Dept. of Anatomy and Histology,
Fac. of Vet. Med., Suez Canal University, Ismailia, Egypt.
Head of Dept. Dr. A.H.K. Osman.

LIVER ULTRASTRUCTURE AND A NEW CELL TYPE IN THE JAPANESE NEWT, Cynops pyrrhogaster

(With 14 Figures)

By

A.H.K. OSMAN; CARL J. PFEIFFER and MAKOTO ASASHIMA***

(Received at 30/6/1990)

التركيب الفوق مجهرى ونوع جديد من الخلايا في كبد النوت اليابانية

عبدالحميد عثمان ، كارل فيفـــر ، ماكوتواساشيمـا

تمت دراسة كبد النوت اليابانية باستعمال الميكروسكوب الضوئي والاليكتروني الماسح والناقـــل. وأوضحت الدراسة أن الكبد يتركب من مجموعات وقنيات وأحبال خلوية تفصلها شبكة من الأوعية الدموية. واحتوت الخلايا الكبدية على مجموعة من العضيات أهمها الميتوكوندريا والشبكة الاندوبلازميه الملســـاء والحبيبة والبيروكسيزومات . وكان هناك مجموعتين من الخلايا الكبدية ، احداهما خازنة للدهون والأخرى خازنة للجليكوجين. تميزت القنيات المرارية بوجود حليمات على اسطح الخلايا الكبدية المكونـــة لجدرانها كما أن تلك الخلايا كانت تلتصق مع بعضها بدسموزومات . أما الشبكة الوعائية الدموية فكانـت جدرانها مبطنة بخلايا اندثيلومية مثقبة وبعض خلايا (كوبفر) . ولقد أوضحت الدراسة وجود نوع جديد من الخلايا في كبد النوت اليابانية يتميز بوجود نوعان من الحبيبات السيتوبلازمية ، النوع الأول: تتميز فيه الحبيبة بكونها متجانسة التركيب ، أما النوع الثاني: تتكون فيه الحبيبة من مجموعـــة مـــن الحبيبات الأقل حجما . وقد أمكن تتبع كلا النوعان في سيتوبلازم الخلايا الاندوثيلومية وكذلك فـــي تجاويف الشبكة الوعائية الدموية الكبدية.

SUMMARY

The liver of the Japanese newt, Cynops pyrrhogaster, has been investigated using light, scanning, and transmission electron microscopy. Hepatic parenchyma was composed exclusively of clusters, cords and tubules of polyhedral cells separated by a sinusoidal net. Hepatocytes had spherical, euchromatic nuclei with one or more nucleoli and stacked mitochondria with an electron-dense matrix and sparse cristae. Rough endoplasmic reticula formed peribiliary stacks and diffusely scattered vesicles and tubules. Smooth endoplasmic reticula were more pronounced in glycogen-rich hepatocytes. Small, peribiliary Golgi complexes and peroxisomes were present. Most hepatocytes contained large numbers of fat droplets distributed evenly throughout the cytoplasm along with glycogen. Some cells were mainly glycogen-storing and contained few or no liposomes. Bile canaliculi had

*: Dept. of Biomedical Sciences, Virginia-Maryland Regional College of Vet. Med., Virginia Polytechnic Institute and State Univ. Blacksburg, Virginia, USA, 24061.
**: Dept. of Biology, Yokohama City University, 22-2, Seto, Kannazawa-Ku, Yokohama, 236, Japan.

short, stout microvilli which were entirely atretic in some canaliculi. Canaliculi were sealed off by junctional complexes including zonulae occludentes and maculae adherentes. The latter showed extraordinary wider desmosomal gaps in thevicinity of the atretic bile canaliculi. The sinusoid wall was lined with fenestrated endothelial cells connected to Kupffer cells by zonulae occludentes. Both the sinusoidal lining and adjacent hepatocytes were void of basal laminae.

A distinctive new cell type was observed in the newt liver. These cells were found individually or in small clusters in proximity with the sinusoidal surfaces. They had small nuclei, a paucity of cytoplasmic organelles, but numerous, unique, osmiophilic granules of two distinct types. Less numerous Type I granules contained homogenous electron-dense material, and a predominant Type II granule contained circumferentially arranged subparticulation. Granules of both types were detected within the cytoplasm of endothelial cells and within sinusoids together with blood elements. The function of this secretory type cell remains obscure.

INTRODUCTION

The ultrastructural organization of the liver of higher vertebrates has been extensively studied by many investigators. In contrast, the liver of lower vertebrates still requires further ultrastructural study in order to help elucidate structure-functional relationships. Ultrastructural investigation of the amphibian liver has been reported by HRUBAN and RECHCIGL (1969), ROELS et al. (1970), BÖCK et al. (1980), JONES et al. (1981), DAUCA et al. (1983) and GORGAS and STORCH (1984). The reptilian liver fine structure has been studied by GOLDBERG (1972), DVORAK et al. (1981), HENNINGER (1982), FERRER et al. (1987), DeBRITO, GITIRANA and STORCH (1988) and STORCH et al. (1989).

The Japanese newt, Cynops pyrrhogaster, has become an increasingly important species for biomedical research in cancer investigation (PFEIFFER et al., 1979; ASASHIMA et al., 1982; PFEIFFER et al., 1989; PFEIFFER and ASASHIMA, 1990), in developmental studies (ASASHIMA et al., 1987; HIRAKOW et al., 1987; UEHARA et al., 1989), and in regeneration biologic studies (EGUCHI and WATANABE, 1973; PFEIFFER et al., 1985). Because of the above reason and the facts that:

a) general ultrastructural information of newt liver is sparse,

and b) both gross and microscopic observations of the Japanese newt liver demonstrated its peculiar morphology, the present communication describes its fine structure for the first time.

MATERIAL and METHODS

Adult male and female Japanese newts, Cynops pyrrhogaster, captured in fields, ponds and creeks in Niigata and Iwate Prefectures were used in this study. Captured newts were maintained in glass aquaria at temperature controlled (12°C) laboratory conditions and were fed Tetra Repto Min (Tetra Werke, Melle, West Germany) commercial newt food twice a week. Specimens were collected from newts in two categories; fed animals with presence of food in the stomach, and animals which had been fasted for at least six days, with food absent in the stomach.

Liver samples were extirpated from approximately 20 newts sacrificed by decapitation and were fixed immediately in a cold solution of 5% glutaraldehyde/3% formalin in 0.1 M sodium cacodylate buffer at pH 7.4. The samples were taken from the proximal 1/3, the mid, and the distal 1/3 of the newt liver, which is a long (approximately 1.5 cm) pointed, bi-lobed organ. After initial fixation specimens were washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h., washed in buffer again and dehydrated in a series of alcohols.

Semi-thin sections (1u) were cut from hepaic tissue embedded in Poly/Bed 812 (Polysciences, Inc.) by standard procedures as described for gastrointestinal tissue (PFEIFFER and KEITH, 1985) and were stained with 1% toluidine blue in 1% sodium borate for 30s, followed by 0.5% safranin O in 0.5% sodium borate for 10s. These sections were used for light microscopy. Thin sections were doubly stained with lead citrate and uranyl acetate and studied with a JEOL CX-II transmission electron microscope operating at 80 kv. Scanning electron microscopy was undertaken on critical-point dried specimens coated with approximately 150 nm of gold. These specimens were examined with a JEOL JSM 35°C scaning electron microscope operating at 10 kv.

