Electron Microscopic Study on Avian Livers with Special Remarks on the Fine Structure of Sinusoidal Cells

By

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---Received for Publication, September 5, 1981---

Key words: Avian liver, Hepatic parenchyma, Endothelial cell, Kupffer cell, Ito cell

Summary: Three of resident cells of the hepatic sinusoidal wall and in connection with these, the hepatic parenchyma were electron microscopically observed in four species of birds.

Avian hepatocytes were characterized by densely packed, abundant large mitochondria probably in accordance with mandatory postulation of high energy for flight. Supra- and paranuclear location of Golgi complexes in avian hepatocytes was characteristic, and this and other cytological signs were suggestive both of emiocytophotic discharge of some bile constituents from the hepatocyte into the bile canalicule and of endocytosis of unknown substance by means of bristle-coated micropinocytotic caveolae and vesicles into the hepatocyte from the content of the bile canalicule.

The most conspicuous feature of the avian sinusoidal endothelium consisted in that the perikaryonal cytoplasm was so rich in organelles, and comparable to that of the Kupffer cell; it contained numerous fine smooth-surfaced tubules filled with dense material, coated micropinocytotic caveolae and vesicles, macropinocytotic vesicles and many lysosomes. The distinction between thinner and thicker portions in the membraneous cytoplasmic extension was not conspicuous and sieve plates limited to the thinner portions were not numerous.

The most conspicuous and reliable cytological differences between the Kupffer cell and endothelial cell were found in cell coat, pseudopodia and mitochondria, the last of which were larger in the former. The worm-like body was found in the Kupffer cells of chicken liver. In the initial stage of the erythrophagocytosis, the Kupffer cell sends out pseudopodia not only to embrace the adhered erythrocyte but also to penetrate into the latter, so that a complex interdigitation of cytoplasm of both cell types was observable.

The avian Ito cell, located in the Disse's space, contained multiple small lipid droplets and exhibited well-known essential cytological features as revealed in the mammalian liver. Segments of the so-called subendothelial processes were found here and there in the Disse's space. The diplosome was demonstrated within the Golgi area and the distal centriole proved to be provided with a cap-like ciliary vesicle, a cross-striated basal foot and a cross-striated rootlet, and a microtubule arose from the tip of the basal foot. The sequent developmental processes of a single cilium from the distal centriole first into the ciliary vesicle and then further into the Disse's space or into the sinusoid through a fenestrum of the endothelial lining were traced. Thus, the single cilium was established to be a common meta-
plasmic structure of the Ito cell in vertebrates. Not only Ito cell, but also endothelial and Kupffer cell of birds contained the spheridy (nuclear body) in the nucleus as reported in mammalian sinusoidal cells.

Besides sporadical lymphocytes and plasma cells, many macrophages of various differentiation stages were found in the hepatic parenchyma of the birds examined, especially of chickens. Those in an advanced differentiation resembled Kupffer cells in ultrastructure. Occasionally morphological sign suggestive of migration of the macrophage into the sinusoid was observed, and the macrophage even in a low differentiation stage was actually found in the sinusoidal lumen. Macrophages derived from the hepatic parenchyme were thought to transform into Kupffer cells after being fixed to sinusoidal endothelial lining. Therefore, the presence of organelle-poor immature Kupffer cells might be explained. Thus, macrophages in the hepatic parenchyma may be an origin or a source of the Kupffer cell in the sinusoid.

In contrast to numberless electron microscopic studies on mammalian livers, those concerning the avian livers are very limited. A series of the light microscopic study on avian livers, however, has already been carried out especially for the purpose of elucidating the morphological feature of the fat-storing cell (Ito cell) in birds which are comparative-anatomically ranked between mammals and lower vertebrates (Kitagawa, 1960; Tanaka, 1960; Ito, Tanaka and Nemoto, 1960; Umahara, 1963). In the present electron microscopic study, the authors will principally investigate three resident cells of the hepatic sinusoidal wall, endothelial cells, Kupffer cells and Ito cells, in chicken, quails, pigeons as well as society finches and in connection with these also fine structures of their hepatic parenchyma.

