Electron Microscopic Studies on the Sinusoidal Cells in the Monkey Liver

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Received November 4, 1982

Summary. The sinusoidal wall was observed by transmission electron microscopy in crab-eating monkey livers. The perikarya of sinusoidal endothelial cells were characterized by numerous macropinocytotic vacuoles and curved smooth-surfaced tubules of high electron density. Size and spacing of fenestrae in endothelial sieve plates corresponded to essentially those in other mammalian species including the human. It was verified that the high concentration of microfilaments was responsible for the electron dense appearance of the sieve plate in tangential sections. Besides occasional overlapping of endothelial sheets, complicated interdigitations of several short lamellae originating from endothelial processes occasionally caused a layered structure of the endothelial lining. Kupffer cells were strikingly rich in lysosomes and contained large mitochondria and phagosomes. They were fixed to the endothelial lining by patches of junctional complexes identical with those between endothelial cells. Ito cells of the monkey liver demonstrated, like those of the human liver, many smooth-surfaced caveolae and vesicles along their perisinusoidal surface, suggesting their micropinocytotic activity. They also contained glycogen β-particles which were partly gathered around lipid vacuoles. The electron dense droplets enclosed by glycogen particles as revealed in human Ito cells and regarded as immature lipid droplets retaining the chemical properties of glycogen, could rarely be confirmed in the present study. Between the Ito cell and hepatocyte there occurred many junctional complexes. The space of Disse contained, besides abundant collagen fibrils, numerous fine filaments forming irregular meshworks or bundles which resembled fibrillar material (precursor of collagen) in appearance in the dilated cisternae of the rough endoplasmic reticulum (RER) of the Ito cell. These filaments often entwined the collagen fibrils in the Disse’s space as if to participate by apposition in their development. The question, whether pinocytosis-like structures of the Ito cell might be involved in the precursor transport from the cisternae of the RER to the Disse’s space, remained unanswered.

As recently pointed out by Kennedy (1979), the monkey liver has hitherto been observed electron microscopically by only a few authors. Moreover, the observations have mostly been confined to the ultrastructure of the hepatocyte and the sinusoidal cells have received little attention (Dyrszka et al., 1976; Forssmann and Ito, 1977; Kennedy, 1979). The present electron microscope study will deal mainly with the sinusoidal cells in the crab-eating monkey liver to supplement this deficiency in the literature.
MATERIALS AND METHODS

Four adult (4-5 year) male crab-eating monkeys (Macaca fascicularis) weighing about 4 kg were used in this study. The animals were fed in the laboratory with solid monkey food supplemented by several kinds of fruits. Under general anesthesia, laparotomy was performed, and after bilateral resection of kidneys for another medical purpose, the liver was perfused in situ via the portal vein with cold (0°C) 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4). Thereafter, the liver was excised. Blocks of the tissue were cut in a drop of the fixative into minute pieces. After 2 hr fixation, they were rinsed several times in a cold 0.1 M phosphate buffer containing 5% sucrose (pH 7.4) and left overnight in the same buffer at 5°C. They were postfixed in a 1% OsO₄ solution in 0.1 M phosphate buffer (pH 7.4) for 90 min. Following dehydration in graded ethanol, the tissue pieces were embedded in Epon 812 and sectioned with the Porter-Blum Ultra-Microtome MT2-B. The ultrathin sections were stained with saturated uranyl acetate and Sato's lead solution. Electron micrographs were taken with a JEM-100C electron microscope.

RESULTS

1. Sinusoidal endothelial cell

The sinusoidal endothelium of the crab-eating monkey liver is composed of the perikaryon and the cytoplasmic extension which occupies the major part of the sinusoidal lining. A continuous distinct basal lamina is lacking also in this animal. The most common configuration of the perikaryon is a spindle-shape, sending out cytoplasmic extensions from both diametric attenuated ends to line the sinusoid (Fig. 3).

Occasionally large perikarya occur lying across the sinusoidal lumen as if to bridge its confronting walls, and along these two walls they extend their cytoplasmic attenuations to line the sinusoidal walls on either side (Fig. 2).

![Fig. 1. Spindle-shaped endothelial perikaryon (EC) rich in organelles, especially in numerous electron dense smooth-surfaced tubules (ST). Along the sinusoidal surface, many coated caveolae and vesicles are seen. D lysosome, FSC Ito cell, LY lymphocyte, M mitochondria, PS Disse's space, SN sinusoid, SPH spheridy. × 16,000](image-url)
The sinusoidal surface of the perikaryon is smooth but many coated caveolae and vesicles measuring about 110 mμ in diameter are revealed along its plasma membrane (Fig. 1), suggesting marked pinocytotic activity. These structures are, however, rare along the perisinusoidal surface, and the micropinocytotic smooth-surfaced caveolae

**Fig. 2.** A large endothelial perikaryon (EC) lying between two sinusoids (SN) as if to bridge the confronting sinusoidal walls extending four fenestrated cytoplasmic extension (EL) to line the sinusoids. E erythrocyte, FSC Ito cell, H hepatocytes, KU Kupffer cell, PS Disse’s space, X subendothelial processes of the Ito cell. Arrow indicates macropinocytotic vacuoles. × 5,000