RESULTS

Light microscopic observations :

The gross anatomy of the newt liver will not be described in detail in this report, but its general size in comparison to that of the adult newt is illustrated in (Fig. 1). Histologically, it was invested along its entire length by a serous coat composed of an outer layer of attenuated cells resting on a prominent basal lamina, and an inner layer predominantly made up of polymorphic mononuclear cells and a few loosely arranged collagenous fibers. The latter layer contained comparatively wide, thin-walled blood vessels of variable size, which divided into smaller ramifications piercing the hepatic parenchyma (Fig. 2).

The architecture of the hepatic parenchyma was characterized by the presence of mainly polyhedral hepatocytes which were arranged in the form of closely adjacent, monolayered tubules encircling minute bile canaliculi. These tubules were mostly in the form of clusters and cordi, and lacked any special configurtion. The wall of each tubule was comprised of 3 to 6 hepatocytes whose narrowest sides bordered bile canaliculi. Neither the tubules nor the clusters were surrounded by an obvious basal membrane.

The individual hepatocyte was almost penta-or hexagonal in outline. The nucleus was spherical, euchromatic, and showed one or two prominent nucleoli. Binuclear cells were occasionally observed. The nucleus was usually peripherally located, depending upon the quantity of the cytoplasmic liposomes (Fig. 2). The nucleus was small in comparison to size of the cell, and therefore, it was absent in some sectional profiles. The hepatocyte cytoplasm was mainly occupied by large number of evenly distributed, spherical liposomes which tended to coalesce, forming droplets of variable size. Fine glycogen granules were concentrated mainly in the form of ribbons at the cell periphery and to a lesser extent between the liposomes and around the nuclei (Fig. 2). Cells with extensive glycogen content showed only a few fat droplets.

A striking feature of the liver of this species was the presence of a special cell type which has not previously been described in the liver of lower or higher vertebrates. These cells were arranged sporadically or in small clusters among the higher population of hepatocytes. Their location was always between the hepatocyte vascular surfaces and the sinusoids, and therefore, they were exclusively perisinusoidal. Most of such cells were comparatively smaller than the adjacent, ordinary hepatocytes but both types of cells were frequently of similar contour. Their cytoplasm was characterized by presence of numerous densely packed, osmiophilic granules which filled most of the cell and pushed the nucleus to the periphery, almost obscuring it. We designate these cells, osmiophilic granulohepatocytes (OG cells) (Fig. 2).

The hepatic parenchymal cells, icluding those forming tubules and those arranged in clusters, were surrounded by an extensive sinusoidal net lined with attenuated endothelial cells and Kupffer cells. Occasional perisinusoidal stellate cells, probably, Ito cells, were also present. They appeared in close proximity to the hepatocyte vascular surface.

Electron microscopic observations:

The external, serosal surface of the liver was characterized by the presence of a large number of microvilli when viewed by scanning electron microscopy (Fig. 3). The surface of the underlying hepatocytes was thrown into irregularly oriented microplicae.

The microplicae of the opposing surfaces of the adjacent cells were interdigitated (Fig. 3).

The nucleus of the hepatocyte, as revealed by transmission electron microscopy, often had a smooth contour without deep infoldings and the majority of its chromatin content was finely dispersed except at its periphery, where circumscribed areas of heterochromatin were observed in close association with the nuclear envelope (Fig. 4). Two relatively large nucleoli were present.

Mitochondria were the most populous cytoplasmic organelle in the newt hepatocyte. They were evenly distributed throughout the cytoplasm, varied greatly in size

and shape (Fig. 4), and were spherical, ovoid, or club-shaped. The mitochondrial cristae were sparse and showed a dwarfed appearance (Fig. 5). Their homogeneous matrix was moderately electron-dense.

Rough endoplasmic reticulum (RER) was usually arranged in parallel stacks in the vicinity of the nucleus and in the peribiliary cytoplasm, but were also randomly scattered throughout the cytoplasm (Fig. 4).

Smooth endoplasmic reticulum (SER) was in the form of numerous vesicular and short tubules in the glycogen storing hepatocytes (Fig. 5). Such cells showed chains or sheets of glycogen granules separating and overlapping the SER and mitochondria. The latter were stacked in some areas (Fig. 5).

Peroxisomes were a characteristic organelle in the cytoplasm. They were ovoid to spherical, membrne-bounded structures whose diameters ranged between 0.170-0.740 um. They contained a finely granular opaque matrix which was either homogeneous or showed extra elecron dense particulate inclusions (Fig. 5). They were mainly found in the perinuclear region in close proximity to the cell membrane, and in association with the mitochondria and fat droplets.

Some hepatocytes (Fig. 6) showed a large number of cytoplasmic liposomes which were usually evenly distributed. The mitochondria compressed between such fat droplets were of smaller size than those in non-lipid storing cells.

The bile canaliculi were circumscribed dilatations of the intercellular space between adjacent hepatocytes. The opposing plasmalemmas forming a canaliculus were thrown into a number of short, stout microvilli with moderately electron-dense cores and denser bases. Some canlaiculi contained intraluminal osmiophilic membraneous debris (Fig. 7), and others showed markedly degenerate microvilli (Fig. 8). The bile canaliculi were sealed off by a number of junctional complexes including zonulae occludentes and, more commonly, desmosomes (Fig. 8). Desmosomal gaps around the degrenerated canaliculi were twice or three times as wide as those around the canaliculi with normal microvilli (0.35 um and 0.096 um, respectively). The rest of the hepatocyte surfaces, particularly those abuting sinusoids, were thrown into comparatively long microvillous folds which were commonly interdigitated with their counterparts of the adjacent cells.

Hepatic sinusoids were lined with a single layer of prominent, attenuated endothelial cells and some Kupffer cells (Fig. 9). The perikaryon of the endothelial cell contained a flattened nucleus which showed massive clumped heterochromatin and little cytoplasm. Cytoplasmic processes of the endothelial cells formed sieve-like, fenestrated plates connecting with those of the adjacent cells by tight juntions. The cytoplasm of the endothelial cells showed sparse organelles but was rich in microvesicular bodies, particularly on the apical surface (Fig. 9). Kupffer cells were observed incorporated in the sinusoidal wall, together with endothelial cells, in the perisinusoidal space of Disse (Fig. 10). The nuclei of Kupffer cells were polymorphic with prominent indentations.

The nuclear chromatin was finely dispersed with some patches of heterochromatin. The cytoplasm showed many mitochondria with well-developed cristae, lysosomes, fragmented debris and large phagocytic vacuoles. The cytoplasm also contained an abundance of microfilamentous strands and some myelin figures. Surfaces of Kupffer, and to lesser extent, endothelial cells, showd many irregular fine cytoplasmic extensions interdigitating with those of the adjacent cells.

Perisinusoidal free phagocytes were also observed in the space of Disse. Phagocytes had a bean-shaped nucleus with a deep wedge-shape indentation and a prominent large nucleolus. They were characterized by the presence of primary and secondary lysosomes.

A novel type of cell, the osmiophilic granulohepatocytes (OG cells), were observed randomly distributed among the hepatic parenchyma. They were distributed sporadically and in small clusters which were almost exclusively situated between the ordinary hepatocytes. The OG cells were often pentagonal or hexagonal in outline, similar to the surrounding hepatocytes (Fig. 11). Neither junctional complexes nor bile canaliculi were observed between adjacent OG cells or between them and adjacent, ordinary parenchymal hepatocytes. The opposing membranes of adjacent OG cells had irregularly interdigitated microplicae (Fig. 12). The nucleus of OG cells was peripheral and small in comparison to the size of the cell and it displayed an irregular outline due to superficial infoldings (Fig. 11). Heterochromain was always restricted to the nuclear periphery and a prominent nucleolus was present. Binuclearity was not observed.

