Electron Microscopic Study on the Hepatic Sinusoidal Wall of the Soft-Shelled Turtle (Amyda japonica) with Special Remarks on the Smooth Muscle Cells*

Yutaka TANUMA

Department of Anatomy (Prof. K. UCHIDA), Teikyo University School of Medicine, Tokyo, Japan

Received December 3, 1986

Summary. The hepatic sinusoids of the soft-shelled turtle (Amyda japonica) were examined by transmission electron microscopy. The sinusoidal wall was composed of endothelial cells, Kupffer cells and Ito cells. The basal surface of the hepatocyte facing the Disse's space was covered by a continuous basal lamina. In addition to the Ito cells, the Disse's space contains a considerable number of smooth muscle cells. Many of these were distributed sporadically, while others appeared as a sphincter circling the sinusoid. The smooth muscle cells in the Disse's space showed the following features: 1) The nucleus was located eccentrically near one end of the cell. 2) The surface vesicles and pits, mitochondria and dense patches along the myofilament bundles were all sparse as compared with those known from mammalian smooth muscle cells. 3) Cytoplasmic processes or ruffles were protruded into the Disse's space. 4) A weak basal lamina could be recognized.

Sinusoidal endothelial cells were characterized by many large electron lucent lysosomes in their perikaryon and by small fenestrae in their attenuated cytoplasm. Ito cells sending out several cytoplasmic processes, possessed a single large lipid droplet on one side of the nucleus. A single cilium budding from the distal centriole into the Disse's space was found in an Ito cell.

Extrasinusoidal macrophages were considerably numerous in the soft-shelled turtle liver. Some of the macrophages were apparently migrating into the sinusoid, there to presumably transform into the Kupffer cells.

It has been established that the hepatic sinusoidal cells comprise the endothelial cells, Kupffer cells and Ito cells (fat-storing cells). In addition to these three cell types, however, SATO and YAMAMOTO (1983) electron microscopically discovered smooth muscle cells dispersed in the Disse's space in the fresh-water catfish. During the course of the present ultrastructural study, the author was able to confirm a similar finding in the liver of a reptilian species, the soft-shelled turtle (Amyda japonica).

MATERIALS AND METHODS

Three adult soft-shelled turtles of both sexes were used. They were sacrificed by decapitation. After dissection, the liver was removed for perfusion via the portal vein with a cold fixative, 2.5% glutaraldehyde in a 0.1M phosphate buffer at pH 7.4, 0°C. The

*This study was supported in part by grants from Ministry of Education, Science and Culture, Japan.
liver was excised and cut into minute blocks. After 2 hr fixation, the tissue blocks were rinsed several times in a cold 0.1 M phosphate buffer containing 5% sucrose at pH 7.4, to be left overnight in the same buffer at 5°C. They were post-fixed with 1% osmium in 0.1 M phosphate buffer containing 5% sucrose, pH 7.4, for 90 min at 0°C. The tissue blocks were dehydrated in an ethanol series and embedded in Quetol 812. Ultrathin sections were stained with saturated uranyl acetate and Sato's lead solution. Micrographs were taken with JEM-100C electron microscope.

For light microscopy, toluidine-blue-stained thick sections of Quetol-embedded tissue or hematoxylin-eosin-stained paraffin sections were used.

RESULTS

Light microscopic observations showed the hepatocytes arranged in masses or cords between which sinusoids of variable shapes and sizes interposed. The hepatocytes contain many lipid droplets (vacuoles) of variable sizes. The large spherical nucleus with its conspicuous nucleolus is shifted eccentrically toward the sinusoidal surface of the cytoplasm (Fig. 1). A solitary large lipid droplet (rarely detectable beneath the endothelial lining) belongs to the fat-storing cell (Fig. 1).

The hepatic parenchyme contains scattered connective tissue masses which correspond to the interlobular connective tissue or Glisson’s sheath, but the lobulation of the hepatic parenchyme was not distinct. In the Glisson’s sheath (portal tract) there appear branches of variable size of the hepatic artery, portal vein and bile duct (Fig. 1). Large branches of the bile duct are provided with a circular smooth muscle layer, with a narrow connective tissue layer corresponding to the lamina propria intervening

---

**Fig. 1.** Hepatic parenchyme as observed with the light microscope. Hepatocytes containing abundant lipid vacuoles form cell masses between which sinusoids (SN) and a portal tract (Glisson’s sheath) (PT) are interposed. In the portal tract are seen a small bile duct (BD), a branch of the hepatic artery (HA) and a portal vein (PV). Arrows indicate single, large lipid droplets in Ito cells. Toluidine blue. ×1,200
between the simple cuboidal epithelium and the muscle layer (Fig. 2). The muscle layer can hardly be distinguished in the wall of small bile duct branches with simple squamous epithelium (Fig. 1). Smooth muscle cells subjacent to the endothelial lining of the sinusoids can often be identified, as was later confirmed by the electron microscopy.