**Fig. 3.** A portion of the spindle-shaped endothelial perikaryon containing many macropinocytotic vacuoles (MV) with an electron-dense material and many electron-dense smooth surfaced tubules (ST). D lysosome, H hepatocyte, M mitochondria, PS Disse’s space, RER rough endoplasmic reticulum, SN sinusoid. Arrow indicates a vacuole bulging in the sinusoid. ×16,000
Fig. 4. Cytoplasmic extension (EL) of endothelial cell composed alternately of thicker (CP) and thinner portions (sieve plates) (SP). Kupffer cell (KU) bears numerous lysosomes (D), large mitochondria (M) and pseudopods. Ito cell (FSC) contains lipid droplets outlined by faint rims of glycogen β-particles and their conglomerates, and sends out processes (X) along the endothelial extension. C diplosome, E erythrocyte, H hepatocyte, PS Disse’s space, SN sinusoid. Arrow indicates a junctional complex between endothelial cells. ×10,000
and vesicles as seen elsewhere in capillary endothelia are almost completely missed along the plasma membranes of both the perikaryon and process. The perikaryonal cytoplasm of the sinusoidal endothelium of the monkey is extraordinarily rich in organelles (Fig. 1). There are thus Golgi complexes composed of stacks of flattened cisternae and vesicles, short and elongated cisternae of the rough endoplasmic reticulum (RER), free polysomes scattered throughout the cytoplasm, a few small mitochondria and some dense bodies (lysosomes) of variable sizes and electron densities. Besides, numerous curved smooth tubules mostly containing electron dense material are irregularly distributed throughout the cytoplasm, and moreover there are variable numbers of smooth membrane-bound vacuoles ranging from 340 to 890 nm in diameter (660nm on an average), which may correspond to macropinocytotic vesicles of Wisse (1972) and the lumina of which are partly filled with variable electron-dense material (Fig. 2, 3, 7a, 12). This dense material often presents a crystal-like appearance (Fig. 3). When these vacuoles are accumulated in the cytoplasm, the limiting membranes of the neighboring vacuoles come in contact with each other to be consequently lost there, thus frequently inducing the fusion of the vacuoles (Fig. 7a). The curved smooth-surfaced

Fig. 5. a and b. Tangential sections of the membranous cytoplasmic extensions of the endothelial cells composed of thicker (CP) and thinner portions (sieve plates) (SP). The thicker portions contain organelles such as mitochondria (M), smooth-surfaced electron dense tubules, microtubules, microfilament bundles etc. The sieve plates appear electron dense. FB collagen fiber, FL filament-meshwork, PS Disse’s space, SN sinusoid, V microvilli. a: x 25,000, b: x 30,000
tubules and macropinocytotic vacuoles characterize the hepatic sinusoidal endothelium of the monkey.

In the nucleus, besides a large nucleolus, a spheridy (nuclear body) is occasionally found showing a concentric fibrillar structure around an electron dense core (Fig. 1).

The attenuated extension of sinusoidal endothelium is divided, as in other mammals, into thicker and thinner portions which roughly alternate with each other (Fig. 2, 4). The thicker portions, called the “cytoplasmic processes” (Wisse, 1970), show variable thickness and configuration and contain the same organelles, such as the perikaryon, excepting the Golgi complex which is confined exclusively to the perikaryon. Microtubules and microfilaments which are hardly demonstrated in the perikaryonal cytoplasm, have clearly been revealed in the cytoplasmic processes being oriented almost parallel to its long axis (Fig. 5a, 6). Microfilaments are especially concentrated in the ectoplasmic layer, making compact bundles (Fig. 5a). The thinner portions are almost devoid of the organelles except the microfilaments. They are provided with a varying number of round or oval fenestrae or pores measuring about 40–290 nm in diameter (100 nm on an average), which make loose clusters, so that the thinner portions exhibit in their tangential sections the appearance of the “sieve plates” (Wisse, 1970) (Fig. 5a, b, 6). The spacings between neighboring fenestrae, as measured from center to center, are 250 nm on an average. Narrow cytoplasmic zones separating fenestrae from each other appear electron-dense on account of the high concentration of the microfilaments (Fig. 5a, b, 6). Neighboring sinusoidal endothelial cells are connected by the “junctional complexes” of Wisse (1970) between the thicker portions of the cytoplasmic extensions. In the perpendicular sections of the sinusoidal wall, the plasma membranes bounding the adjacent thicker portions are juxtaposed across a space about 20 nm in width, which is bridged by a transversely striated material of high electron density. The apposed plasma membranes and adjacent narrow cytoplasmic zone show an increased electron density. Thus, the junctional complexes appear as electron-dense dots or short bars (Fig. 4, 13). In the tangential sections of the sinusoidal endothelial lining, the contact margins of the membraneous thicker portions of the neighboring endothelial cell extensions exhibit a more or less long wavy line of high electron density induced by the junctional complexes connecting the contact margins. As shown in Figure 6, the junctional complexes connecting the margins of the neighboring endothelial cell extensions are discontinuous (Wisse, 1970). If the

![Image](image_url)

**Fig. 6.** Junction between neighboring endothelial cells as seen in a tangential section. Long margins of the thicker portions (CP) are closely apposed across a narrow space and connected by several patches of the junctional complex (arrows). In the thicker portion microtubules, electron dense smooth-surfaced tubules etc., are seen. PS Disse’s space containing many microvilli (V) of hepatocyte, SN sinusoid, SP sieve plates. ×30,000
closely apposed plasma membranes diverge in places where the junctional complexes are interrupted, there may appear more or less wide intercellular gaps other than intracellular fenestrae in the endothelial lining.

In the present study, loops of fine cytoplasmic cord have often been observed on the endothelial perikaryon bulging into the sinusoid. The cytoplasmic cord is discontinuous, being rather regularly interrupted by gaps, and its two ends are continuous with the perikaryonal cytoplasm (Fig. 2, 3). The nature of these unusual structures is obscure, but it may not be completely unreasonable to suppose that they would be vacuoles bulged into the sinusoid and formed of a thin fenestrated cytoplasmic layer of the sinusoidal perikaryon. The second unusual structure shown by the sinusoidal endothelial cell of the crab-lating monkey is interdigitations, or more or less complex overlapping of lamellae extending from the cytoplasmic extension in which the sieve plates always intermingle. In the most simple case, two thin cytoplasmic lamellae closely overlap for an indefinite distance (Fig. 7a). But as shown in Figures 7a and b, more complex overlappings or interdigitations of four or five short lamellae are occasionally seen in the endothelial lining. Between these lamellae, some of which represent sieve plates, patches of the junctional complex are observed.