The OG cell cytoplasm showed a striking paucity of organelles. It was characterized mainly by the presence of two discrete populations of cytoplasmic granules having a considerable osmiophilic affinity, hence the name, osmiophilic granulohepatocytes. The granules of the first type (type I granules) were of a homogeneous electrondensity and they varied in shape and size. They were oval, ovoid, sherical or rod-shaped (Fig. 13) and their diameter ranged between 0.176 to 0.470 um. The granules of the second type (type II granules) were more common (Fig. 11) and their shape was predominantly spherical. Their average diameter was 0.530 um, but occasional granules with an average diameter of 1.150 um were also observed. These granules were characterized by a unique substructure in which each granule was made up of a cluster of circumferentially arranged finely grained osmiophilic particles (Figs. 12 & 13). A few granules showed a. homogeneous electron-dense core and a finely grained rim. Each granule was surrounded by a moderately opaque homogeneous matrix which either conformed to the size of the granule or appeared larger. Some of the granules of both types were membrane-coated. In addition to the specific granules, the cytoplasm of the OG cell contained sparse mitochondria and some microfilaments which were mainly concentrated perinuclearly.

The specific granules of both types (I and II) were also observed in the extracellular spaces and inside the cytoplasmic processes of the adjacent perisinusoidal phagocytes. They were also strikingly detected in the cytoplasm of the sinusoidal endothelium as well as free in the open sinusoidal space (Fig. 14).

LIVER NEW CELL TYPE, JAPANESE NEWT

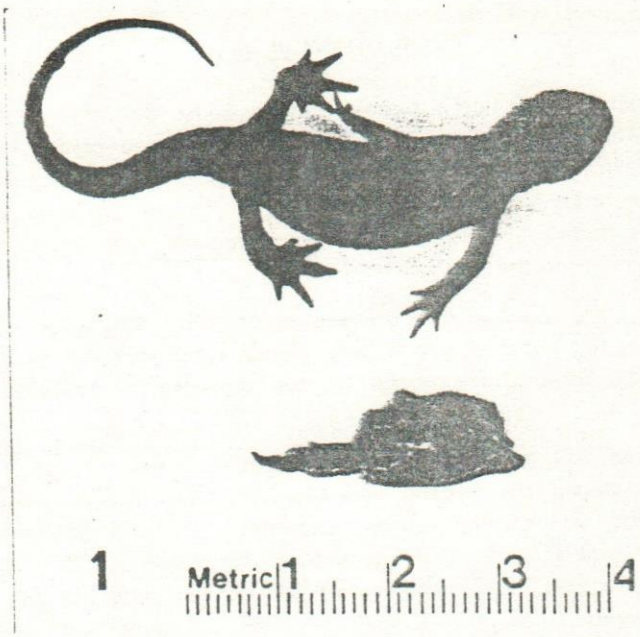

Fig. (1): Dorsal view of adult female Japanese newt, and its liver (below). Note the pointed lobe of the liver.

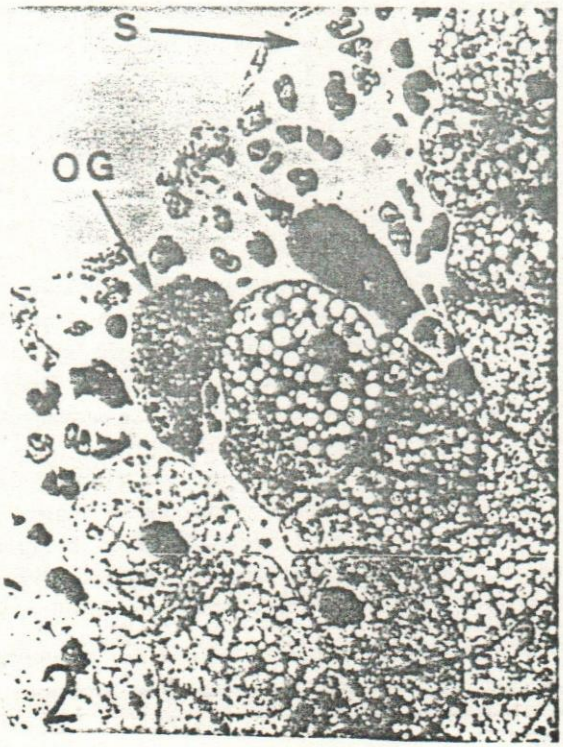

Fig. (2): Light micrograph of peripheral aspect of newt liver, illustrating the serous coat(s) and hepatic parenchymal cells. Fat droplets (F) and glycogen (G) can be observed at this level, as well as osmiophilic granulohepatocytes (OG). X= 212.

Fig. (3): Scanning election micrograph of serosal surface of newt liver, showing microvilli (arrow). In this illustrtion breaks in the serosal covering (upper and lower left) reveal the underlying hepatic parenchymal cell surfaces. X= 2,000.

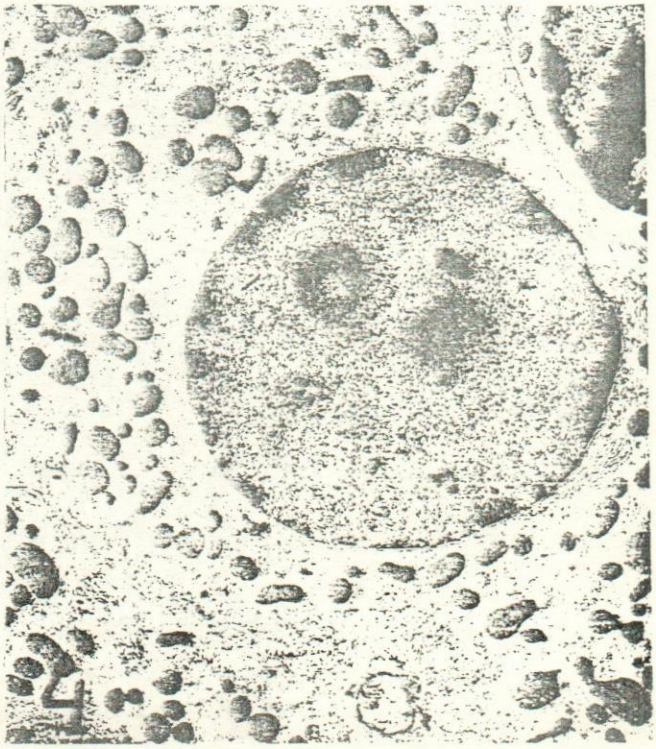

Fig. (4): Transmission electron micrograph of newt hepatocyte, showing the typical spherical nucleus with two nucleoli, and numerous mitochondria of variable shape in the cytoplasm. X= 4,100.