In the connective tissue of the portal tract, small lymphoid tissue (infiltration type) is often distributed around the portal vein branch spreading out into the parenchyme (Fig. 3).

The so-called central vein, the terminal branch of the hepatic vein, can not be definitively identified.

As observed with the electron microscope, the parenchyme of the soft-shelled turtle liver is composed of polygonal or irregularly shaped hepatocytes which contain considerable amounts of lipid-droplets usually forming groups in the peripheral portion of the hepatocytes. Large mitochondria with matrix granules and glycogen particles are distributed throughout the cytoplasm, and small numbers of small lysosomes are scattered at random. Cisterns of the rough endoplasmic reticulum are frequently detected elongated around the mitochondria.

The interhepatocytic spaces which communicate with the perisinusoidal Disse's space are relatively wide and show alternately expanded portions and constrictions, the former containing tortuous microvilli protruded from the hepatocytes, so that the distinction of the bile canaliculi—usually surrounded by narrow apical surfaces of
three or four hepatocytes with sparse short finger-shaped microvilli from the expanded portions—is often difficult.

The wide basal surface of the hepatocyte facing the Disse's space is covered by a conspicuous basal lamina which does not extend on the lateral surface bordering the

---

**Fig. 3.** Two large branches (PV) of the portal vein in the portal tract. Lymphoid tissue (LT) spreads along their walls. SN sinusoid. Hematoxylin and eosin. ×1,200

**Fig. 4.** Two sinusoidal endothelial perikarya found on opposite poles of a sinusoid (SN). The perikaryon (PK) on the right hand is located in a depression between hepatocytes; the other (PK) on the left hand bulges into the sinusoid. Both perikarya contain only sparse electron lucent lysosomes with a few spots of high electron density. A thin cytoplasm extends between the two perikarya, making the endothelial lining (EL) of the sinusoid in which thicker and thinner portions are distinguished. E erythrocyte, H hepatocytes, L lymphocyte, PS Disse's space. ×5,400
interhepatocytic space. From the basal surface of the hepatocyte, microvilli protrude
into the Disse's space; these vary in number from place to place. At times, many
microvilli form conglomerates. The microvilli and their conglomerates are covered by
the extension of the continuous basal lamina of the basal surface of the hepatocyte
(Fig. 8, 9).

Fig. 5. a–c. Three perikarya (PK) of the sinusoidal endothelial cells bulging into the sinusoid
(SN). They contain varying numbers of large lysosomes of low electron density
containing highly electron dense spots. In addition, there are smooth-surfaced
tubules, sparse cisterns of rough endoplasmic reticulum, mitochondria, coated ves-
icles and pits, some of which open into the sinusoid or Disse's space (arrowheads). In
c a centriole (arrow) protruding a cross striated rootlet is seen. In b a macrophage
(MP) with lysosomes and a cross section of a smooth muscle cell (SM) sending out
a thick process to form a close junction (arrow) with a hepatocyte are seen in the
Disse's space. H hepatocyte, PS Disse's space. a: ×11,100, b: ×14,000, c: ×13,300
1. Sinusoidal endothelial cell

It has been long established that the sinusoidal endothelial cells which line the sinusoids are composed of a cell body containing the nucleus (perikaryon) and an attenuated membranous cytoplasmic extension. The perikaryon fits either in a cavity between hepatocytes or bulges into the sinusoidal lumen (Fig. 4). In the latter case it often elongates along the sinusoidal wall (Fig. 5a-c). The most conspicuous feature of the sinusoidal endothelium of the turtle is the abundance of large, presumable lysosomes of low electron density which for the most part are gathered in the perikaryonal cytoplasm. These vary in amount from cell to cell; endothelial cells depicted in Figure 4 possess only sparse lysosomes in contrast to those depicted in Figure 5a–c. These relatively large lysosomes frequently contain a few small, basically round spots of higher electron density.

Besides the large lysosomes are revealed coated pits and vesicles along the sinusoidal surface, short cisternae of the rough endoplasmic reticulum, smooth surfaced curved tubules and small mitochondria distributed mostly near the nucleus; the Golgi complex is almost never detected. In one perikaryon a centriole is demonstrated on the opposite side far away from the eccentric nucleus and a fine cross-striated rootlet is protruded from the centriole (Fig. 5c). The spacial relation between the

![Figure 6](image_url)

Fig. 6. Thinner portion of the cytoplasmic sheet of the sinusoidal endothelial cell. a. Perpendicular section. Fenestrae (arrows) are hardly discernible. b. Tangential section. Many small fenestrae are shown. F collagen fibrils, FSC Ito cell, H hepatocyte, PS Disse's space, SM smooth muscle cell, SN sinusoid. a and b: ×13,300
centriole and the Golgi complex is not clear.