2. Kupffer cell

Kupffer cells are cytoplasm-rich and contain abundant organelles, especially numerous dense bodies of variable sizes, probably lysosomes (Fig. 4, 8, 9). A large Golgi apparatus is found on one side of the nucleus and occupies a wide cytoplasmic area containing a diplosome. Mitochondria are numerous and larger than those in the sinusoidal endothelial and fat-storing cells of Ito, and tend to be clustered at random (Fig. 8). The RER is relatively well-developed, and flattened cisternae studded with ribosomes are

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**Fig. 7.** a and b. Overlapping or interdigititation of two or several cytoplasmic lamellae originating from thicker portion (CP) of the endothelial extension or perikaryon (EC). Some of the lamellae are fenestrated. Between the lamellae, patches of the junctional complex (arrows) are seen. M mitochondria, MV macropinocytic vacuoles, PS Disse’s space, SN sinusoid, V microvilli, X subendothelial process of the Ito cell. a and b: x 30,000
distributed throughout the cytoplasm, many of them being elongated closely along the surface of the mitochondria. Free polysomes are also distributed throughout the cytoplasm, and even in the pseudopods. Curved smooth-surfaced tubules containing an electron dense material are detectable also in the Kupffer cells, though less numerous than in the endothelial cells (Fig. 9). Along the plasma membrane facing the sinusoid, there occur coated caveolae and vesicles measuring about 120 nm in diameter on an average (Fig. 9). However, the fuzzy cell coat known to be a common surface structure of the Kupffer cell, is completely missed, probably on account of the inadequate prefixation with glutaraldehyde (Wisse, 1974). Also, the so-called worm-like structure is invisible in the Kupffer cell of the monkey. Pseudopods of variable shapes and sizes are a conspicuous morphological feature of the Kupffer cell distinguishing it from the sinusoidal endothelial cell. Large phagocytic vacuoles, almost empty or containing ingested erythrocytes, polymorphnuclear leucocytes or blood platelets (Fig. 8) have occasionally been observed. In the cytoplasm which is occupied by numerous organelles, microfilaments and solitary microtubules are hardly detectable (Fig. 9), but the ectoplasmic layer exhibits microfilaments which are oriented parallel to the cell surface.

The Kupffer cells lie on the sinusoidal endothelial lining bulging into the sinusoid and are connected to the latter by a number of patches of junctional structures identical with those between neighboring endothelial cells (Fig. 8, 9). The question as to the
Fig. 9. A portion of the Kupffer cell (KU) characterized by many lysosomes (D) and pseudopods. It contains cisternae of RER, electron dense smooth-surfaced tubules (ST), microtubules and coated caveolae as well as vesicles. Between the endothelial lining (EL) of the sinusoid (SN) and the Kupffer cell, patches of junctional complex (arrows) are seen. MP a portion of macrophage, PS Disse’s space. × 25,000

Fig. 10. A macrophage (MP) containing many large mitochondria (M) as large as those of the Kupffer cell (KU) and abundant polysomes, migrating from the Disse’s space (PS) into the sinusoid (SN) by penetration through the endothelial perikaryon (EC). H hepatocyte. × 10,000
existence of the spheridy (nuclear body) in the Kupffer cell nucleus of the monkey liver has not exactly been answered, since the nucleated profiles of the Kupffer cells are not sufficiently numerous in the present study. In the parenchyma of the monkey liver, macrophages and other cell types have scarcely been revealed, except a few macrophages in the Disse’s space. One of them contains numerous large mitochondria and abundant free polysomes and is encountered on its migration from the Disse’s space to the sinusoid (Fig. 10).

3. Fat-storing cell (lipocyte, Ito cell)

As widely known, Ito cells located in the Disse’s space are lacking in a continuous basal lamina and are cytologically characterized by well-developed RER and lipid droplets (vacuoles) in the cytoplasm (Fig. 11). Their shapes are varying probably according to the direction of the section plane. They are frequently elongated along the endothelial lining (Fig. 11, 12, 14), though they may often show rounded configurations (Fig. 17). The Ito cells send out unknown numbers of cytoplasmic processes of various thicknesses and lengths which can ramify chiefly along the endothelial lining (Fig. 13, 14, 17). Their isolated segments of different lengths are frequently detected closely subjacent to the endothelial lining, hence the designation of “subendothelial processes” (Ito and Shibasaki, 1968) (Fig. 2, 4, 8, 17). Besides these long ones, a few short microvillous processes are occasionally projected into the Disse’s space often interlacing with microvilli protruding from hepatocytes.

The number of lipid droplets in one Ito cell varies from one to fourteen, depending on its profile. In a cell containing multiple lipid droplets, the size of the droplets is not uniform (Fig. 12). As measured in electron micrographs, they range from 0.7 to 4 \( \mu \text{m} \) in diameter with the average of 2 \( \mu \text{m} \). Because of the lack in the limiting membrane (Fig. 20), closely adjacent droplets frequently fuse with each other. The lipid droplet can occur even in the peripheral portion of the cytoplasmic process far

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Fig. 11. Ito cell (FSC) characterized by abundant cisternae of RER, lipid droplets (LD) and sparse small mitochondria (M). Kupffer cell (KU) is fixed to the endothelial lining with large gaps (EL) by patches (arrow) of the junctional complex. Between hepatocyte (H) and upper left part of the Ito cell, patches of the junctional complex (arrows) are seen. Arrowhead indicates coated caveola. \( \times 16,000 \)
away from the cell body. In the monkey liver the “empty fat-storing cells” (Ito and Shibasaki, 1968), i.e., those devoid of the lipid droplets, have occasionally been revealed (Fig. 13). They are in general small and appear atrophic because of the paucity of the cytoplasm, but they nevertheless maintain morphological characteristics of the Ito cell. Numerous cisterns of RER are distributed throughout the cytoplasm together with free polysomes, and they are often more or less dilated, containing variable amounts of lipids.