Materials and Methods

In this study, livers from chickens (male white Leghorns), quails (Coturix coturix), homing pigeons (Columba livia var.) and society finches (Jushimatsu) (Lonchura striata var. domestica) were used. They were anesthetized by inhalation of ether vapor, and the livers were fixed by perfusion via portal vein with a cold (0°C) fixative containing a 0.1 M phosphate-buffered 2.5% glutaraldehyde (pH 7.4). After perfusion fixation, the liver were excised and cut under a drop of fixative into minute blocks. 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 the dehydration in graded ethanol, the tissues 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. Hepatic parenchyma

As seen with the electron microscope, the hepatic cell cord of the bird agrees in structure with the terminal portion of the tubular gland, and is called “hepatic cylinder or tubule” (Kingsbury et al., 1956). In cross sections, 4-6 conical hepatocytes are radially arranged surrounding a central bile canalicule corresponding to the glandular lumen (Fig. 1). The narrow apical surface of the hepatocytes facing the bile canalicule is provided with
short finger-shaped microvilli and so is also their wide basal surface bordering the perisinusoidal or Disse's space. The interhepatocytic space varies in width and lacks almost completely microvilli that protrude from bordering hepatocytes except in the recessus at the exit to the Disse's space. The adjacent hepatocytes are connected each other by junctional complex, abutting on the canalicular lumen. Desmosomes are scarcely found between them. Only in society finch, gap junctions (communicating junctions) have often been found for variable distances between closely apposed hepatocytes; such close junctions have only exceptionally been detected in chickens. Along the basal or perisinusoidal surface of the hepatocyte there appear, besides ordinary smooth-walled and bristle-coated micropinocytotic invaginations and vesicles, many large hollows or indentations of the plasma membrane, which in profile frequently simulate vacuoles of variable sizes and shapes in the basal cytoplasm of the hepatocyte. Perisinusoidal microvilli of the hepatocyte are generally irregular in appearance, though in the chicken, they are slender and relatively regular in shape and size, resembling those of the mammalian hepatocyte. In the society finch, they are most scarce and in some locations almost missed. In the quail, sparse and irregularly shaped ones are short, bulky and fungiform or drumstick-shaped. In the pigeon, they are sparse and irregular in shape, with occasional mingling of bullar cytoplasmic extensions of the hepatocyte.

In birds the hepatocyte is generally smaller than that of mammals, and is mononucleated, having a relatively large nucleus. Binucleated hepatocytes may be exceptional. A single round nucleus with a large nucleolus is usually located eccentrically, being rather deviated toward the perisinusoidal surface (Fig. 1, 2). As shown in the survey electron microscopic pictures of the avian hepatocyte (Fig. 1, 2), it contains abundant large mitochondria which are closely packed in the cytoplasm, occupying about one half of its entire volume. They are ovoid, rod-shaped and filamentous and roughly display apico-basal orientation. Cristae mitochondriales are moderately numerous and arranged mostly in the transverse direction. The mitochondrial matrix is generally less dense than the cytoplasmic matrix and contains sparse intramitochondrial granules.

Single membrane-bound microbodies (peroxisomes) are far less numerous than mitochondria, appear more electron-dense than the latter, and possess no peculiar structures such as the marginal plate and core. Flattened cisternae of the rough endoplasmic reticulum (RER) and free polysomes are distributed between mitochondria, and the former are for the most part elongated, closely along the mitochondrial surface, surrounding the organelles (Fig. 3). The free polysomes are distributed even in the apical ectoplasmic layer, bordering the bile canaliculus.

Dense bodies, probably lysosomes, are usually found in the apical cytoplasm, but among them lipofuscin granules are hardly discerned. Cytolysosomes (autophagic vacuoles), bounded by the limiting membrane are occasionally identified (Fig. 3, 4a). Dense accumulations of glycogen α-granules are found in random locations of the cytoplasm in all species of birds examined. In the glycogen accumulation, cisternae of the smooth endoplasmic reticulum (SER) can not be identified, and the mitochondria are also excluded from the accumulation. Lipid vacuoles (droplets) of variable sizes are detected in avian hepatocytes, but in the chicken they are small in size and amount, and often hardly detectable.

The Golgi apparatus is distributed in
supra- and paranuclear area of the hepatocyte. It is composed of several Golgi complexes disposed at variable intervals (Fig. 1, 3), and each complex is composed of a stack of 4-5 flattened cisternae and many vesicles (Fig. 3). The cisternae are frequently dilated as a whole, or partially at the end part or at random portions to make vacuoles, some of which are filled with electron lucent granules or vesicles measuring about 400 Å in diameter. These electron lucent vesicles may correspond to the so-called very low density lipoprotein (VLDL) particles. As shown in Figure 4a, vacuoles of variable sizes, which contain partially amorphous material of moderate electron density, occur frequently in the apical or pericanalicular cytoplasm of the avian hepatocyte, occasionally presenting pictures of emiocytoplast dischage of their content into the bile canaliculus (Fig. 4b). On the other hand, along the apical surface of the hepatocyte facing the bile canaliculus, bristle-coated micropinocytotic caveolae and vesicles (600Å in diameter) have not infrequently been detected in intermicrovillous areas as shown in Figure 5.