The cytoplasmic extension of the sinusoidal endothelium is divided into thicker and thinner portions, but the thicknesses are irregular and an alternate distribution is often obscure (Fig. 4). In perpendicular sections of the thinner portions the fenestrae can be identified, although rarely (Fig. 6a); in tangential sections, however, they can be clearly seen (Fig. 6b), appearing as small round pores measuring about 90 nm in diameter on the average.

As is well established, the perisinusoidal surface of the endothelial lining confronting the basal surface of the hepatocyte lacks the basal lamina, in contrast to the latter.

2. Fat-storing cell (Ito cell)

So-called empty fat-storing cells are occasionally found in the Disse’s space. They possess no lipid-droplet, and sparse organelles, especially cisterns of the rough endoplasmic reticulum are underdeveloped. They do retain, however, the characteristic morphologic feature, i.e., the subendothelial processes extended along the sinusoidal endothelial lining (Fig. 7). In the Ito cell containing a small lipid droplet, the cisternae of the rough endoplasmic reticulum are better developed, often encircling the mitochondria (Fig. 8). It is unique finding that the mitochondria contain intramitochondrial granules (matrix granules) as do those of the hepatocytes in the same animals. Along with the enlargement of lipid droplets, Ito cells containing two or three large lipid droplets (Fig. 9) appear sporadically in the Disse’s space. These finally become mature

Fig. 7. Undifferentiated empty Ito cell (FSC). Attenuated cytoplasmic processes are protruded just beneath the endothelial lining (EL) of the sinusoid (SN) from both ends of the cell body which contains a spindle-shaped nucleus long. Besides a small lysosome, cisternae of the rough endoplasmic reticulum are barely distinguishable. H hepatocytes, PS Disse’s space. ×6,000
Ito cells containing a single extraordinarily large lipid droplet on one side of the nucleus, thus inducing a large indentation on the nuclear membrane (Fig. 10). The lipid droplets, of variable sizes in the different stages of development, are provided with no limiting membrane, though in some cases they possess a faint rim of β-glycogen particles (Fig. 10). Figure 11a depicts an Ito cell with a large single lipid droplet projecting an electron dense, short, pointed process into the Disse’s space. As seen in a higher magnification (Fig. 11b), the bud-like cytoplasmic process extends into the Disse’s space from a basal body (distal centriole). Beneath the basal body a proximal centriole is faintly visible. Golgi complexes are distributed around the diplosome.

Collagen fibers are generally sparse in the Disse’s space (Fig. 11a, 13a, 16, 18).

**Fig. 8.** An Ito cell possesses only one or two small lipid droplets (LD) and fairly well-developed cisterns of the rough endoplasmic reticulum around the mitochondria (M). Along the basal surface of the hepatocytes (H) together with microvilli, a continuous basal lamina (arrows) is shown which is lacking, however, along the microvillous processes (P) protruded from the Ito cell (FSC). EL endothelial lining of the sinusoid (SN), MV microvilli of hepatocyte, TH thrombocyte. ×14,700

**Fig. 10.** An Ito cell (FSC) in a mature stage, containing a single huge lipid droplet (LD) which exhibits a faint glycogen-particle rim and makes a large depression on the nuclear membrane. In the cytoplasmic layer around the nucleus and the lipid droplet, mitochondria and associated rough endoplasmic reticulum are seen. H hepatocytes, PK perikaryon of the sinusoidal endothelial cell being attenuated toward the left side, PS Disse’s space, SN sinusoid. ×12,100
Fig. 9. An Ito cell (FSC) with two large lipid droplets (LD) protruding cytoplasmic processes (P) into the Disse's space (PS). The basal surface of hepatocytes (H) is covered by a continuous basal lamina (arrows) together with microvilli protruded from the hepatocytes. E erythrocyte, EL endothelial lining of the sinusoid (SN). ×11,700

Fig. 10. Legend on the opposite page.
3. Kupffer cells and extrasinusoidal macrophages

Kupffer cells are encountered rather frequently in the sinusoid of the turtle liver. Besides many lysosomes, relatively large mitochondria which tend to make groups in random portions, rough endoplasmic reticulum and free ribosomes are scattered throughout the cytoplasm, except for the ectoplasmic layer which protrudes pseudopodia (Fig. 12a). Centrioles are occasionally detected, but the Golgi apparatus is

Fig. 11. a. An Ito cell (FSC) with a single lipid droplet (LD) elongated along the endothelial lining (EL) of the sinusoid (SN). From the sinusoidal surface of the Ito cell a bud-like process (arrow) is protruded into the Disse's space, suggesting a single growing cilium. A long tortuous and branching cytoplasmic process (P) is sent out from the sinusoidal surface on the opposite side into the Disse's space (PS) along the endothelial lining. E erythrocyte, F collagen fiber, H hepatocyte. b. Higher magnification of the single budding cilium arising from the distal centriole (arrow) of the diplosome which is located within the Golgi apparatus (G). The proximal centriole is indicated by a short arrow. EL endothelial lining of the sinusoid, LD lipid droplet. a: ×8,300, b: ×28,600
missing for an unknown reason (Fig. 12a, b, 14). Kupffer cells in the soft-shelled turtle liver are fixed by junctions to the endothelial lining (Fig. 12a). For example, the endothelial cell body send out cytoplasmic processes toward the Kupffer cell, and their ends connect with the Kupffer cell body by the so-called junctional complexes of Wisse (1970) (Fig. 12a, b).