LIVER NEW CELL TYPE, JAPANESE NEWT

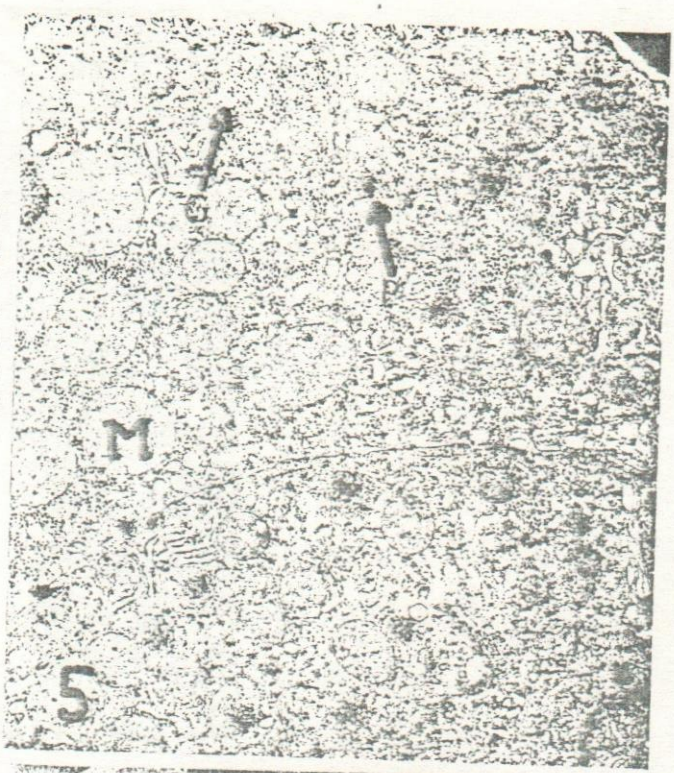

Fig. (5): Cytoplasm of two newt hepatocytes, illustrating peroxisomes (P), both rough and smooth endoplasmic reticula, and diffusely distributed glycogen (G), Note small dense bodies in mitochondria (M). A portion of a nucleus is in the upper right corner. X= 9,100.

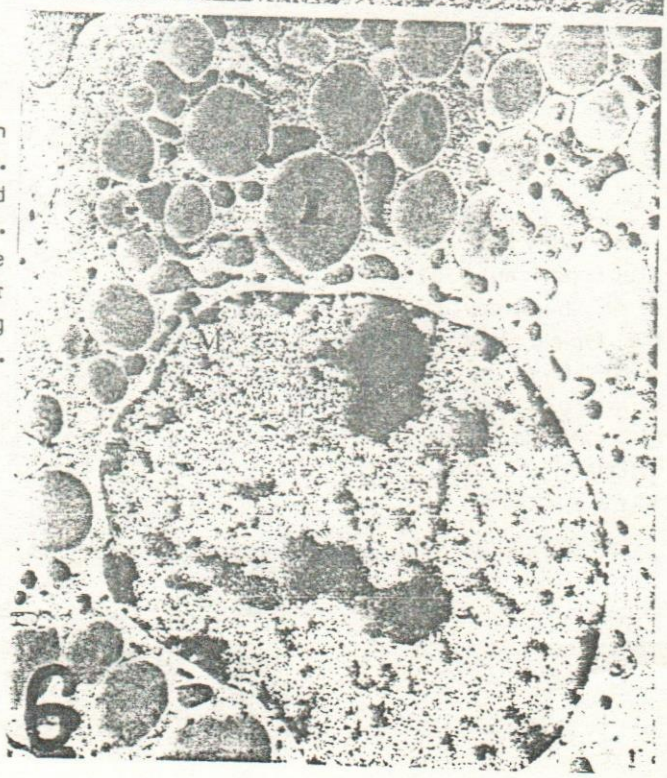

Fig. (6): Illustration of fat laden newt hepatocyte. Liposomes (L) are scattered throughout the cytoplasm. Mitochondria (M) in these lipid rich cells were smaller than in non-lipid storing hepatocytes. X= 3,900.

Fig. (7): Bile canaliculi (arrows) between adjacent hepatocytes sometimes contained osmiophilic membranous debris. X= 6,050.

Fig. (9): Endothelial cells (arrow) lining the hepatic sinusoids showing flattened nucleus and thin cytoplasmic plate-like processes. Stubby microvilli were observed on their apical surfaces, extending into the sinusoidal space (S). X= 9,000.

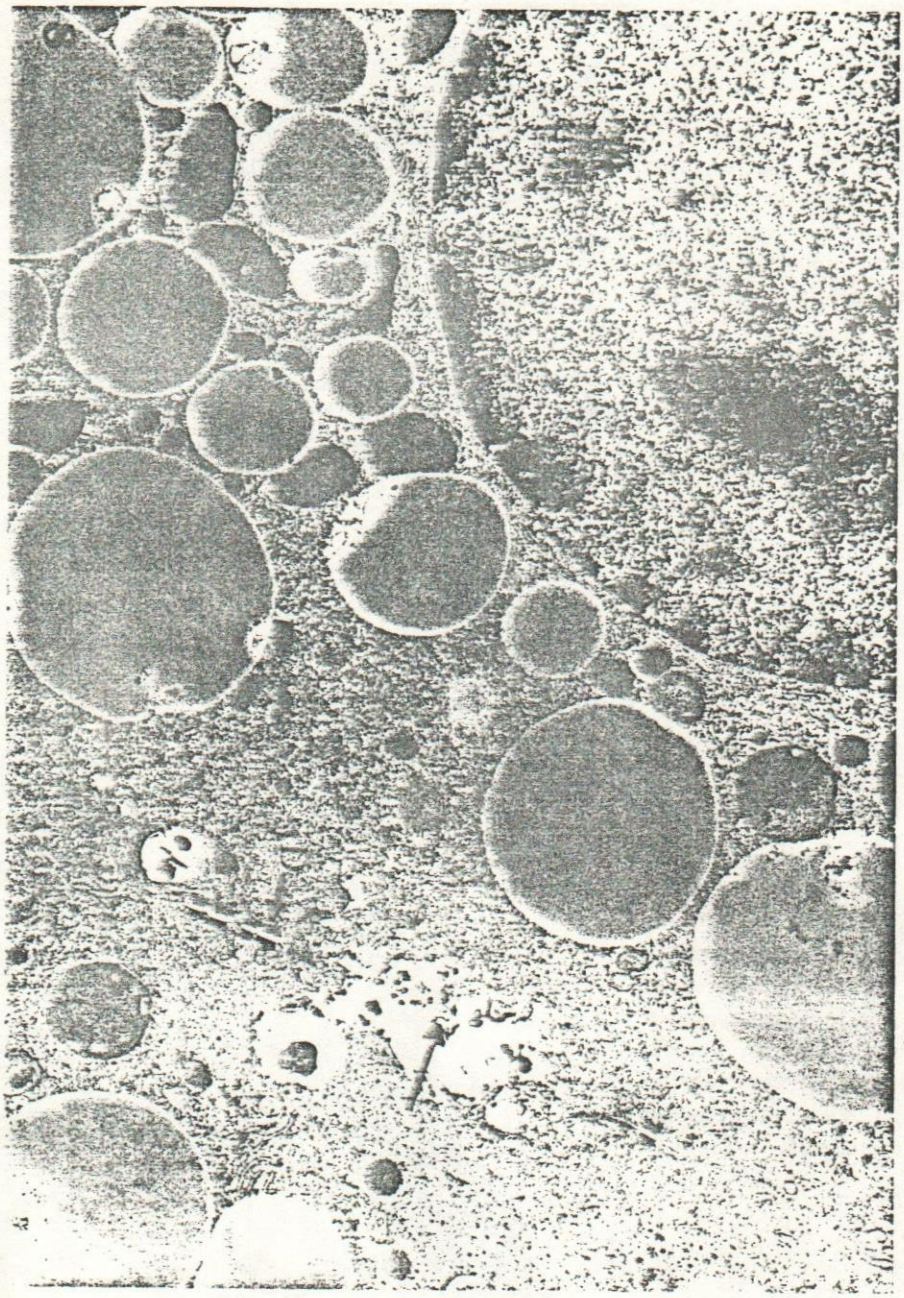

Fig. (8): In some instances microvilli of bile canaliculi (arrow) were markedly degenerate. Desmosomes (D) were commonly noted in the vicinity of bile canaliculi. Note also prominent liposomes (L). X= 7,800.