2. Sinusoidal endothelial cell

The sinusoidal endothelial cell is composed of the cell body or perikaryon that contains a nucleus and the thin membranous cytoplasmic extension, the latter of which occupies the major part of the partition wall between the perisinusoidal or Disse's space and the sinusoidal lumen. In contrast to the Kupffer cell, the cell body of the endothelial cell does not strongly bulge toward the sinusoidal lumen, but is rich in cytoplasmic organelles, so that it is often difficult in the avian liver to distinguish the two sinusoidal cell types from each other. However, the sinusoidal surface of the endothelial cell is almost smooth and lacking, in contrast to the Kupffer cell, in pseudo-podia and cell coat (fuzzy coat or glyco-calyx). Cytoplasmic organelles are for the most part gathered in the perikaryonal cytoplasm. Most conspicuous organelles are fine tortuous or curved smooth-surfaced tubules containing electron dense material (Fig. 1, 2, 6, 8), which are distributed throughout the perikaryonal cytoplasm and the adjoining thicker portion of the cytoplasmic extension. These electron dense tubular structures occur in the majority of endothelial perikaryon in a considerable amount and give to the avian sinusoidal endothelium a characteristic feature. The numerous micropinocytotic pits and vesicles of the avian sinusoidal endothelium are almost exclusively bristle-coated and measure approximately 130 m in diameter on an average. They are especially numerous in the chicken liver (Fig. 1, 2, 7, 21) and found also in the adjoining thicker portion of the cytoplasmic extensions. In the chicken, there occur, besides numerous coated micropinocytotic vesicles, a considerable number of the so-called macropinocytotic vesicles (Wisse, 1972), measuring on an average 630 m in diameter, and some of them contain moderately electron dense material (Fig. 1, 2, 7, 21).

Sparse small mitochondria are round, oval and filamentous in profile and are scattered randomly. The Golgi complex is located on one side of the nucleus and composed of stacks of flattened cisternae and vesicles. In some cases, the flattened cisternae are elongated, more or less tortuous and show a conspicuous regular lamellar array (Fig. 1, 2, 6, 8). In avian sinusoidal endothelial cells, the centriole could not be detected. Cisternae of RER and free ribosomes are distributed throughout the cytoplasm and the former occasionally show an intimate spatial relation with the mitochondria (Fig. 10). Dense bodies, probably lysosomes, vary in number and size from cell to cell. In the
endothelial cell depicted in Figure 2, a large number of medium-sized and small lysosomes are found, and in those cells in Figure 6 and 8, strikingly large ones are conspicuous, while in that of Figure 9, organelles are all poorly developed and sparse. Microtubules run almost straight in random directions in the perikaryon without making bundles, and microfilaments are revealed mainly in ectoplasmic layer, showing a tendency to make bundles. These two filamentous structures extend into the cytoplasmic extensions, being oriented almost parallel to their long axis.

The nucleus of the sinusoidal endothelium is oval or spindle-shaped in profile and occasionally contains, besides one or two conspicuous electron dense nucleoli, a small spheridy (nuclear body) (Fig. 7, 9, 10), which is less electron dense than nucleoli, exhibits finely filamentous structure (Fig. 10) and is characterized by a light halo.

The membraneous cytoplasmic processes of avian sinusoidal endothelial cells vary in thickness. But the regular alternate arrangement of thicker and thinner portions (areas) is not confirmed (Fig. 9). Since the perikaryonal cytoplasm attenuates gradually toward the membraneous process, portions of the latter adjacent to the perikaryon are frequently thick (Fig. 7, 8). Not rarely, however, the perikaryon may be transformed abruptly into thin portions of the process (Fig. 1, 8, 9). Pores or fenestrae of the sinusoidal endothelial lining are found as a rule in the thinner portions of the cytoplasmic process as proposed by Wisse (1970), and their small cluster makes the so-called "junctional complex" of Wisse (1970). The narrow cleft (200Å wide) between the apposed plasma membranes may be filled with an electron dense material, and the electron density is increased in the apposed plasma membranes and adjacent narrow cytoplasmic areas, so that the junctions appear, at a low magnification, as electron dense dots or minute bars. As widely accepted, the hepatic sinusoidal endothelial lining is lacking the continuous basal lamina in the majority of animals, although some exceptional cases are known. This is also the case in avian livers examined in the present study. But upon detailed observations with the electron microscope, it has been revealed that short segments resembling the basal lamina are present especially in locations where the endothelial lining runs closely adjacent to structures of the Disse's space, such as Ito cell including its subendothelial process and microvilli of hepatocytes protruding in this space.