Extra- sinusoidal macrophages are frequently found in the interhepatocytic or

![Fig. 12. a and b. Kupffer cells (KU) of the soft-shelled turtle bulging into the sinusoid (SN) and fixed to the endothelial lining (EL) of the sinusoid. In a the pseudopods protrude not only into the sinusoid, but also into the Disse's space (PS). Arrow indicates a centriole within the Golgi apparatus (G). In b the perikaryon (PK) of the endothelial cell sends out two slender processes to anchor the Kupffer cell by means of the junctional complexes (arrows) of Wisse. Cisternae of the rough endoplasmic reticulum, dense bodies of variable sizes and vacuoles are scattered throughout the cytoplasm except for the ectoplasmic layer, but large mitochondria show a tendency to accumulate in random areas of the cytoplasm. a: ×6,800, b: ×10,000]
Disse’s space. They are characterized by mitochondria as large as those of the Kupffer cells of the sinusoids (Fig. 5b, 13a, 15a). They frequently contain lysosomes of variable sizes as well as an occasional a clump of highly electron dense small particles. Morphological signs suggestive of their migration through gaps of the endothelial lining into the sinusoid are frequently observed (Fig. 13b). These macrophages likely transform into Kupffer cells after complete transposition in the sinusoid. Occasionally a lymphocyte is also found in the interhepatocytic space.

Fig. 13. a. An extrasinusoidal macrophage (MP) with numerous mitochondria, sparse small dense bodies and a centriole (arrow) in the hepatic parenchyme. EL endothelial lining, F collagen fiber, H hepatocytes, SN sinusoid. b. An extrasinusoidal macrophage (MP) with large mitochondria and lysosomes undergoing migration through the endothelial lining (EL) into the sinusoid (SN). The cytoplasmic portion has already moved into the sinusoid. On the left side of the sinusoid a smooth muscle cell (SM) is seen in the Disse’s space. H hepatocytes. a and b: ×6,800
4. Smooth muscle cells in the Disse’s space

Smooth muscle cells are frequently found in the Disse’s space of the soft-shelled turtle liver. As shown in Figure 14, smooth muscle cells surround a sinusoid almost completely, and at the bottom are overlapped in two layers. Between the closely overlapping muscle cells there are a few possible nexuses. The above findings strongly suggest that the hepatic sinusoid of the turtle is provided with real sphincters composed of smooth muscle cells at random locations as proposed by SATO and YAMAMOTO (1983) in the catfish liver. Besides the circularly arranged smooth muscle cells, there appear longitudinally oriented ones (Fig. 15a) which, by contraction, may possibly stretch the sinusoidal wall in a longitudinal direction. From observations of cross, longitudinal and oblique sections of the smooth muscle cells in the Disse’s space,

Fig. 14. Circularly oriented smooth muscle cells (SM) in the Disse’s space (PS) around a small sinusoid (SN) just beneath the endothelial lining (EL). They probably participate in the sphincter mechanism of the sinusoid. The nucleus of a smooth muscle cell is located extremely eccentrically. Mitochondria are sparse and myofilaments are abundant in the cytoplasm. A cytoplasmic process is projected through the endothelial lining into the sinusoid (arrow). E erythrocyte, H hepatocytes, KU Kupffer cell. × 8,300
however, it is difficult to visualize their accurate orientation against the sinusoids. The number of profiles of smooth muscle cells in the Disse’s space of the turtle liver seems to exceed that in the sinusoidal wall in the cat-fish.

The spindle-shaped nucleus of the smooth muscle cells in the Disse’s space appears to be located eccentrically; it contains a moderate amount of heterochromatin (Fig. 14, 15a, 18).

The nuclear membrane shows no striking indentations. The cytoplasm is packed by longitudinally oriented myofilaments. In rare cases myofilaments deviate from one

![Figure 15](image_url)