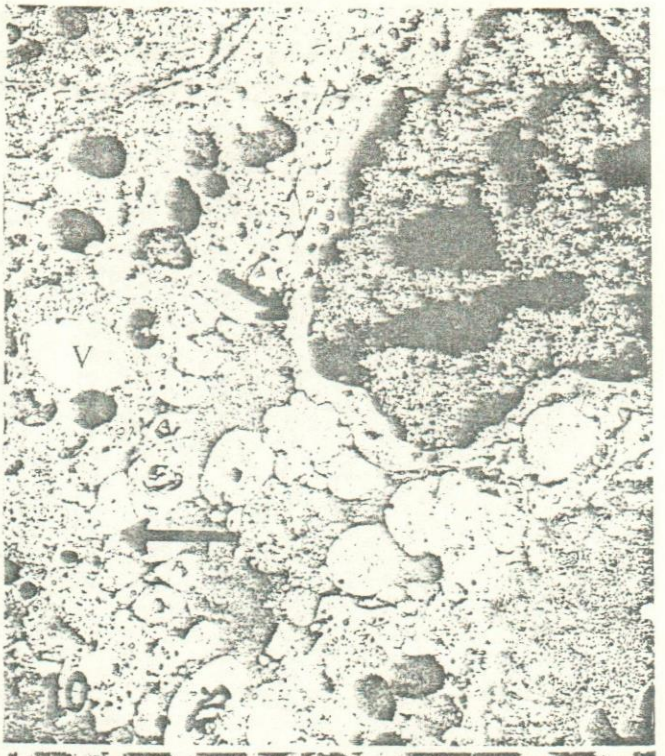

Fig. (10): Kupffer cells (arrow) were intermingled with sinusoidal endothelial lining cells. Kupffer cell cytoplasm demonstrates macrophage abundant vacuoles (V), phagosomes, lysosomes, and cellular debris. small portions of hepatic parenchymal cells are also shown in the lower right and upper left corners of this figure. X= 4,100.

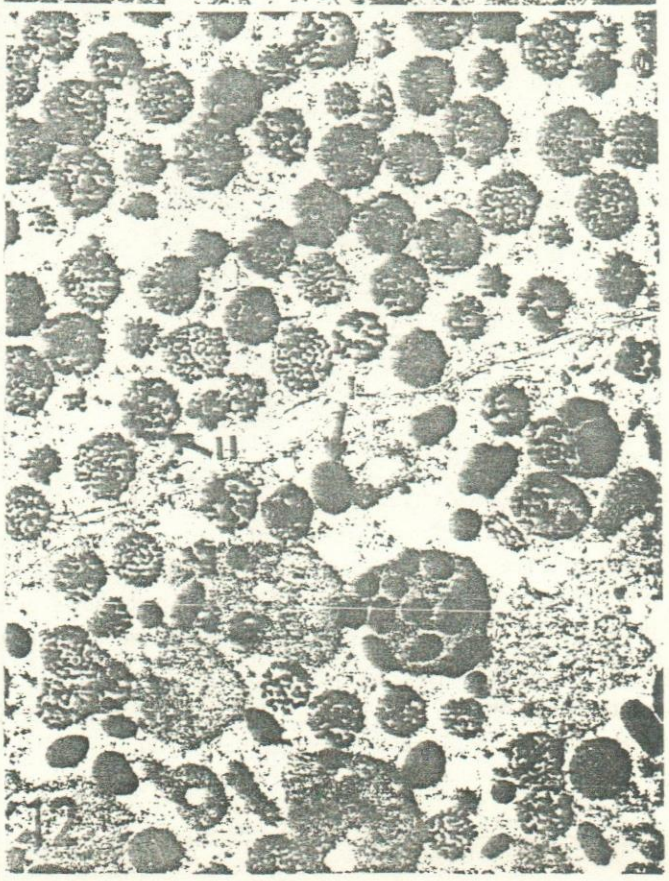

Fig. (12): At higher resolution than shown in Fig. 11., the internal structure of Type I granules (I) and Type II granules (II) are illustrated. Also, a composite of Type I granules (C) can be seen, as well as the subgranulation of Type II granules. X= 12,900.

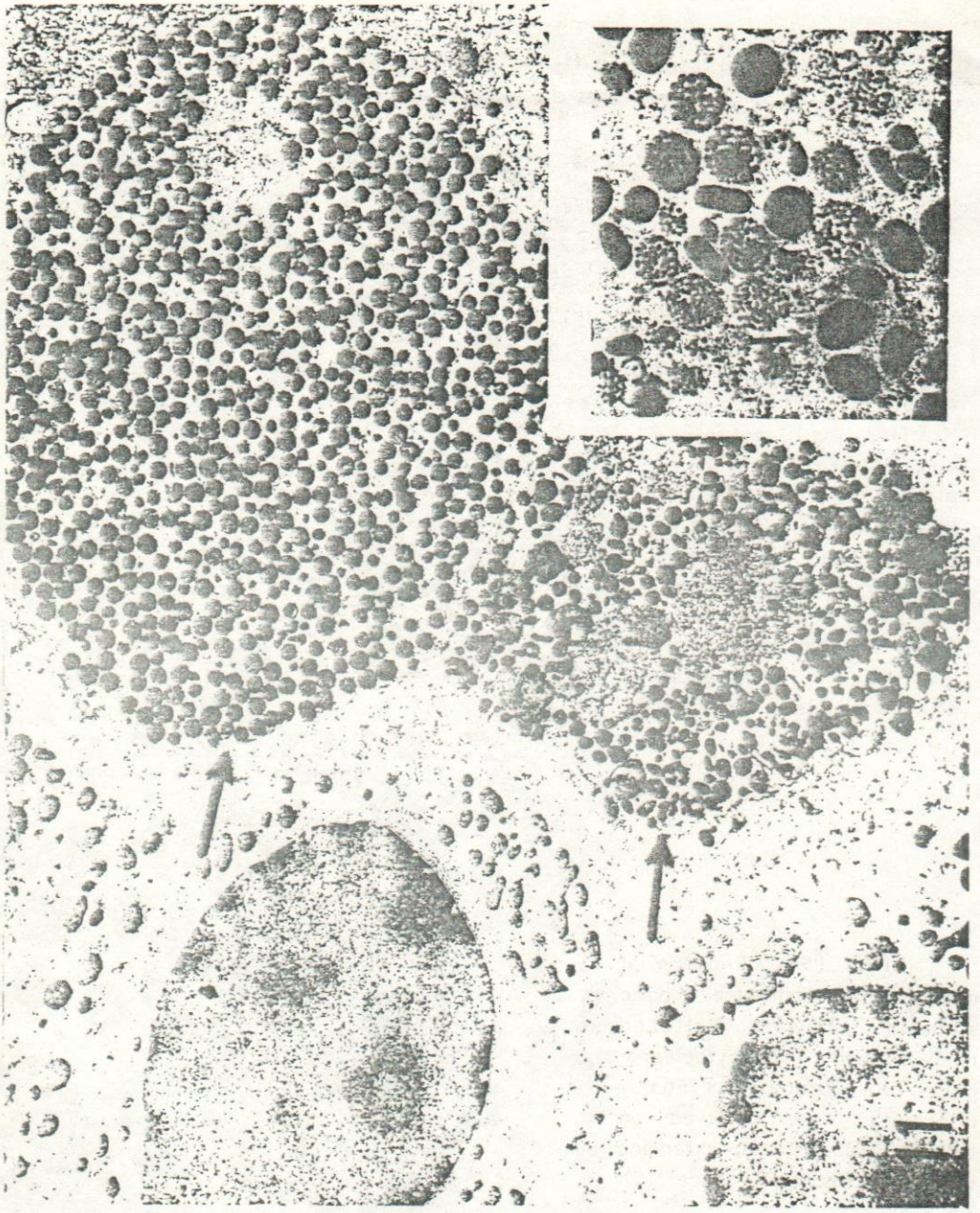

Fig. (11): Cross section of two adjacent osmiophilic granulohepatocytes (OG cells) of the Japanese newt, showing extensive packing of the cytoplasm (arrows) with electron dense granules. The lower cell shows greater diversity in form of granules; most granules in the upper cell are Type II. X= 3,800.