**Fig. 12.** A profile of the Ito cell (FSC) containing many lipid droplets (LD). Some of them fuse with each other. EC endothelial perikaryon containing a lysosome (D) and macropinocytic vacuoles, EL endothelial lining of sinusoid (SN), H hepatocyte, PS Disse’s space containing many collagen fibers (FB). ×8,000

**Fig. 13.** An empty Ito cell (FSC) appearing atrophic. Along the plasma membrane facing the Disse’s space (PS), many micropinocytotic caveolae and vesicles are seen. EL endothelial lining of the sinusoid (SN), FB collagen fibers, H hepatocyte, M mitochondrion, arrow indicates a junctional complex between endothelial cells. ×16,000
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a finely fibrillar material (Fig. 14, 18). Usually they show close spatial relationships with mitochondria. The sparse mitochondria of the Ito cell are as small as those of the sinusoidal endothelial cell; namely they are smaller than those of the Kupffer cell (Fig. 11). Their distribution in the cytoplasm is not uniform, making small clusters in random places. A few small dense bodies (lysosomes) are scattered disorderly in the cytoplasm (Fig. 14, 16, 17) A multivesicular body is occasionally observed. A well-developed Golgi apparatus composed of several stacks of short flattened cisternae is found on one side of the nucleus, accompanied by many vesicles. The large Golgi area contains a diplosome (Fig. 15a, b).

The present study failed to demonstrate exactly a longitudinal section of a single cilium developing from the distal centriole of the diplosome into the Disse's space. However, cytological signs suggesting such a development has occasionally been obtained (Fig. 15a). In Figure 15b a cross-striated rootlet and microtubules radiating from the diplosome are observed. The microfilaments are detected in the cytoplasmic areas between cisternae of the RER and lipid droplets, often making bundles oriented in random directions. They are, however, usually concentrated along the ectoplasmic layer both of the cell body and cytoplasmic processes. Especially the microfilaments distributed in the endoplasm are converged toward the base of the processes to enter longitudinally in their axial cytoplasm together with a few solitary microtubules (Fig. 16). The so-called subendothelial processes are also rich in longitudinally disposed microfilaments.

The nucleus of the Ito cells containing a relatively large nucleolus shows manifold shapes and locations and often possesses one or more depressions on the nuclear membrane produced by lipid droplets. The nucleus-cytoplasm ratio is smaller than that in the Kupffer cell. Noteworthily, a remarkable spheryd (nuclear body) has often been detected in the nucleus of the Ito cell of the crab-eating monkey. It is a moderately electron-dense spherical body, measuring about 550 nm in diameter, and in the majority of cases it occurs singly but sometimes in a pair in an indefinite location of the nucleoplasm, without showing any intimate spatial relationship to the nucleolus (Fig. 14, 17). The most reliable marker for distinguishing exactly this nuclear inclusion seems to be a light halo. The spheryd is composed of a finely fibrillar material, which usually

![Fig. 14. An Ito cell (FSC) elongated along the sinusoid (SN). Between lipid droplets (LD) fusions are seen. Among cisternae of the RER dilated ones are intermingled. D lysosome, EL endothelial lining, FB collagen fibers in the Disse's space (PS), H hepatocyte, M mitochondria, SPH spheryd. Arrow indicates a junctional complex between endothelial cells. ×10,000](image-url)
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shows, in the nucleus of the Ito cells of the monkey liver, a concentric lamellar arrangement surrounding a central, finely granular, core of high electron density. The central core resembles a small heterochromatin mass in its structure and high electron density (Fig. 17 inset a, b). Occasionally electron-dense particles are scattered in the spheridy independently of or together with the central core (Fig. 14, 17 inset c).

In the monkey livers examined in the present study, particulate glycogen (β-particles) revealed in the Ito cells was not conspicuous in amount and the cells containing this inclusion were frequent. The glycogen β-particles are detected both in the cell body and in cytoplasmic processes, and in the former they are mostly gathered on to the surface of the lipid droplets to form a thin and faint rim or small dense conglomerates (Fig. 4, 17). Among ordinary electron lucent lipid vacuoles, smaller electron dense droplets sometimes appear, being closely surrounded by a thick rim composed of many glycogen particles as well as by their dense conglomerates (Fig. 18). Such droplets may surely differ from lysosomes because they fail in the limiting membrane.

Besides a few coated caveolae and vesicles (about 100 nm in diameter) (Fig. 11) the majority of the examined Ito cells of the monkey livers show, along their perisinusoidal surface, variable numbers of smaller microcinocytotic caveolae and vesicles (about 47 nm in diameter) and some of the vesicles are often fused together (Fig. 13, 16, 19 and inset). These microcinocytotic pictures are not confined to the surface of the cell body, but they also spread over that of the cytoplasmic processes (Fig. 13, 16). They occasionally show the intimate topographical relationship with glycogen particles (Fig. 16 inset).

4. Junctions between the Ito cell and hepatocyte

At places where the plasma membranes of the hepatocyte and Ito cell are closely apposed across a narrow intercellular space about 20 nm wide, electron dense junctional structures resembling desmosomes are often recognized (Fig. 11). In higher magnification, the apposed plasma membranes and the adjacent narrow areas of the cytoplasm of the both cell types are conspicuously increased in electron density and the inter-
cellular space is filled with a fibrillar material which appears to bridge the apposed plasma membranes (Fig. 20).

5. Collagen fibers in the Disse’s space

The Disse’s space of the monkey generally contains a considerable amount of collagen microfibrils which make bundles in the majority of locations, thus presenting collagen fibers of variable thickness (Fig. 12–14, 16, 17). Besides, many microfibrils run in irregular directions without forming bundles (Fig. 16). The thicker microfibrils measure about 60 nm in diameter and thinner ones about 25 nm, on average. There are furthermore fine filaments (about 5–8 nm in thickness) oriented in different directions forming irregular meshworks (Fig. 5, 16, 20, 21), which often entwine collagen fibrils (Fig. 16, 20, 21a). These filaments, however, may occasionally form small bundles (Fig. 21b). The similar fine filaments can be detected in the more or less dilated cis-

![Fig. 16. A thick cytoplasmic process of the Ito cell in the Disse’s space (PS). Microfilament bundles and solitary microtubules run parallel to the long axis of the process. Clusters of glycogen β-particles (GL) are scattered over the entire length of the process. Along the plasma membrane, micropinocytotic vesicles and caveolae are seen. D lysosome, FB collagen fiber, in the collagen fiber on the left side fibrils are entwined by filaments, FL filament meshwork, H hepatocyte, V microvill. × 25,000. Inset. Intimate relation between pinocytotic caveolae (arrows) and clusters of glycogen β-particles (GL). × 60,000]
terns of the RER of the Ito cell, exhibiting a finely fibrillar or flocculent appearance in low power view (Fig. 18, 20, 21b). Such dilated cisterns of the RER as found in the Ito cells, however, have never been revealed in the hepatocytes bordering the Disse’s space. The Ito cells situated in the Disse’s space are enclosed both by collagen fibers and microvilli of the hepatocytes. Directions and arrangements of the collagen fibers surrounding the surface of these cells are manifold. In the narrow subendothelial space intervening between the endothelial lining of the sinusoid and the Ito cells, the amount and thickness of collagen fibrils are generally small (Fig. 13, 14). The microvilli of the hepatocytes are decreased in number and length where collagen fibers occupy a large area of the Disse’s space, the former being confined to the narrow space adjacent to the hepatocytes.