**Fig. 15.** a. Longitudinal section of a smooth muscle cell (SM) extending between two sinusoids (SN). The smooth muscle cell sends out several processes or ruffles, two of which connect with an extrasinusoidal macrophage (MP). EL endothelial lining, FSC Ito cell with single large lipid droplet (LD), H hepatocytes, PK perikarya of endothelial cells, PS Disse’s space. b. Higher magnification of the smooth muscle cell (SM) shown in Figure a: two cytoplasmic processes connect (arrows) with the macrophage (MP) in the Disse’s space (PS). In the cytoplasm densely packed with myofilaments, long inconspicuous dense patches are detected. Subsarcolemmal dense areas occupy the major part of the cell surface, but surface vesicles and pits are only rarely encountered. The basal lamina is hardly identifiable as its outline is indistinct. a: ×5,000, b: ×13.300
pole of the nucleus, leaving a clear area (Fig. 15a, b). The so-called sublemmal dense area occupies the major part of the cytoplasmic surface (Fig. 15b, 16), while the so-called dense areas (dense patches or bodies) scattered along the myofilament bundles are neither numerous nor conspicuous (Fig. 15b, 16). The basal lamina is detectable by careful observation, but its outer border is not distinct (Fig. 15b, 16). The most striking ultrastructural feature of the smooth muscle cell in the Disse’s space of the turtle liver is the paucity of the surface (subsarcolemmal) vesicles and pits (Fig. 15b). In addition, the surface of the smooth muscle cells sends out small cytoplasmic processes or folds (ruffles) of variable shapes and sizes, which are especially numerous in the perinuclear region (Fig. 13a, 14, 15a, b, 17). Among the processes, there are such ones as project into the sinusoid through the endothelial lining (Fig. 14, 16) or toward the neighboring hepatocyte (Fig. 5b) or macrophage (Fig. 15a, b) to connect with the hepatocyte or macrophage by a close junction or to terminate freely in the Disse’s space. Furthermore, simple branchings of the attenuated end part of the smooth muscle cell are occasionally observed.

Small, round or oval mitochondria are sparsely scattered throughout the cytoplasm, occasionally concentrated in the perinuclear cytoplasm (Fig. 17). Cisterns of the rough endoplasmic reticulum are distributed randomly or in association with mitochondria.

Fig. 16. A highly magnified section of a smooth muscle cell (SM) in the Disse’s space (PS). Myofilament bundles are nearly longitudinally sectioned. In the cytoplasmic areas between the bundles are seen accumulations of glycogen particles. The subsarcolemmal dense areas occupy the major part of the cell surface, but dense patches along the bundles are sparse and inconspicuous. Surface vesicles and pits are barely detectable. The basal lamina (arrowheads) can be identified, but its outlines become vague toward the connective tissue. The upper cytoplasmic process penetrates the endothelial lining (EL) to extend into sinusoid (SN): the bottom left one extends into the Disse’s space. Arrows indicate the basal lamina covering the basal surface of the hepatocyte (H) together with its microvilli and a small process. F collagen fiber. ×25,000
A diplosome accompanied by the Golgi apparatus was demonstrated in one case of the perinuclear cytoplasm (Fig. 17). Glycogen particles are demonstrated frequently in the smooth muscle cells of the turtle liver. Their amounts undergo individual variation. They are often concentrated near the mitochondria and one nuclear pole (Fig. 16, 18).

Fig. 17. A cross section of the nucleated portion of a smooth muscle cell (SM). The perinuclear cytoplasm containing several mitochondria and a diplosome (C) accompanied by a Golgi apparatus (G) protrudes many complicated cytoplasmic processes or ruffles into the Disse's space (PS). The subsarcolemmal dense area and basal lamina are visible, but the surface vesicles and pits are almost indiscernible. Endothelial lining (EL) covering the nucleated portion is composed of closely overlapped fenestrated thinner portions of the endothelial extention. F collagen fiber, SN sinusoid. × 14,800

Fig. 18. Longitudinal section of a smooth muscle cell (SM) containing abundant glycogen particles accumulated around the nucleus. The spindle-shaped nucleus is located extremely excentrically. The subsarcolemmal dense area is interrupted at long intervals. A few surface vesicles and pits can be detected. Faint basal lamina can be indentified (arrowhead). A small centriole-like circular body is found in the dense area near the nucleus (arrow). EL endothelial lining of the sinusoid (SN), F collagen fiber, FSC Ito cell, PS Disse's space. ×13,300
5. Smooth muscle cells of the intrahepatic portal vein

Small branches of the portal vein in the portal tract (Glisson's sheath) contain a thin smooth muscle layer adjacent to the endothelial lining and distributed at variable intervals. The smooth muscle cells composing this layer show some ultrastructural differences as compared with those found in the Disse's space and resemble the ordinary smooth muscle cells, for example, of the intestinal muscle coat (YAMAMOTO, 1977). As an example of this, surface vesicles and caveolae are more numerous here than in the smooth muscle cells in the Disse's space, and the dense areas along the myofilament bundles are more numerous and conspicuous (Fig. 19a, b). The spindle-shaped nucleus is located in the thickened central part of the muscle cell (Fig. 19b). Numerous small cytoplasmic processes or ruffles are protruded into the connective tissue from the entire surface of the cell. The majority of these contains the surface vesicles.
DISCUSSION

Few reports are available on the ultrastructure of the sinusoidal wall of the reptile livers except for an electron microscopic study carried out recently by TAIRA and MUTOH (1981). Theirs was a study of mainly the fat-storing cell (Ito cell) of the lizard (Takydromus tachydromoides), snake (Rhabdophis tigrinus) and the tortoise (Clemmys japonica); though obtaining interesting findings, they left other sinusoidal cells untouched.