The most common configuration of the hepatic sinusoidal endothelium consists of a spindle-shaped perikaryon located in the concavity between hepatocytes and two cytoplasmic extensions originating from the
both diametric ends of the spindle-shaped perikaryon to line a sinusoid (Fig. 6, 7). The perikaryon bulges toward the sinusoidal lumen more or less extensively according to the section plane (Fig. 1, 6). Besides these common patterns of the configuration, more complicated one as seen in Figure 8 is rarely observed in the chicken and quail liver. In this pattern, the perikaryon lies across a sinusoid as if to bridge the opposite walls of the sinusoid. Along these walls, the perikaryonal cytoplasm sends out to the both directions each two membraneous cytoplasmic processes to embrace two sinusoids on both sides of the perikaryon. As shown in Figure 9, a perikaryon occasionally intervenes between two hepatic cylinders and both of its free surfaces facing two neighboring sinusoids protrude thin cytoplasmic processes in two respective directions to surround two sinusoids on both sides of the perikaryon. The most complex and unusual pattern of the configuration of the sinusoidal endothelium is depicted in Figure 2; there, three surfaces of a massive perikaryon are attached to three neighboring hepatic cylinders and send out 6 cytoplasmic extensions along the surface of the hepatic cylinders to line three sinusoids, intervening between the three cylinders.

3. Kupffer cell

Since Kupffer cells are most frequently encountered in the hepatic sinusoid of the chicken liver, the present study is designed to observe them mainly in this avian species. As elucidated in this observation, avian sinusoidal endothelial cells are so rich in cytoplasmic organelles as the Kupffer cell, but the most reliable morphological characteristics to be used for the distinction of the two cell types, from each other, consist in that the Kupffer cell is provided with the fuzzy (cell) coat and conspicuous pseudopodia instead of the fenestrated cytoplasmic extensions of the endothelial cell (Fig. 11, 12), although the above mentioned coat, covering the entire sinusoidal surface inclusive of the pseudopodia, can not be preserved satisfactorily due to unsuitable fixation with glutaraldehyde (Wisse, 1972).

Development and feature of the pseudopodia are different from cell to cell. Strikingly elongated pseudopodia (filopodia) can approach the opposite sinusoidal wall to adhere to the endothelial lining or penetrate the fenestra (Fig. 12). In contrast to the sinusoidal endothelium, cytoplasm-rich Kupffer cells bulge more or less markedly into the sinusoidal lumen, and the location of a oval or spindle-shaped nucleus is indefinite, probably due to the ameboïd movement of the cytoplasm. The cytoplasm/nuclear ratio is larger in the Kupffer cell than in the endothelial and Ito cell. Kupffer cell nuclei agree in the chromatin disposition and electron density of the matrix with those of the other two types of the sinusoidal cell, and they contain, besides conspicuous electron dense nucleoli, one or a few less dense small spheridies (nuclear bodies) (Fig. 13). The Golgi complex, usually located in the proximity of the nucleus, is composed of stacks of several flattened cisternae and vesicles (Fig. 14, 15), and often spreads over a wide area of the cytoplasm, in which occasionally one of the paired centrioles of the diplosome is encountered (Fig. 15). Mitochondria of the Kupffer cell are round, oval and more of less elongated in profile and larger than those of the endothelial and Ito cell. They are distributed randomly throughout the cell body (Fig. 11, 14, 15). Also abundant polysomes are distributed all over it and further in thicker portions of the pseudopodia excepting the Golgi area (Fig. 11, 14). Short as well as long curved flattened cisternae of the rough endoplasmic reticulum (RER) exhibit the same fashion of distribution as the free polysomes, but elongated cisternae often
make clusters in random areas of the cytoplasm (Fig. 14, 15), while their lamellar array is seldom. Against the good development of the RER, tubular cisternae of the smooth surfaced endoplasmic reticulum (SER), containing electron dense material, are only rarely identified.

Dense bodies, probably lysosomes, somewhat outnumber those in the endothelial cell and are generally larger, though their shape, size and electron density are widely variable (Fig. 11, 14, 16, 17). Small vacuoles comparable with macropinocytotic vesicles of the endothelial cell are often found also in Kupffer cells (Fig. 12, 14, 18), and small scanty lipid vacuoles are occasionally observed, being distinguished from the former (Fig. 16). Bristle-coated micropinocytotic pits and vesicles are frequently seen along the plasma membrane covering both the perisinusoidal and sinusoidal surfaces (Fig. 11, 12, 17), but they are less numerous than in the endothelium. Microtubules and microfilaments are revealed in the Kupffer cell cytoplasm (Fig. 17), the latter being rich in pseudopodia. In the chicken liver, erythrophagocytosis of Kupffer cells is relatively often observed, and simultaneous ingestion of two or more erythrocytes were not infrequent. In the beginning of the erythrophagocytosis, the red cell adheres on the sinusoidal surface of the Kupffer cell (Fig. 16) and pseudopodia are protruded from the latter to embrace the erythrocyte on the one hand and to penetrate into it on the other (Fig. 15), and thus a peculiar complex profile of interdigitation of processes from the both cell types is exhibited (Fig. 18); finally the erythrocyte is taken in the phagocytic vacuole and digested gradually, presenting a variety of dissolution pictures (Fig. 19).