Fig. (13): This insert shows another type of composite of Type I granules, contained within a "membrane" (arrow) composed of smaller granules similar to the subgranulation of Type II granules. X= 14.100.

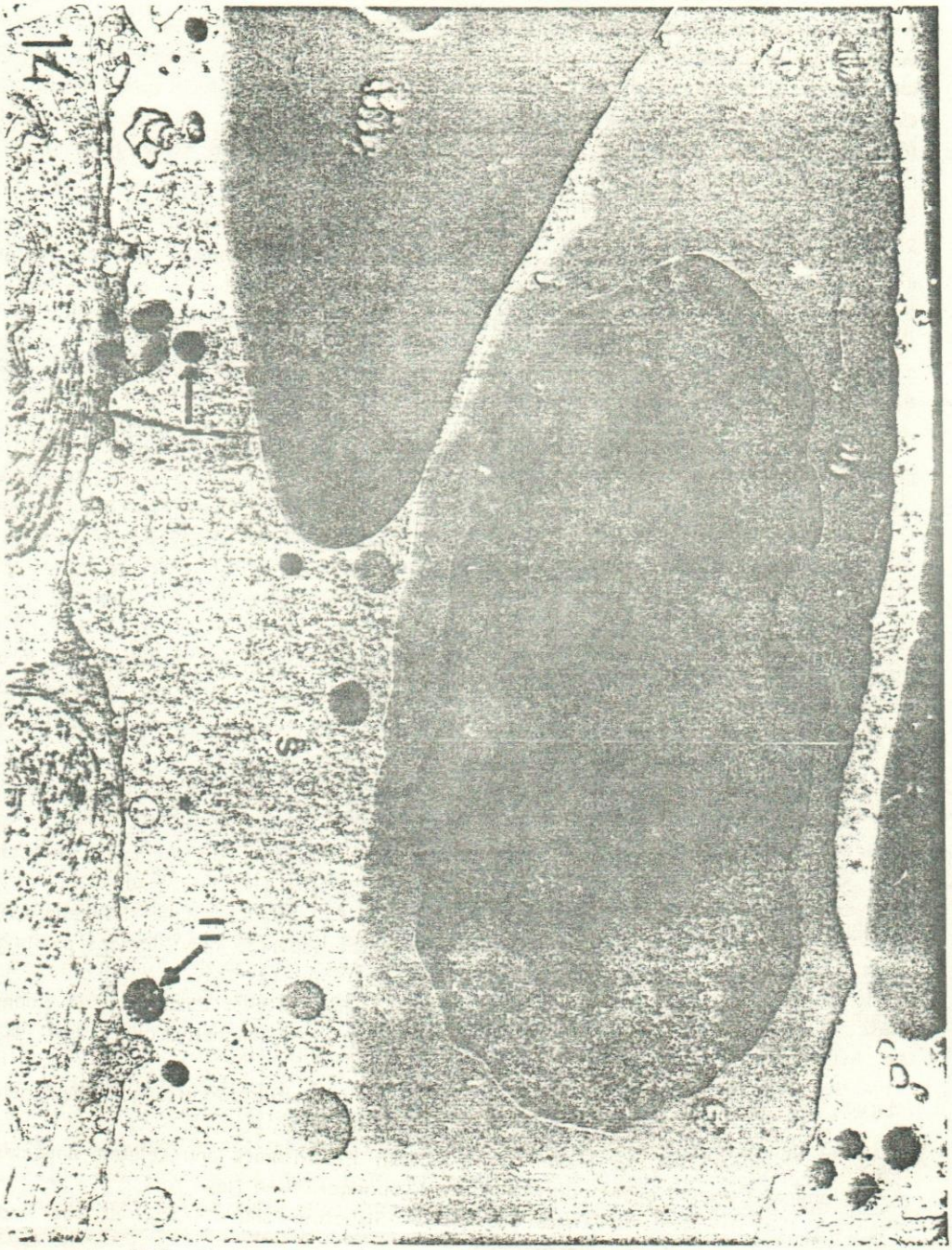

Fig. (14): Electron micrograph of the sinusoidal space (S), showing presence of both nucleated red blood cells (RBC) and freely floating OG cell granules of Type I and Type II (arrows). X= 9,750.

DISCUSSION

The present study demonstrated that the Japanese newt liver was invested by a thin serosa, loosely attached to the underlying parenchymal structures. The apical mesothelial surface was studded with numerous fine microvilli which were comparatively smaller in size and of lesser density than those described for the visceral peritoneum of various higher vertebrates (BARADI and HOPE, 1964; ANDREWS & PORTER, 1973; FURUBAYASHI et al., 1984 and PFEIFFER et al., 1987).

The hepatic parenchyma of this species was usually organized in the form of clusters, cords and to a lesser extent, single-layered tubules separated by blood sinusoids, in contrast to the liver of teleost fishes (WELSCH andSTORCH, 1973), larval and adult sea lampreys (PEEK et al., 1979; SIDON and YOUSON, 1983), some reptiles (TAIRA and MUTOH, 1981) and crocodiles (STORCH et al., 1989), which have entirely a tubular architecture. On the other hand, YOUSON et al. (1985) have declared the ability of the lamprey liver to lose its tubular organization at sexual maturity.

A characteristic feature of the hepatocyte was its folded surface which was interdigitated with that of the adjacent cells. It is suggested that this folded and interdigitated surface configuration not only functions to provide some sort of fixation and maintenance of cellular relations, but it might also be necessary to enable the cell to undergo distention with fat storage required for winter survival. Evidence for cellular distention in the present investigation was indicated by the presence of some fat-laden hepatocytes which had lost their polygonal appearance, and their surfaces were more or less smooth. In the present study major differences were not observed in hepatocytes of recently fed versus newts.

The majority of bile canaliculi observed in the present study were in the form of circumscribed dilatations of the intercellular space between two adjacent cells; however, at least one surface of the hepatocyte does not share in the formation of such canaliculi and it usually faces a blood sinusoid (vascular surface). The opposing surfaces forming a bile canaliculus were thrown into a number of microvilli which were different from the microvillous folds characterizing the remainder of the cell surfaces. They were shorter, non-anastomosing, did not form interdigitations with adjacent cells, their cores were more opaque due to the presence of homogeneous electron-dense material, their bases showed a dense cytoplasm, and finally, they projected into a bile canaliculus which was always sealed off by junctional complexes. These elaborate intercellular bile canaliculi were similar, in respect to position, to those described in the liver of teleosts (YAMAMOTO, 1965; WELSCH and STORCH, 1973) but differed from the telecosts in which the hepatic parenchyma consisted of tubules encircling the bile canaliculi. Progressive loss of bile canaliculi, intrahepatic ducts, common bile duct and gall bladder was observed in sea lampreys during sexual maturation (YOUSON, 1981). A corresponding feature of the Japanese newt liver was the massive degeneration among the microvilli of some bile canaliculi and the presence of osmiophilic debris in the lumina of others.