The most characteristic feature of the hepatocytes of the soft-shelled turtle is that their basal surface is covered by a conspicuous basal lamina together with the microvilli protruded into the Disse's space. The same finding was recorded by TAIRA and MUTOH (1981) in the lizard liver. It therefore seems likely that the basal lamina covering the hepatocytes is a common feature of the reptile liver. This basal lamina is lacking in mammalian, avian and piscine livers. It is noteworthy that it appears again in the lamprey liver (SHIN, 1977; PEEK et al., 1979).

In agreement with evidence from the catfish liver (SATO and YAMAMOTO, 1983), lobulation of the hepatic parenchyme is also rarely observed also in the soft-shelled turtle. The connective tissue masses corresponding to the Glisson's sheath (portal tract) are scattered from place to place containing branches of the hepatic artery, portal vein and bile duct; the lymphoid tissues are distributed occasionally around the portal vein branches as reported by KANESADA (1956) in reptiles and birds (cf. reference of OHATA et al., 1982). An interesting and unique finding is that in the wall of the thicker branch of the bile duct with simple cuboidal epithelium there occurs a relatively thick circular smooth muscle layer. The occurrence of this muscle layer in the wall of the intrahepatic part of the bile duct system has never been reported in the mammalian liver. Furthermore, thin smooth muscle layers are distributed discontinuously in the wall of the portal vein in the portal tract just beneath the endothelial lining.

The sinusoidal endothelial cell of the turtle liver is characterized by large electron lucent bodies filling the perikaryonal cytoplasm; the majority of them contain small electron dense spots or granules. These lysosome-like bodies may be organelles corresponding to the macropinocytic vacuoles (vesicles) (WISSE, 1972) which have been detected by OHATA et al. (1982) in the perikaryonal cytoplasm of the sinusoidal endothelial cells of the chicken liver. TAIRA and MUTOH (1981) observed many similar bodies in the lizard sinusoidal endothelial cells, but they regarded them as lipid-droplets. In their experimental electron microscopic study on chicken livers, OHATA and ITO (1986a, b) demonstrated that, after intravenous perfusion of India ink, the carbon particles are taken up by pinocytotic mechanism in sinusoidal endothelial perikaryon and are ingested by macropinocytotic vacuoles as early as 30 min after perfusion to then form large vacuoles filled with particles. In the soft-shelled turtle liver, the possible large, electron lucent lysosomes may participate in the endocytosis or phagocytosis of foreign substances from the blood, thus the sinusoidal endothelial cells of the soft-shelled turtle liver are thought to have an avid endocytic activity.

In one case where the perikaryon was filled with the lysosomes, a centriole was detected among them far away from the eccentric nucleus. That this centriole protruded a cross striated rootlet was a somewhat curious finding. A similar, peculiar finding had been earlier revealed by UMARAHARA (1968) in the capillary endothelial cell of bat brown adipose tissue.
The fenestrae of the endothelial cells seemed to be the smallest ones among those in the hepatic sinusoids of vertebrates, measuring 90 nm on the average, so that they were usually missed in perpendicular sections of the thinner portion.

The Ito cells of the mature soft-shelled turtle contain a single large lipid droplet on one side of the nucleus making a large indentation on the nuclear membrane. From a viewpoint of the development of their lipid droplets, these pass through an "empty stage" with no lipid droplet, then a stage with only one or two small droplets, followed by a stage with two or three larger droplets which probably fuse finally into one extraordinarily large droplet in the mature stage.

In his light microscopic study on snake livers, WATARI (1959) reported that the Ito cells of the snake liver possessed a single large lipid droplet except for empty cells and that the size of the single droplet underwent seasonal changes. Recently, TAIRA and MUTOH (1981) electron microscopically demonstrated that in lizards (Takydromus tachydromoides) and snake (Rhabdophis tigrinus) caught from natural habitats the Ito cells of the liver possessed a single large lipid droplet—in agreement with findings in the soft-shelled turtle and in the snake observed light microscopically by WATARI.

In the tortoise (Clemmys japonica) the natural lipid droplet pattern was disturbed due to experimentally induced hypervitaminosis A (KOBAYASHI and TAKAHASHI, 1971).

A single cilium budding from the distal centriole of the diplosome within the Golgi apparatus was demonstrated in one of the Ito cells observed in the soft-shelled turtle. This finding suggests that in the reptile species also do the Ito cells possess the single or solitary cilium projecting into the Disse's space, which is regarded as a sensory cilium or chemoreceptor and has been demonstrated by many authors in a variety of vertebrate species including man (OHATA et al., 1982).

