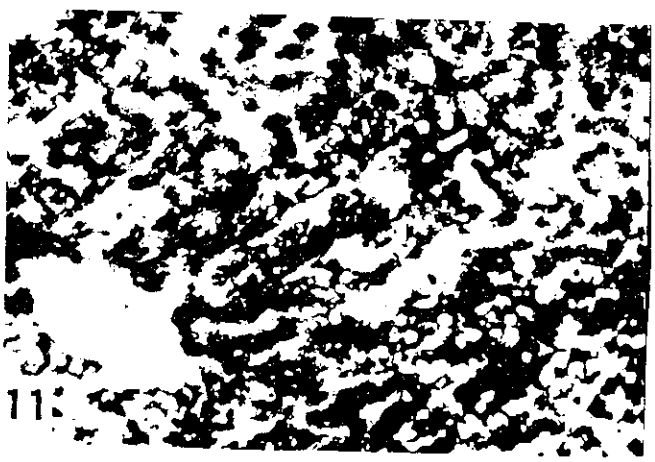

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دراسات هستولوجية وهستوكيمائيه على كبد حيوان ابن عرس (العرسه)

سلام السيد أبو شافعى

قسم علم الحيوان ـ كلية العلوم ـ طنطا ـ مصر

يتضمن هذا البحث دراسة التركيب الهستولوجى لنسيج كبد العرسة وكذلك دراسة بعض المحتويات الهستوكيمائية فى هذا النسيج مثل المواد الكربوهيدراتية والمواد البروتينية ونشاط كل من انزيمى الفوسفاتيز القاعدى والفوسفاتيز الحامضى.

وقد تبين من الدراسة والفحص الاتى:

- أن التركيب الهستولوجى لكبد حيوان التجارب يشبه الى حد كبير التركيب الهستولوجى لكبد الثدييات الاخرى الا أن كمية النسيج الضام فى الحيز بين الفصوص الكبدية قليلة.
- ارتفاع محتوى الخلايا الكبدية من المواد البروتينية والواضح من الصبغة المحبة لسيتوبلازمها.
- محتوى الخلايا الكبدية من المواد الكربوهيدراتية (الجليكوجين) عال وخاصة فى المنطقه المركزيه لكل فصيص كبدى حول الوريد المركزى.
- ارتفاع معدل نشاط انزيم الفوسفاتيز الحامضى فى الخلايا الكبدية وخلايا كوفر (Kupffer) والخلايا البطانية المبطنة للجيبيات الدموية وكذلك فى جدر الاوعية الدموية.
- أما انزيم الفوسفاتيز القاعدى فيكون نشطا على حدود الجيبيات الدموية فى أغشية الخلايا وخاصة فى المنطقه المركزية للفصيصات الكبدية.