The lumina of bile canaliculi were sealed with junctional complexes including zonulae occludentes and, mainly, maculae adherentes (desmosomes). These structures were only apparent where the opposing surfaces of the adjacent cells formed bile canaliculi, and were absent on the vascular surfaces of the hepatocytes. Therefore, it is suggested that they formed a barrier between bile and th intercellular tissue fluids. YOUSON et al. (1987), who studied biliary atresia in the sea lamprey, suggested that gap junctions and zonulae occludentes function as a bile-blood barrier which is readily disrupted during metamorphosis, at which time bile canaliculi and bile ducts disappear. The present study has revealed that desmosomes sealing bile canaliculi with degenerative microvilli also show enlarged intercellular gaps which were two or three times the width of gaps in desmosomes which seal non-degenerating canaliculi. This might be an early sign of junctional complex disruption or it might be attributed to an increase in the intraluminal pressure after the degenerated microvilli have lost their absorptive activity.

Mitochondria were the most numerous membranous organelle in the newt hepatocytes. Generally, they were characterized by an electron dense matrix and cristae were scarce. These features are also common in mammals (NOVIKOFF and HOLTZMAN, 1987), and crocodiles (STORCH et al., 1989). In contrast to these features, the mitochondria in fish hepatocytes exhibited markedly developed tubular cristae (WELSCH and STORCH, 1973). Moreover, in the present study a reverse correlation between the mitochondrial number and size and magnitude of stored lipid (cytoplasmic liposomes) was observed. The cells storing large numbers of fat droplets showed a reduction in the number and size of mitochondria, but nonfat-storing cells showed the reverse, and these mainly stored glycogen. These observations might be interpreted on the basis that the hepatocytes initiating the process of fat storage, required for winter survival, are not required for supporting major oxidative activity which might accelerate fat mobilization.

The fat droplets were a main cytoplasmic inclusion in the majority of hepatocytes. This energy-rich stored material has been observed in the hepatocytes of all ectothermic animals, including fish, amphibia and reptiles (NICHOLLS et al., 1968; GOLDBERG, 1969; WELSCH and STORCH, 1973; SIDON and YOUSON, 1983; STORCH et al., 1989). The role of the hepatic fat in vitellogenesis has been confirmed in the females of Uta (HAHN, 1967) and another amphibian, Xenopus laevis (NICHOLLS et al., 1968).

Ovoid to spherical peroxisomes were a constant feature of the newt hepatocytes. Their size, 0.17 to 0.74 um diameter, was similar to the diameter of peroxisomes in hepatocytes of other amphibia, such as the toad (JONES et al., 1981) and Ichthyophis glutinosus (GORGAS and STORCH, 1984). In respect to the presence of electron-dense inclusions in the less dense peroxisomal matrix, our observations concur with those described by REDDY and SVOBODA (1973) in newt liver. Nucleoids have been demonstrated in hepatic peroxisomes of Rana pipiens and Bufo marinus (SCOTT et al., 1969; JONES et al., 1981). Marginal plates were also reported in hepatic peroxisomes of Ichthyophis (GORGAS and STORCH, 1984).

In the Japanese newt the perisinusoidal space of Disse showed a variety of strucutres including; cytoplasmic processes of endothelial and Kupffer cells, the microvillous folds of hepatocytes, free phagocytes, endothelial-like cells which were connected with each other by tight junctions, and microfilament-rich cells (probably Ito cells). Collagen fibers were encountered in some areas. STORCH et al. (1989) also reported the preence of Kupffer cells in the perisinusoidal space of Disse in the crocodile. It is suggested that all of the perisinusoidal cell types contribute primarily to the following functions: phagocytosis of tissue debris from the perisinusoidal spaces and prevention of their passage to the circulation via the endothelial fenstrations, regulation of the passage of materials between the hepatocytes and sinusoids, and finally, provision of the sinusoidal wall and prevention of its occlusion. FUJITA et al. (1986) have suggested that the perisinusoidal cytoplasmic processes constitute a hepato-skeletal system.

The most striking feature of the Japanese newt hepatic parenchyma observed here was the presence o a unique cell type which can be designated on the basis of its morphology as an osmiophilic granulohepatocyte (OG cell). While the OG cells possess some similarity with other cell types, such as mast cells and pigment cells, their general morphology and specific granule structure is distinctive from both Japanese newt pigment cells (PFEIFFER et al., 1989) and mast cells. Their extensive granulation and high population in the liver of this species suggests an important secretory or hormonal role for the OG cell. The presence of released granules of the OG cell, such as observed free in the hepatic sinusoids, and the recent but rare finding of such a granule embedded in a cardiomyocyte of the Japanese newt (PFEIFFER et al., 1990) supports the hypothesis that these granules are transported out of the liver into the general circulation. A specific physiologic role(s) of these secretory granules and the OG cell will be clarified by future investigations in which the chemical content of OG cell granules is identified.

REFERENCES

Andrews, P.M. and Porter, K.R. (1973): The ultrastructural morphology and possible functional significance of mesothelial microvilli. Anat. Res., 177, 408-426.

Asashima, M.; Komazaki, S.; Satou, C. and Oinuma, T. (1982): Seasonal and geographical changes of spontaneous skin papillomas in the Japanese newt Cynops pyrrhogaster. Can. Res. 42, 3741-3746.

Asashima, M.; Mogami, Y.; Okuno, M. and Baba, S.A. (1987): Embryonic development of the newt Cynops pyrrhogaster in very weak magnetic fields. Inst. Space Astronaut. Sci. Res. Note. 357, 1-14.

Baradi, A.F. and Hope, J. (1964): Observations on ultrastructure of rabbit mesothelium. Exp. Cell REs., 34: 33-44.

Böck, P.; Kramar, R. and Pavelka, M. (1980): Peroxisomes and related particles in animal tissues. Cell Monogr 7. (1st ed). Springer, Wien New York.

Bouwens, L.; A. Geerts, E. Wise (1988): 2- Structure and function of the hepatic sinusoidal wall. In: Biopathology of the liver. An ultrastructural approach (P.M. Motta, ed.). Kluwer Academic Pub., Dordrecht. 13-22.

Dauca, M.; Calvert, R.; Menard, D.; Hugon, J.S.; Houdry, J. (1983): Development of peroxidases in amphibians. III- Study on liver, kidney, and intestine during thyroxine-induced metamorphosis. J. Exptl. Zool., 227: 413-422.

De Brito Gitirana, L. (1987): The influence of environmental factors (nutrition, temperature) on the ultrstructure of hepatocytes in reptiles. Ph.D. Thesis, University of Heidelberg.

De Brito Gitirana, L. and Storch, Y. (1988): The fine structure of the hepatocytes in Brazilian land tortoise during thermal adaptation. Zool. Anz., 220: 1-7.

Dvorak, M.; Stankova, J. and Koneca, H. (1981): Fine morphology of liver cell surfaces in different animal species. Sci. Med., 54: 147-156.

Eguchi, G. and Watanabe, K. (1973): Elicitation of lens formation from the ventral iris epithelium of the newt by a carcinogen, N-methyl-N'-nitro-N-nitrosoguanidine. J. Embryol. Exp. Morph. 30: 63-71.