Whether or not Kupffer cells may be present in fish livers has remained a controversial subject (TAMARU, 1979; TANUMA and ITO, 1980; FUJITA et al., 1980; SATO and YAMAMOTO, 1983). In reptiles, however, they are thought to occur consistently in the hepatic sinusoids (TAIRA and MUTOH, 1981). In soft-shelled turtle livers they are frequently found in sinusoids, being fixed to the endothelial lining (ITO et al., 1980). The worm-like bodies which are considered as a marker structure of mammalian Kupffer cells have been demonstrated in those of chicken liver (OHATA et al., 1982), but not in the soft-shelled turtle liver. No trace of glycocalyx (a fuzzy cell coat) could be found in soft-shelled turtle Kupffer cells.

As in the chicken liver (OHATA et al., 1982), extrasinusoidal macrophages were also often found in the Disse's and interhepatocytic space in the soft-shelled turtle liver. They often exhibited images suggesting migration through the endothelial lining into the sinusoid to there transform into the Kupffer cells. These extrasinusoidal macrophages occasionally contained, besides large mitochondria and lysosomes, a densely packed clump of highly electron dense small granules probably ingested from the melanocytes found frequently in the interhepatocytic space of the soft-shelled turtle liver. The existence of pigment cells (melanocytes?) was confirmed by TAIRA and MUTOH (1981) in the Disse's space of the tortoise (Clemmys japonica). The above finding may suggest that the extrasinusoidal macrophages have already acquired their phagocytic ability in the hepatic parenchyme before transformation into Kupffer cells (OHATA and ITO, 1986a, b). The frequent occurrence of extrasinusoidal macrophages in the hepatic parenchyme of the soft-shelled turtle may be related to the existence of the lymphoid tissue in the Glisson's sheath spreading into the adjacent parenchyme (OHATA and ITO, 1986a, b).

In their electron microscope observation of freshwater catfish livers, SATO and
YAMAMOTO (1983) were able to ascertain smooth muscle cells in the Disse's space, and came to the conclusion that the catfish sinusoids are provided with sphincters of smooth muscle nature. Subsequently, the present author demonstrated smooth muscle cells in the Disse's space of the soft-shelled turtle liver. This would seem to be the first report of smooth muscle cells in the Disse's space of a reptile.

In the present study, a circularly arranged smooth muscle layer—which may be referred to as a real sinusoidal sphincter—was actually revealed just beneath the endothelial lining. From observations of the profiles of individual smooth muscle cells scattered in the Disse's space, it is difficult to presume their orientation against the sinusoid. In contrast to the smooth muscle cells in the wall of the portal vein branches which resemble in ultrastructure the ordinary smooth muscle cells such as the intestinal musculature (YAMAMOTO, 1977), those found in the Disse's space exhibited some ultrastructural peculiarities: 1) An oval nucleus is usually located eccentrically close to one end of the long spindle-shaped cell. 2) From the cell surface cytoplasmic process or folds (ruffles) of variable shapes and sizes are projected into the Disse's space, especially numerous from the perinuclear cytoplasm. Some of these penetrate the endothelial lining of the sinusoid or attach with their wide surface to the neighboring hepatocyte or macrophage existing in the Disse's space. These attachment devices differ from the gap junctions between hepatocytes. Incidences of attachment between the process of the smooth muscle cell and the hepatocyte was also noted by SATO and YAMAMOTO (1983) in the catfish liver. 3) A weak basal lamina wraps the entire surface of the smooth muscle cells, but its outer surface fades away into the connective tissue of the Disse's space showing no distinct outline. According to SATO and YAMAMOTO (1983) the smooth muscle cells in the Disse's space of the catfish liver are embedded in an amorphous material. 4) Smooth muscle cells in the Disse's space of the turtle liver are characterized by their extreme paucity of surface (subsarcolemmal) vesicles and pits. They are also thought to be less numerous than those of the smooth muscle cells in the catfish liver. 5) The so-called sublemmal dense area occupies the major part of the surface of the smooth muscle cell, while the dense patches (areas) distributed along the densely packed myofilament bundles are sparse and inconspicuous. These structures were left untouched by SATO and YAMAMOTO (1983) in the smooth muscle cells in the Disse's space of the catfish liver. 6) Small mitochondria are scattered in small numbers throughout the cell, showing a tendency to be most numerous in the perinuclear cytoplasm, together with the diplosome within the Golgi area. These mitochondria are far less numerous as compared with the many mitochondria in the smooth muscle cells in the mouse small intestine (YAMAMOTO, 1977). This difference in the amount of mitochondria is most likely derived from the difference in activity between the cells in question. The paucity of the surface vesicles and pits in the smooth muscle cells in the Disse's space may also be related to their low functional activity.

The soft-shelled turtle liver is rich in smooth muscle cells, which are distributed not only in the Disse's space, but also in the smooth muscle layer of the intrahepatic bile ducts as well as those of the portal vein branches.