DISCUSSION

1. Sinusoidal endothelial cell
The perikarya of the endothelial cells of the crab-eating monkey are characterized by a conspicuous richness in macropinocytotic vacuoles and vesicles and by abundant

Fig. 17. Ito cell (FSC) containing a spheridy (SPH) in the nucleus. It sends out cytoplasmic processes (X) along the endothelial lining (EL). D lysosome, FB collagen fiber, G Golgi complex, H hepatocyte, LD lipid droplet with a thin rim of glycogen β-particles, M mitochondria, MV macropinocytotic vacuoles in the endothelial cell, PS Disse’s space, SN sinusoid. ×10,000. Inset a-c. Spheridies with a central electron dense granular core of variable sizes. Note electron lucent halo. Spheridy occurs singly (a, b) but occasionally in pairs (c). a: ×36,000, b: ×25,000, c: ×20,000
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curved smooth-surfaced tubules containing electron-dense material. The latter organelles were given special attention for the first time by Tanuma and Ito (1978) in observing the bat liver and were referred to by them as components of "smooth endoplasmic reticulum." Recently, Ohata et al. (1982a) noticed in some birds that the sinusoidal endothelial cells were conspicuously rich in both of the above-mentioned organelles. They are presumed to participate, together with many coated micropinocytic caveolae and vesicles, in the uptake and metabolism of the finely particulate

Fig. 18. Ito cell (FSC) containing lipid vacuoles (LD) and an electron dense droplet (DD) outlined by many glycogen particles and their dense conglomerates. EL endothelial lining of the sinusoid (SN), FB collagen fiber, H hepatocyte, M mitochondria, PS Disse's space, cisternae of RER, X cytoplasmic processes of the Ito cell. ×18,000

Fig. 19. Many micropinocytic caveolae and vesicles along the surface of an Ito cell (FSC). A small conglomerate of the vesicles is seen. PS Disse's space, cisterna of RER. ×40,000. Inset. Intimate spatial relation between cisternae of RER and micropinocytic caveolae and vesicles. A large conglomerate of the vesicles is seen. D lysosome, PS Disse's space. ×60,000
material of an unknown nature (Wisse, 1972) and to clean up the sinusoidal blood, as proposed by Tamaru (1979). Many vacuoles or phagosomes observed by Ito and Shibasaki (1968) in the normal human sinusoidal endothelial cells might surely correspond to the macropinocytotic vacuoles of the sinusoidal endothelial cell of the monkey liver.

In the present transmission electron microscopic (TEM) study, roughly regular alternation of the thicker and thinner portions was confirmed in the cytoplasmic process of the sinusoidal endothelial cell. The thinner fenestrated portions, called sieve plates, appeared considerably electron dense as seen in their tangential sections in spite of their lack of organelles. The present study could verify that this high electron density was induced by a high concentration of microfilaments in conformity with the description of Wisse (1970). As measured in the tangential sections of the sieve plates, sizes of the fenestrae were widely variable, ranging from 40 to 290 nm in diameter (about 100 nm on an average) and the spacings of the fenestrae from center to center were 250 nm on an average. According to Wisse (1970), the fenestrae of the rat sieve plates measured approximately 100 nm in diameter and were uniformly distributed in every sieve plate with spacings of about 150–250 nm from center to center. He maintained, however, that the larger gaps observed by various authors were nothing but artifacts. TANUMA and ITO (1978) observed by TEM the sieve plates in the bat and reported that the pore sizes of the sieve plates were varied from 80 to 250 nm and that the spacings of the pores were irregular. They demonstrated, though rarely, wide gaps measuring about 300–800 nm in conformity with the report of ORCI et al. (1971). MUTO et al. (1977) observed by TEM and SEM endothelial cell of the normal human liver in three biopsy cases and demonstrated that the endothelial fenestrations could be

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**Fig. 20.** Patches of the junctional complex (arrow) between Ito cell (FSC) and hepatocyte (H). D lysosome, FB collagen fiber, its microfibrils are entwined by fine filaments, LD lipid droplets provided with no surface structure, M mitochondria, PS Disse's space, V microvilli. × 40,000
divided into two types, small and large. The small ones were less than 0.1 μm in diameter and about 25 of them occurred in clusters within areas of one μm² called “sieve plates.” The large ones about 0.5–2 μm in diameter occurred in many places of sinusoid, intermingling with the small ones. The present result indicates that the fenestrations of the hepatic sinusoidal endothelial lining in the crab-eating monkey are not so widely variable in size and spacing as demonstrated with the SEM and TEM in several mammalian species including the monkey and human.

As to the existence of the double-layered endothelial lining of the hepatic sinusoid, many investigators have discussed it with reference to the literature (Ito, 1973). In their TEM study on the normal human liver, Ito and Shibasaki (1968) frequently encountered in the sinusoidal wall the cytoplasmic sheets stratified in two layers. They evidenced, however, that the innermost layer was identical with the sinusoidal endothelial lining, while all others outside of it were the “subendothelial processes” of Ito cells located in the Disse’s space. Thus, they concluded that the hepatic sinusoidal endothelial lining is principally single layered. An authentic double layered sinusoidal endothelial lining was demonstrated, however, by Tanuma and Ito (1980), although only for a short distance, in the sinusoidal wall of the crucian liver. Occasional overlappings or interdigitations of two or several cytoplasmic lamellae originating from

**Fig. 21.** a. Collagen fibers (FB) in the Disse’s space (PS). Their microfibrils are entwined by fine filaments. FSC Ito cell, H hepatocyte, V microvilli. × 36,000. b. High ultrastructural resemblance between fibrillar material in dilated cisternae of RER and fine-filament bundle and meshwork (FL) in the Disse’s space (PS). Note intimate relationship between the pinocytotic caveolae of the Ito cell and fine-filaments bundle in the Disse’s space. × 40,000
the cytoplasmic extensions of the endothelial cell were observed, though for a short distance, in the monkey liver. Among the lamellae, which were partly connected by patches of the so-called "junctional complex," the fenestrated ones intermingled. These hitherto unknown lamellar structures could be regarded as reinforcing the endothelial lining of the sinusoid.