Ferrer, C.; Zaust, A.; Ballesta, J.; Hernandez, F. and Pastor, L.M. (1987): The liver of Testudo graeca (Chelonia). A comparative study of hibernating and non-hibernating animals. J. Submicrosc. Cytol., 19: 275-282.

Fujita, H.; Tatsumi, H.; Ban, T. and Tamura, S. (1986): Fine stuuctural characteristics of the liver of the cod (Gadus morhua macrocephalus), with special regard to the concept of a hepatoskeletal system formed by Ito cells. Cell Tissue Res., 244: 63-67.

Furubayashi, R.; Miyamoto, H.; Tanii, T. and Ishibashi, T. (1984): Scanning electron microscopic observations of serosal surfaces of the internal orgns in the calf. Jpn. J. Zootech. Sci., 55: 20-24.

Goldberg, S.R. (1972): Seasonal weight and cytologicl changes in the fat bodies and liver ofthe lguanid lizard Sceloporus jarrovi Cope. Copeia, 227-232.

Gorgas, K. and Storch, V. (1984): Marginal plates in hepatic peroxisomes o Ischthyophis glutinosus (Amphibia: Gymnophiona). A cytochemical study. Cell Tissue Res., 238: 413-416.

Hahn, W.E. (1967): Estradiol-induced vitellogenesis and concomitant fat mobilization in the lizard Uta stansburiana. Comp. Biochem. Physiol., 23: 83-93.

Henninger, J.M. (1982): Histology of the liver in the box turtle. Anat. Rec., 202, 79A (Abstract).

Hirakow, R.; Komazaki, S. and Hiruma, T. (1987): Early cardiogenesis in the newt embryo. Scanning Microscopy 1: 1367-1376.

Hruban, Z. and Rechcigl, M. (1969): Microbodies andrelated particles. Int. Rev. Cytol. Suppl. 1 (Abstract).

Jones, R.G.; David, W.L. and Goodman, D.B.P. (1981): Microperoxisomes in the epithelial cells of the amphibian urinary bladder: An electron microscopic demonstration of catalase and malate synthease. J. Histochem. Cytochem. 29: 1150-1156.

Nicholls, T.J.; Follett, B.K. and Evennett. P.J. (1968): The effects of oestrogens and other steroid hormones on the ultrastructure of the liver of Xenopus laevis Daudin. Zellforsch. 90: 19-27.

Novikoff, A.B. and Holtzman, E. (1970): Cells and organelles (1st ed.). New York, Holt, Rinehart andWinston, Inc.

Peek, W.D.; Sidon, E.W.; Youson, J.H. and Fisher, M.M. (1979): Fine structure of the larval liver in lamprey. Petromyzon marinus L. Hepatocytes and sinusoids. Am. J. Anat. 156: 231-249.

Pfeiffer, C.J.; Nagai, T.; Fujimura, M. and Tobe, T. (1979): Spontaneous regressive epitheliomas in the Japanese newt, Cynops pyrrhogaster. Can. Res. 39: 1904-1910.

Pfeiffer, C.J.; Nagai, T.; Fujimura, M. and Tobe, T. (1985): Teratogenic effects of carcinogenic agents on limb regeneration in the Japanese newt, Cynops pyrrhogaster. Teratogenesis, Carcinogenesis, Mutagenesis, 5: 137-147.

Pfeiffer, C.J. and Keith, J.C., Jr. (1985): Ultrastructure of the stomach of the small short-tailed shrew, Blarina brevicauda. C.J. Submicrosc. Cytol. 17: 315-326.

Pfeiffer, C.J.; Pfeiffer, D.C. and Misra, H. (1987): Enteric serosal surface in the piglet. A scanning and transmission electron microscopic study of the mesothelium. J. Submicrosc. Cytol., 19: 237-246.

Pfeiffer, C.J.; Asashima, M. and Hirayasu, K. (1989): Ultrastructural characterization of the spontaneous papilloma of Japanese newts. J. Submicrosc. Cytol. Pathol. 21: 659-668.

Pfeiffer, C.J. and Asashima, M. (1990): Gstric leiomyomas in the Japanese newt, Cynops pyrrhogaster; ultrstructural observations. J. Comp. Path. 102: 79-87.

Pfeiffer, C.J.; Asashima, M.; Cho, C.H. and Shimada, K. (1990a): Observations on cardiac ultrstructure of the adult Japanese newt, Cynops pyrrhogaster. J. Submicrosc. Cytol. Pathol. 22: 17-26.

Pfeiffer, C.J.; Pyle, H. and Asashima, M. (1990b): Blood cell morphology and counts in the Japense newt, Cynops pyrrhogaster. J. Zoo. Wildlife Med. (in press).

Reddy, J. and Svoboda, D. (1973): Microbody (peroxisome) matrix: transformation into tubular structures. Virchows Arch. B. Zell-path., 14: 83-92.

Roels, F.; Schiller, B. and Goldfischer, S. (1970): Microbodies (peroxisomes) in the toad, Bufo marinus. A cytochemical study. Z. Zellforsch., 108 139-149.

Scott, P.J.; Visentin, L.P. and Allen, J.M. (1969): The enzymatic characteristics of peroxisomes of amphibian and avian liver and kidney. Ann. N.Y. Acad. Sci. 169: 244-264.

Sidon, E.W. and Youson, J.H. (1983): Morphological changes in the liver of the sea lamprey, Petromyzon marinus L., during metamorphosis II. Canalicular degeneration and transformation of the hepatocytes. J. Morph., 178 225-246.

Storch, V.; Braunbeck, T. and Waitkuwait, W.E. (1989): The liver of the West African crocodile Osteolamaemus tetrapis. An ultrastructural study. J. Submicrosc. Cytol. Pathol., 21: 317-327.

Taira, K. and Mutoh, H. (1981): Comparative ultrastructural study of the Ito cells in the liver in some reptiles. Arch: histol. Jap., 44: 373-383.

Uehara, M.; Taguchi, M.; Asashima, M. and Pfeiffer, C.J. (1989): Developmental physiology of cardiac contraction in the Japanese newt in vivo and in vitro. J. Mol. Cell. Cardiol. 21: 709-718.

Welsch, U. and Storch, V. (1972): Elektron mikroskopische Untersuchungen and der Leber von Ichthyophis kohtaoensis (Gymnophiona). Zool. Jhrb. Anat., 89: 621-635.

Yamamoto, T. (1964): Some observations of the fine structure of the liver cells in the starved goldfish (Carassius auratus) with special reference to the morphology of fat mobilization during starvation to the liver. Arch. Histol. Jap., 24: 335-345.

Yamamoto, T. (1973): Enzyme histochemical and ultrastructural observations on the liver of teleost fishes. Arch Histol. Jap., 36: 21-37.

Youson, J.H. (1981): Theliver. In: Hardisty, M.W. and Potter, I.C. (eds.). The Biology of Lampreys (1st ed.). Vol. 3, pp. 263-332.

Youson, J.H.; Ellis, L.C.; Ogilvie, D. and Shivers, R.R. (1987): Gap junctions and zonulae occludentes of hepatocytes during biliary atresia in the lamprey. Tissue and Cell. 19: 531-548.

Youson, J.H.; Sidon, E.W.; Peek, W.D. and Shivers, R.R. (1985): Ultrastructure of the hepatocytes in a vertebrate liver without bile ducts. J. Anat., 140: 143-158.