Junctions between the Kupffer cell body and the endothelial lining have been revealed also in avian livers examined in the present study. As seen in Figure 16, between diametrical marginal portions of the perisinusoidal surface of the Kupffer cell and the apposed end parts of the sinusoidal endothelial lining, two junctional complexes are detected. Detailed fine structure of the junctional complexes, as seen in Figure 15, agree with that revealed between neighboring sinusoidal endothelial cells (Wisse, 1970).

As described above, the Kupffer cells are rich in cytoplasmic organelles and characterized by the most complex cytoplasmic structure. But occasionally the sinusoidal wall contains Kupffer cells which differ from the ordinary organelle-rich Kupffer cell by their paucity of organelles. They possess, as shown in Figure 13, a number of short cisternae of the RER, many free polysomes, and a few large mitochondria in the cytoplasmic layer surrounding a relatively large nucleus. They can be identified as Kupffer cells merely by the scanty pseudopodia protruded from cell body, which fails completely in the endothelial cell. By careful observation of Figure 13, it may be clarified that the marginal portions of the perisinusoidal surface of this organelle-poor, probably immature Kupffer cell, connect with the end parts of the endothelial extensions as those of the ordinary cells do.

Out of four avian species examined, the so-called worm-like structure, which is considered to be a characteristic ultrastructure of the Kupffer cell, has been revealed only in the chicken. In its random area adjacent to the sinusoidal surface, more or less curved tubules measuring about 1000–1200 Å in diameter are occasionally demonstrated, which are bounded by the limiting membrane and characterized by a median dense line (Fig. 11, 12). These tubules may be referred to as segments of the worm-like structure, and some of them communicate at a random place with the cell surface where the limiting membrane connects with the surface plasma.
membrane covered by the fuzzy or cell coat (Fig. 11, 17). This finding may support the view that the worm-like structure had been derived from the tubular invagination of the plasma membrane of the Kupffer cell, and the median dense line might result from the fusion of the surface of the cell coat entering into the invagination with accompaniment of the plasma membrane. The Kupffer cell of the chicken does not exhibit any large, complex worm-like structures that occupy large area of the cytoplasm, but instead a number of curved tubular segments are sometimes detected making a loose cluster. The occurrence of a single segment is not rare (Fig. 11, 15). The worm-like structures have no spatial relations with the phagosomes, Golgi complex and endoplasmic reticula.

But along the limiting membrane, bristle-coated pits and vesicles are as frequently observed as along the surface plasma membrane (Fig. 17).

4. Ito cell (fat-storing cell, lipocyte)

The incidence of Ito cells is higher in the avian liver than in the mammalian liver. They are located in the Disse's space, being separated from the sinusoidal lumen by the endothelial lining. They show variable configurations, consisting of the cell body, which is often situated in the concavity between hepatocytes and cytoplasmic extensions (Fig. 20, 21) inclusive of subendothelial processes (Fig. 1, 2, 13–15). Ito cells of chicken, quail, pigeon and society finch liver have electron microscopically been proved to contain multiple lipid droplets of small size in the physiological state (Fig. 20–22). The so-called empty Ito cells devoid of lipid vacuoles are found also in avian livers examined, but they are distinguished from the pericyte by the absence of the basal lamina (Ito and Shibasaki, 1968). Numerous flattened cisternae of the RER together with free polysomes are distributed throughout the cytoplasm except in the Golgi area (Fig. 20–23), and they contain a moderately electron dense flocculent material, making in random sites more or less conspicuous dilated portions (Fig. 23, 25).

The Ito cell possesses, in the proximity of the nucleus, relatively well-developed large Golgi complexes which consist of stacks of flattened cisternae and small vesicles mingled with sparse coated ones (Fig. 22, 23). In the Golgi area, one or paired centrioles of the diplosome is often revealed (Fig. 22). Abutting on one of the paired centrioles (the distal centriole), a cap-like vesicle which may probably correspond to the ciliary vesicle, is present, covering the distal end of the centriolar cylinder (Fig. 24, 25). Further, from the lateral wall and the proximal end of the cylinder, a conical basal foot (Fig. 24) and a rootlet-like structure (Fig. 25), both having cross striations are projected, respectively, into the cytoplasm. Although these structures which are regarded as uniform accessory components of the basal body of the motile cilia, have only rarely been detected in the Ito cell of the chicken, but they may indicate a possibility that the distal centriole of the Ito cell of the chicken may be destined to turn into the basal body of the single cilium. Upon careful observation of Figure 26-a, b, c, the developmental process of a solitary cilium from the distal centriole (basal body) may be assumed as follows: From the distal end of the distal centriole, a single cilium is protruded into the ciliary vesicle (Fig. 26a) and then into the Disse's space (Fig. 26b) and occasionally further into the sinusoid through one of the endothelial fenestra (Fig. 26c).