Another curious structure was represented by the probable vacuoles protruded from the endothelial cytoplasm into the sinusoid which seemed to be formed by a thin fenestrated cytoplasmic layer. The functional significance of this unique structure was quite unknown. The SEM may be a more suitable means as an approach to this question. In the livers of some birds, Ohata et al. (1982) recently demonstrated the profiles of large sinusoidal endothelial perikarya which embraced two or three sinusoids between their fenestrated cytoplasmic extensions. In the monkey livers we could rarely observe a large endothelial cell which embraced two sinusoids with four fenestrated cytoplasmic extensions on both sides of its perikaryon. In disagreement with Kennedy (1979), we could not confirm the presence of large endothelial cells completely lacking in fenestrations which he revealed in the sinusoids of the cynomologus and rhesus monkey.

2. Kupffer cell
Since the endothelial perikarya of the monkey liver were strikingly rich in organelles, distinction between the organelle-rich Kupffer cells and sinusoidal endothelial perikarya was often difficult. The essential criteria for the distinction consisted in 1) that the Kupffer cells were provided with pseudopods instead of the fenestrated cytoplasmic extensions, 2) that they usually contained numerous lysosomes and large phagosomes enclosing often ingested blood cells, and 3) that they possessed mitochondria larger than those of the endothelial and Ito cells. The fuzzy cell coat and the worm-like structure known to be important cytophological characteristics of the Kupffer cell, were not revealed in the present study. The worm-like structure (micropinocytosis vermiciformis) was recently investigated in detail by Tanuma (1978) in the bat liver Kupffer cell with reference to abundant literature. Thereafter, this tubular structure was confirmed in kitten liver (Tanuma et al., 1981), in chicken liver (Ohata et al., 1982a) and in normal human liver (Tanuma et al., 1982a). The reason why we failed to reveal the worm-like structure in the Kupffer cells of the monkey is probably due to in our specimen preparation procedures which might be inadequate to the preservation of Kupffer cells. The same might also be the case in the failure to demonstrate the nuclear body (spheridy) in monkey Kupffer cells.

Recently, Ito et al., (1980) discussed the junctional mode between the Kupffer cell and the endothelial lining of the sinusoid on the basis of the findings of their TEM study on human (Ito and Shibasaki, 1968) and bat liver (Tanuma and Ito, 1978) with reference to several SEM studies. They explained the reason why their TEM preparations disclosed the "junctional complexes" between the Kupffer cell body and the endothelial sheet commonly to lie in a pair at two diametrical sites of the perisinusoidal surface of the former. The same finding has been confirmed by Tanuma et al. (1981) in a kitten liver, by Ohata et al. (1982a) in avian liver and by Tanuma et al. (1982a) again in human liver. In the present TEM study, however, we could not confirm in the monkey liver such two diametrical junctional areas, but we could instead reveal only a number of patches of the junctional complex randomly between the endothelial lining and the perisinusoidal surface of the Kupffer cell.

Free macrophages, lymphocytes and plasma cells were detected by TEM in variable
numbers in the interhepatocytic space of the normal bat liver (Tanuma and Ito, 1978), kitten liver (Tanuma et al., 1981) and avian liver (Ohata et al., 1982a) and some of them were found migrating from the Disse's space into the sinusoid through the endothelial lining. However, these findings were hardly confirmed in human livers (Ito and Shibasaki, 1968; Tanuma et al., 1982a). In the monkey livers, a few macrophages have been encountered in the Disse's space and they were occasionally found migrating into the sinusoid by penetrating the endothelial lining.

3. Fat-storing cell (lipocyte, Ito cell)

Ito cells in the monkey liver bore several essential features, namely deficiency of basal lamina, lipid droplets, well-developed rough endoplasmic reticulum, large Golgi apparatus containing a diplosome, sparse small lysosomes, sparse small mitochondria and several cytoplasmic processes, which occasionally contained lipid droplets (Ito, 1969, 1978). In his extensive light microscopic studies on livers in a variety of vertebrate species Ito (1956, 1969, 1978, 1980) has reported that the human and monkey Ito cells likewise possess multiple small lipid droplets. As observed by TEM, several profiles of the Ito cells of monkey liver contained one to fourteen droplets ranging in diameter from 0.7 to 4 μm with the average of 2 μm. According to Ito and Shibasaki (1968), Ito cells contained variable numbers of lipid droplets up to ten, varying in diameter from 0.8 to 2.9 μm. It has thus been evidenced by TEM that the lipid droplets were roughly conformable in size and number in human and monkey Ito cells. Fat droplet containing Ito cells were reported in the livers of several kinds of monkeys, i.e., the tupaias and cynomologus (Forssmann and Ito, 1977) and rhesus (Dyrska et al., 1977; Kennedy, 1979). Only Forssmann and Ito revealed in them several fat vacuoles of small and medium sizes.

In a series of TEM studies on the hepatic sinusoidal cells, it has been verified in a variety of vertebrate species including the human, that a diplosome is always present in the center of the Golgi area of the sinusoidal endothelial cell, Kupffer cell and fat-storing cell (Ito and Shibasaki, 1968; Tanuma and Ito, 1978, 1980; Tanuma et al., 1981; Ohata et al., 1982a). Of three types of the sinusoidal cells, only Ito cells have been proved by the above authors to protrude a single cilium into the Disse's space. The cilium developed from the distal centriole of the diplosome and was supposed to be sensory in nature and probably a chemoreceptor (Ohata et al., 1982b). In this study on the monkey liver we could not encounter a longitudinally sectioned, perfect image of this single cilium. Nevertheless, we could gain a morphological sign suggestive of its development from the diplosome.