It has recently been proved by means of the immunofluorescence and immunoperoxidase method that there exist action filaments both in the sinusoidal endothelial cell and Ito cell of the rat liver (De Leeuw et al., 1984; Tsuchiya et al., 1985). TANUMA and ITO (1978) have reported in a TEM study on the bat liver that cytoplasmic processes of Ito cells occasionally extend into the Disse's space to circle the sinusoid in like manner as the sphincter. Thus, for an understanding of the sphincter mechanism in the hepatic sinusoidal wall, the existence of the smooth muscle cells may be considered an
indispensable morphological condition. However, in livers of the lower vertebrates such as reptiles, the existence of the actin filaments in the sinusoidal endothelial cell and Ito cell has yet to be demonstrated.

Acknowledgement. The author would like to thank Professor T. Ito for his generous discussion and advice.

REFERENCES

De Leeuw, A. M., S. P. McCuskey, A. Geerts and D. L. Knook: Isolated fat-storing cells divide and contain collagen. Hepatology 4 : 392-403 (1984).

Fujita, H., T. Tamaru and J. Miyagawa: Fine structural characteristics of the hepatic sinusoidal walls of the goldfish (Carassius auratus). Arch. histol. jap. 43 : 265-273 (1980).

Ito, T., Y. Tanuma and S. Shibasaki: Junction between Kupffer cells and hepatic sinusoidal endothelium. A review. Okajimas Fol. Anat. jap. 57 : 145-158 (1980).

Kanesada, A.: Lymphoid tissues occurring in the liver and bone marrow of reptiles and birds (Japanese text with English abstract). Arch. histol. jap. 10 : 471-481 (1956).

Kobayashi, K. and Y. Takahashi: Effect of the administration of large doses of vitamin A on the fine structure of rat liver with special reference to changes in the fat-storing cell. Arch. histol. jap. 33 : 421-443 (1971).

Ohata, M., Y. Tanuma and T. Ito: Electron microscopic study on avian livers with special remarks on the fine structure of sinusoidal cells. Okajimas Fol. Anat. jap. 58 : 325-368 (1982).

Ohata, M. and T. Ito: Experimental study on the fine structure of chicken liver parenchyma with special references to extrasinusoidal macrophages and sinusoidal blood cells. Part 1. Sinusoidal cells and macrophages in the normal and India ink-perfused livers. Part 2. Sinusoidal blood cells in normal and India ink perfused livers. Arch. histol. jap. 49 : 83-103, 199-209 (1986a, b).

Peek, W. D., E. W. Sidon, J. H. Yousun and M. M. Fisher: Fine structure of the liver in the larval lamprey, Petromyzon marinus L.; Hepatocytes and sinusoids. Amer. J. Anat. 156 : 231-250 (1979).

Sato, H. and T. Yamamoto: Fine structure of the sinusoidal wall in the liver of fresh-water catfish (Parasilurus asotus), with special reference to the smooth muscle cells. Arch. histol. jap. 46 : 125-130 (1983).

Shin, Y. C.: Some observations on the fine structure of lamprey liver as revealed by electron microscopy. Okajimas Fol. Anat. jap. 54 : 25-60 (1977).

Taira, K. and H. Mutoh: Comparative ultrastructural study of the Ito cells in the liver in some reptiles. Arch. histol. jap. 44 : 373-384 (1981).

Tamaru, T.: Electron microscopic studies of Kupffer stellate cells (Japanese text with English abstract). Med. J. Hiroshima Univ. 27 : 235-279 (1979).

Tanuma, Y. and T. Ito: Electron microscope study on the hepatic sinusoidal wall and fat-storing cell in the bat. Arch. histol. jap. 41 : 1-39 (1978).

Tsuchiya, M., M. Oda and N. Tsukada: Capillaries in the liver—Characteristics of the hepatic sinusoids and their abnormalities—(In Japanese). Cell 17 : 12-17 (1985).
Umahara, Y.: Light and electron microscopic studies on the brown adipose tissue in the bat. Arch. histol. jap. 29: 459-509 (1968).

Watari, N.: Morphologische Studien über die jahreszeitlichen Veränderungen von Mitochondrien, Fett- und Glykogengehalt der Leberzellen bei den Schlangen nebst Bemerkungen der Fettspeicherungszellen (fat-storing cells). (Japanese text with German abstract). Arch. histol. jap. 16: 369-423 (1959).

Wisse, E.: An electron microscopic study of the fenestrated endothelial lining of the rat liver sinusoids. J. Ultrastr. Res. 31: 125-150 (1970).

———: An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. J. Ultrastr. Res. 38: 528-562 (1972).

Yamamoto, M.: Electron microscopic studies on the innervation of the smooth muscle and the interstitial cell of Cajal in the small intestine of the mouse and bat. Arch. histol. jap. 40: 171-201 (1977).