Ito cells of the birds contain, as in mammals, relatively sparse mitochondria as small as those of the endothelial cell and also sparse small lysosomes (Fig. 23), although among the latter large ones are
also found, though infrequently (Fig. 24). Microtubules run in the cell body solitarily in random directions (Fig. 24), and among them are such as connect either with the tip of the basal foot or with the centrioles themselves. Microfilaments are mainly distributed in the ectoplasmic layer, making bundles (Fig. 22). Both filamentous structures course in the cytoplasmic processes, inclusive of the subendothelial ones, roughly in parallel with their long axis. A few bristle-coated micropinocytic pits and vesicles (900 Å in diameter) are occasionally found scattering along the plasma membrane (Fig. 22, 27), but ordinary smooth-walled ones of smaller size have hardly been detected except in one case of the Ito cell detected in the chicken liver in which a small amount of glycogen β-particles has been demonstrated closely along the boundary of lipid vacuoles (Fig. 27).

Nuclei of Ito cells are oval or more or less elongated in profile, and they possess one or two electron dense nucleoli and occasionally a small, less electron-dense spheridy, which is surrounded by an electron lucent halo (Fig. 28a). As shown in Figure 28a and b, the nucleus of the Ito cell in chicken has rarely revealed a nucleolus containing a membrane-bounded vacuole, in which one or multiple vesicles filled with a moderately electron dense amorphous material are found.

5. Mesenchymal cells other than the three resident sinusoidal cells found in the avian hepatic parenchyma
a) Plasma cell and lymphocyte

In the present electron microscopic study on avian livers, clusters of lymphocytes and plasma cells have been demonstrated in the Glisson's sheath especially in chicken and quail. Further they were occasionally detected solitarily in the interhepaticocytic space adjacent to the Disse's space (Fig. 29).

b) Macrophage

As seen in Figure 7, 9, 19, and 30, macrophages are found in the avian livers examined, especially frequently in the chicken liver. They are located in the interhepaticocytic space mostly adjacent to the Disse's space, solitarily or occasionally, forming a small cluster. They are characterized by abundant free polysomes distributed throughout cytoplasm, a few short cisternae of the RER, sparse dense bodies (lysosomes), mitochondria as large as those of Kupffer cells, a small Golgi complex, a diplosome and sparse short pseudopodia. These features may speak for the low differentiation due to the immaturity of these macrophages. In rare instances, the immature macrophages are also visible within the sinusoidal lumen (Fig. 18). A macrophage depicted in Figure 31, however, possesses, like the Kupffer cell, numerous elongated flattened cisternae of RER and a well-developed Golgi complex, suggesting its higher cytological differentiation, and a conspicuous pseudopod protruding through a fenestrum or gap of the endothelial lining into the sinusoidal lumen may be regarded as the sign of its migration into the latter.

Discussion

1. Hepatic parenchyma

Avian hepatic parenchyma is for the most part composed of the so-called hepatic cylinders (Kingsbury et al, 1956) comparable to the terminal portion of the tubular gland (Kitagawa, 1960; Tanaka, 1960; Ito et al., 1960; Umahara, 1963). By electron microscopic observation of cross sections of the cylinder, it has been elucidated that 4–6 conical hepatocytes surround a central bile canaliculus. The canalicular surface of the hepatocyte is provided with regular, slender, finger-shaped microvilli, while the wide basal area facing the Disse's space exhibits a com-
plicated and irregular appearance induced by irregular microvilli protruded into the Disse's space and by many indentations of the plasma membrane. In the avian liver, hepatocytes are exclusively mononucleated, and binucleated ones are invisible in contrast to the mammalian liver. A relatively large nucleus is eccentrically located, being somewhat inclined to the perisinusoidal surface. These morphological properties of the nucleus may support the view that the avian hepatocyte would rather resemble that of lower vertebrate as proposed by Kitagawa (1960).