It is also a noteworthy finding that the spheridy (nuclear body) often appeared in the nucleus of the three types of the sinusoidal cell (Ito and Shibasaki, 1968; Wisse, 1972; Tanuma and Ito, 1978, 1980; Tanuma et al., 1981; Ohata et al., 1982a). In the monkey liver, the spheridy was demonstrated both in the endothelial and Ito cell, but especially in high frequency in the latter. It was composed of a finely fibrillar material which showed concentric lamellar structures around an electron dense granular core resembling a small heterochromatin mass. The nature, origin and significance of the spheridy are unknown and in the present study, the view proposed by Wisse (1972) supporting its intimate relation with the nucleolus was not confirmed.

Ito and Shibasaki (1968) classified the processes of the Ito cells into the sparse microvillous and thicker cytoplasmic processes, the latter of which became elongated along the sinusoidal endothelial lining. They designated the cytoplasmic processes,
or their branches elongated closely along the endothelial lining, as "subendothelial processes." They are rich in microfilaments and are thought to be involved in the reinforcement of sinusoidal lining (Ito and ShibaSaki, 1968; Wisse, 1970; Muto et al., 1977). In the monkey liver, the subendothelial processes were also observed as in many other vertebrate species (Tanuma and Ito, 1978, 1980; Tanuma et al., 1981; Taira and Mutoh, 1981; Ohata et al., 1982a), being distributed here and there in the Disse's space as isolated fragments subjacent to the endothelial lining.

As remarked by Ito (1973) and Tanuma et al. (1982a), relatively few investigators have revealed glycogen in Ito cells. Recently, however, Yamamoto and Enzan (1975), Enzan and Kawakami (1978) and Enzan and Yamamoto (1980) disclosed in the Ito cells of human fetuses, and Tanuma et al. (1981) in those of a 67-day-old kitten, glycogen particles around the lipid vacuoles, or inversely lipid vacuoles embedded in the accumulation of the glycogen particles. These studies suggested that the Ito cells may contain glycogen particles more frequently and in larger amounts in fetuses and in immature young individuals. Tanaka et al. (1981) carried out histochemical studies on the Ito cells in human livers and demonstrated abundant glycogen and a weak lactate dehydrogenase activity, presuming that an active glycogenolysis, rather than glycogenogenesis, might probably take place in these cells. Tanuma et al. (1982a) reinvestigated biopsy specimens from normal human adult livers, and could reveal in Ito cells lipid vacuoles enclosed by glycogen particles. ShibaSaki (1982) could demonstrate a large amount of glycogen particles in Ito cells of the normal rabbit liver, making for the most part thick rims around the lipid vacuoles. In recent TEM studies, Tanuma et al. (1981, 1982a) and ShibaSaki (1982) have discussed fat synthesis from carbohydrate in the Ito cell, referring at the same time to fat synthesis in the fat cell and the role played by the glycogen in this metabolic process. It was not only in Ito cells but also in other cell types which synthesize fat droplets from carbohydrates, that the glycogen usually appeared prior to the fat droplet in their cytoplasm (Ito, 1973). It is thus suggested that the glycogen must first be synthesized from the glucose ingested by the cells to be stored in the cytoplasm and then fat droplets appear in a glycogen accumulation causing such a spacial structure that glycogen β-particles form a rim around the fat droplets. This structure indicates that the glycogen particles might be preserved mainly on the surface of the preformed fat droplet, to be further utilized there for the synthesis of triglyceride, which might proceed through the well-known processes of the biosynthesis of triglyceride from glucose. In this context, the present study on adult monkey livers principally confirmed the findings gained by the above authors in kitten, human adult and rabbit livers. The electron-dense droplets associated with glycogen particles and their conglomerates which Tanuma et al. (1982a) often encountered among lipid droplets of the human Ito cells and which they regarded as immature ones retaining chemical properties of the glycogen were also revealed, though rarely, in the Ito cells of the monkey liver.

Along the perisinusoidal surface of both the cell body and cytoplasmic processes of the Ito cells of normal human livers, caveolae and vesicles of the plasma membrane were revealed by Ito and ShibaSaki (1968), Muto et al. (1977) and recently again by Tanuma et al. (1982a). Ito and ShibaSaki (1968) and Tanuma et al. (1982a) have considered them to be structures suggesting the micropinocytotic activity of the Ito cell. Until quite recently, it has been believed that the micropinocytotic pictures might be almost restricted to the human Ito cells. It seems one of the most worthy findings in the present study that we could actually reveal in the monkey liver, besides rare coated
caveolae and vesicles, numerous smooth-surfaced micropinocytotic caveolae and vesicles over the entire surface of the Ito cells because it has thus, become probable that they could occur in the Ito cells of the primate liver.

ITO and SHIBASAKI (1968) presumed that the smooth-surfaced tubules derived from the pinocytotic vesicles might be involved in the synthesis of glycogen particles in the human Ito cell. In the recent reinvestigation of the human Ito cell, TANUMA et al. (1982) have found that glycogen β-particles occurred abutting on the micropinocytotic caveolae and vesicles, and they came to the conclusion that the glycogen might be synthesized by utilizing the glucose ingested by the pinocytosis in the cytoplasm adjacent to the site of the pinocytotic activity. Similar findings suggesting the intimate relation between the glycogen synthesis and micropinocytotic caveolae and vesicles were occasionally confirmed in the Ito cell of the monkey liver.