The most conspicuous cytological feature of the avian hepatocyte consists in that it contains abundant closely packed large mitochondria which occupy almost one half of the entire cytoplasm. This has already been proved by light microscopic observation in variety of birds carried out by the above named authors. The abundant large mitochondria of the bird hepatocyte may be responsible for the production of large amount of energy required for flight, though in the chicken which does not fly, this cytological feature may still be maintained unaltered. The above assumption is supported by the cytological evidence that the bat which flies like bird, possesses hepatocytes packed by vast amount of large mitochondria as demonstrated by light and electron microscopic studies carried out by Tanuma (1980) as well as by Takahashi (1959) as well as by Tanuma and Ito (1978). The usual location of the Golgi complex in the apical cytoplasm close to the bile canaliculus, as widely established in the mammalian hepatocyte, has not been confirmed in the avian hepatocyte; in it, several Golgi complexes are distributed from supra- to paranuclear regions, being composed of the stack of flattened cisternae and vesicles. Some of the dilated portions of the cisternae are filled with electron lucent granules or vesicles about 400-500 Å in diameter, which may correspond to VLDL-particles of the mammalian hepatocyte. The similar supra- and paranuclear location of the Golgi complexes was reported recently in hepatocytes of a immature kitten (Tanuma, Ohata and Ito 1981). Cytological findings, actually suggesting the bile secretion of the hepatocyte are only rarely found in the literature; Ma and Biempica (1971) revealed in their electron microscopic study on biopsied specimens from normal human livers, relatively large electron lucent vacuoles (100-150 müler) in the vicinity of the bile canaliculus, and assumed their involvement in the secretion of some bile constituents. Tanuma (1980) also demonstrated small vacuoles in the cytoplasm of the crucian hepatocyte bordering the intrahepatic bile canaliculus. He observed the emiocytotic discharge of these vacuoles derived probably from the Golgi complex into the bile canaliculus, and assumed the bile secretion in the crucian hepatocyte. Morphological basis supporting possible bile secretion of avian hepatocytes, so far as revealed in the present electron microscopic study, consist, first, in the occurrence of vacuoles of variable shapes and sizes in the pericanalicular cytoplasm and, second, in pictures indicating the emiocytotic discharge of their content into the bile canalicule. These vacuoles partially contain low electron dense material and may probably originate from the Golgi complex.

In the present study, it has on the other hand been revealed that along the plasma membrane bordering the bile canalicule, there often occur bristle-coated micropinocytotic invaginations and vesicles, known as structures suggestive of micropinocytotic ingestion of macromolecules. Thus, it has been presumed that in avian liver some macromolecular constituents may be taken up from the bile into the hepatocyte by micropinocytotic mechanism.
2. Sinusoidal endothelial cell

Several problems concerning the hepatic sinusoidal endothelial cell have been thoroughly discussed by Wisse (1970, 1972), Tanuma and Ito (1978) in rat and bat, respectively, and by Tamaru (1979), Tanuma and Ito (1980) and Fujita et al. (1980) in lower vertebrates (fishes). As revealed in mammals and fishes, the hepatic sinusoidal endothelium of birds is composed of fenestrated cytoplasmic process. The avian hepatic sinusoidal endothelium, especially that of chicken, is strikingly rich in perikaryon and thin membranous fenestrated cytoplasmic process. The avian hepatic sinusoidal endothelium, especially that of chicken, is strikingly rich in organelles, so that the distinction of the endothelial perikaryon from the Kupffer cell is in survey pictures occasionally difficult. Most striking organelles are fine tortuous or curved smooth-surfaced tubular structures mostly containing an electron dense material, which are distributed throughout the perikaryonal cytoplasm and in thicker portions of the cytoplasmic extension. The smooth-surfaced tubules, possibly referred to as the constituents of the SER, were first remarked by Tanuma and Ito (1978) in the normal bat and then by Tanuma, Ohata and Ito (1981) in the kitten liver. The present electron microscopic study has elucidated that these smooth tubular structures occur in a remarkable amount in avian hepatic sinusoidal endothelium, so it may become an important problem to solve the functional significance of these questionable structures which remain open.

The second conspicuous organelles of the sinusoidal endothelial cell of the avian liver are large bristle-coated micropinocytotic pits and vesicles along the plasma membrane of the perikaryon and of the thicker portion of the cytoplasmic process. According to Wisse (1972), micropinocytotic pits and vesicles of the sinusoidal endothelial cell of rat liver are almost exclusively provided with the bristle coat. This was confirmed also in bat and kitten liver by Tanuma and Ito (1978) and by Tanuma, Ohata and Ito (1981), respectively. Bristle-coated caveolae and vesicles are in general strikingly numerous in the chicken endothelial cell, and they, together with the numerous smooth tubular structures of high electron density, constitute the complex characteristic feature of the sinusoidal endothelial cell of the avian liver which is comparable to that of the Kupffer cell.

Macropinocytotic vesicles, which were first described by Wisse (1972) in rat liver sinusoidal endothelium, represent larger vesicles or vacuoles containing sometimes more or less electron dense fluffy material. Similar large vesicles have been described in the normal mammalian liver and designated as vacuoles, phagosomes or phagosome-like vacuoles (Ito and Shibasaki, 1968; Tanuma and Ito, 1978; Tanuma, Ohata and Ito, 1981). In avian livers examined, distinct macropinocytotic vacuoles measuring about 630 μm have been demonstrated in a considerable number in the endothelial perikaryon, and they played a role in making the avian liver endothelial complex ultrastructure.