4. Junction between the Ito cell and hepatocyte

Recent authors engaged in TEM studies on teleost livers have revealed desmosomal connections between sinusoidal cells themselves and between sinusoidal cell and hepatocyte (NOPANITAYA et al., 1979a, b; TANUMA and ITO, 1980; FUJITA et al., 1980; SHIN, 1981; SAKANO and FUJITA, 1982; TANUMA et al., 1982b). These desmosomal connections around the hepatic sinusoids were not reported, however, in the TEM studies on the liver of the lampreys (SHIN, 1977; PEEK et al., 1979), of the amphibia (SPORNITZ, 1975), and of the reptiles (TAIRA and MUTSUKO, 1981). Nor were they reported in any of the numerous TEM studies on the mammalian livers carried out by previous authors. Recently, WAKE (1980) has claimed that the Ito cells in the Chinese hamster liver connect at many places with the hepatocyte as well as with the sinusoidal endothelial cell. He did not, however, show in detail the fine structures of these connections. In the present study on the monkey liver, electron-dense junctional structures were observed at places where the plasma membrane of the hepatocyte and of the Ito cell were closely juxtaposed across a narrow space of about 20 nm. It was elucidated by high power observations that their high electron densities were induced by increased electron densities of juxtaposed plasma membranes and adjacent narrow cytoplasm of the both cell types, and that the narrow intercellular space was filled with a transversely striated fibrillar material which appeared to bind the apposed plasma membranes. From these findings it has been clarified that these maculae of junctional structures must in principle be identical with the so-called “junctional complexes” between the sinusoidal endothelial cells themselves and between the endothelial and Kupffer cell, but they are distinguished from the desmosome by lacking both the attachment plaque and microfilament bundles converged to the latter.

5. Collagen fibers in the Disse’s space

In the monkey livers, a considerable amount of collagen fibers (bundles of collagen microfibrils) was revealed in the Disse’s space, which might be comparable to that in the human adult liver. Distribution, arrangement and direction of the collagen fibers and microfibrils in the Disse’s space and their relations to the hepatocytic microvilli protruded into the Disse’s space, as reported by Ito and SHIBASAKI (1968) in the human liver, have roughly been confirmed in the monkey liver. The thickness of the collagen fibrils in the Disse’s space of the human liver measured 30–70 nm according to Ito and SHIBASAKI (1968), the average being about 50 nm and according to TANUMA et al. (1982a), varied from 15–20 nm to 50–60 nm. In the monkey liver, the thickness of the microfibrils in the Disse’s space varied from 25 nm up to 60 nm, thus the intralobular col-
lagen fibrils in the human liver almost equaled in thickness with those in the monkey liver, although the frequency of the thinner ones was thought to be far larger than that of the other.

Recently, the view that the Ito cell is responsible for the intralobular fibrogenesis has been proved by enzyme histochemical studies (Tanaka et al., 1974, 1976a, b, 1981) and by autoradiographic studies in 3H-proline-administered animals (Patrick and McGee, 1967; McGee and Patrick, 1972). Also purely morphological studies by means of the TEM had been attempted by many authors to verify that the Ito cells (perisinusoidal cells) might participate in the intralobular fibrogenesis in the liver. Following the TEM study of Wood (1963), Wewalka et al. (1966), Schnack et al. (1966, 1967) and Stockinger (1969) proposed in the studies on the biopsy specimens from the human livers that RER of the "ACT-cells" (Ito cells) must be responsible for the intralobular fibrogenesis in the liver. Ito and Shibasaki (1968), Ito (1973) and most recently Tanuma et al. (1982a) confirmed, in human biopsy specimens, the findings of the above Viennese authors, thus coming to the same conclusion. Tanuma et al. (1981) observed, in the Disse's space of a 67-day-old immature kitten liver, bundles of collagen fibrils ranging 27-46 nm to 50 nm in diameter, and also fine filaments which resembled the fine fibrillar or flocculent material in the dilated cisternae of the RER of the Ito cells. They could further obtain the finding that the collagen fibrils were often entwined or accompanied by the fine filaments, suggesting further development of the collagen fibrils by apposition of the RER-derived precursor substance. In the monkey livers similar findings concerning the fibrogenesis have been confirmed. In this animal, in the Disse's space contained, besides collagen fibrils of variable thickness, especially numerous filaments measuring about 5-8 nm in thickness, making a fine meshwork or occasional bundles which strikingly resembled in appearance the above mentioned fibrillar material in the diluted cisternae of the RER of the Ito cells. They could further obtain the finding that the collagen fibrils were often entwined or accompanied by the fine filaments, suggesting further development of the collagen fibrils by apposition of the RER-derived precursor substance. In the monkey livers similar findings concerning the fibrogenesis have been confirmed. In this animal, in the Disse's space contained, besides collagen fibrils of variable thickness, especially numerous filaments measuring about 5-8 nm in thickness, making a fine meshwork or occasional bundles which strikingly resembled in appearance the above mentioned fibrillar material in the diluted cisternae of the RER of the Ito cell. It was, thus, suggested that the fibrillar material or precursor substance (procollagen of Millar and Matukas, 1974) synthesized by the RER of the Ito cell might be discharged in the Disse's space to be converted there to collagen by the procollagen peptidase, an extracellular enzyme (Miller and Matukas, 1974). The findings observed in the kitten liver, that the fine filaments entwined the preformed collagen fibrils, have often been revealed also in the monkey liver. An assumption that the pinocytotic vesicles and caveolae of the Ito cells in the monkey liver would be involved in the transport of the procollagen from the cisternae of the RER to the Disse's space, could not be completely denied. Ohuchi and Tsurufuji (1972), Sakakibara et al. (1978) and Hata et al. (1979) have proposed that the hepatocyte might also participate in fibrogenesis in the Disse's space. No cytological signs which might support this view could be found in the present study.

Recently, Shin (1981) emphasized, in the electron microscopic study on the goldfish liver, that the RER of the Ito cell might not be considered as the organelle for the fibrogenesis, but that it might be responsible for the formation of the ground substance of the Disse's space, since he could hardly reveal collagen fibers there. In the Disse's space of crucian liver Tanuma and Ito (1980) could not demonstrate collagen fibrils (Type III collagen), but instead sparse fine fuzzy filaments (possibly type IV collagen). The presumption proposed by Shin (1981) that the RER of the Ito cells might be involved in the synthesis of the retinol-binding protein would be highly evaluated.

Acknowledgements. The authors wish to express their appreciation to Dr. T. Fujiwara and Dr. M. Tatsumi of the Department of Veterinary Science, The National Institute of Health for their assistance in the collection of animals used in this study.
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