It has been well established that micro- and macropinocytosis are involved in the endocytosis of fine particulate materials such as Thorotrast, carbon particles, etc by the hepatic sinusoidal cell (Wisse, 1972; Naito, 1976; Tamaru, 1979; Fujita et al., 1980). The pinocytotic activities of cell may play an important role in the clean-up of the blood (Tamaru, 1979), and on the basis of the above ultrastructural characteristics, it may be concluded that this activity must be strong in the avian liver. But the question as to whether the smooth-surfaced electron dense tubules may participate in the same activity or in some other unknown functions remains currently unanswered.

As in mammalian livers, membranous cytoplasmic process of the sinusoidal
endothelial cell of birds is composed of thicker and thinner portions, but distinction between them by the thickness and their alternate disposition are not so clearly established as in the mammalian liver. Fenestrated thinner portions called "sieve plates" were demonstrated not only in mammals (Wisse, 1970; Muto, et al., 1970; Tanuma and Ito, 1978; Tanuma, Ohata and Ito, 1981) but also in lower vertebrates, for example in fishes (Nopanitaya et al., 1979; Tanuma and Ito, 1980; Fujita et al. 1980). In the four kinds of bird examined, sieve plates have been detected but, so far as observed with the transmission electron microscope, frequency of sieve plates seems to be small, since thinner portions without interruptions (fenestrae) are encountered not rarely, and from a small number of interruptions it may be assumed that fenestrae grouped in each sieve plate may be less numerous than in mammals. Junction between neighboring endothelial cells by means of the so-called junctional complex (Wisse, 1970) is found between thicker portions of the endothelial processes also in birds, but the desmosome reported by Fujita et al. (1980) and by Tanuma and Ito (1980) in the gold fish and in the crucian liver, respectively, has been completely missed.

Although isolated segments of the subendothelial process of the Ito cell frequently simulate the double-layered endothelial lining in mammalian liver, the sinusoidal endothelial lining is thought to be principally simple-layered in the latter (Ito and Shibasaki, 1968; Wisse, 1970; Kawanami, 1973; Muto, et al., 1977; Tanuma and Ito, 1978). This is also the case in hepatic sinusoidal wall of birds in contrast to that of lower vertebrates in which double-layered areas were actually revealed in addition to pseudo-double-layered portions (Haar and Hightower, 1976; Tanuma and Ito, 1980). As widely known, the hepatic sinusoidal endothelium is devoid of a continuous basal lamina in mammalian liver, with some exceptional cases, for example, pathological cases and ruminant livers, as noted by Ito (1973), Popper (1977), Tanikawa and Ikejiri (1977) and Tanuma and Ito (1978). In lower vertabrate liver, this has been evidenced by Tanuma and Ito (1980), Fujita et al. (1980) and Nopanitaya et al. (1979). Also in birds, the absence of the continuous basal lamina has actually been confirmed, but nevertheless segments of the basal lamina or basal lamina-like structure have been revealed in places where the endothelial lining is closely juxtaposed to the structures such as Ito cell, its subendothelial process and hepatocytic microvilli in the Disse's space, as proposed by several authors in their studies on mammalian livers (see Tanuma and Ito, 1978). Recently, a discontinuous basal lamina of the sinusoidal endothelium was demonstrated by Haar and Hightower (1976) in the normal adult newt.

Besides the common configuration of the sinusoidal endothelial cell in which a spindle-shaped perikaryon in profile protrudes two cytoplasmic extensions from the diametrical poles to embrace, together with adjacent endothelial cells, a sinusoid, there occur, especially in the chicken and quail liver, a more complicated configuration of the endothelial cell which possesses a larger perikaryon with a complex outline, sending out several cytoplasmic extensions to surround two or more sinusoids. So far as the present authors know, such a large complex configuration of the hepatic sinusoidal endothelium has been reported for the first time in the bird liver.

3. Kupffer cell

In the avian livers examined, the sinusoidal endothelial cell is in general
rich in cell organelles, and often appears cytoplasm-rich in profile, so that it is sometimes difficult to distinguish the sinusoidal endothelial cell from the Kupffer cell. The most reliable morphological features useful for the distinction between these cell types consists in that the Kupffer cell which strikingly bulges into the sinusoidal lumen is provided with the pseudopodia and that the unsatisfactorily preserved cell coat is seen in the glutaraldehyde-fixed preparation instead of the fenestrated cytoplasmic extensions of the endothelial cell. Apart from these important features, the Kupffer cell possess larger mitochondria as well as Golgi complex and moreover numerous elongated flattened cisternae of the RER. Lysosomes are in general larger and more numerous than in the endothelial cell, but this difference may scarcely be applicable for distinction of the Kupffer cell in the avian liver.

Spheridies are demonstrated not only in the avian Kupffer cell but also in endothelial and Ito cell nuclei in accordance with the view that they might be a common ultrastructural feature of the hepatic sinusoidal cell nuclei. Spheridy (nuclear body) was first described by Ito and Shibasaki (1968) in human resident sinusoidal cell types and then by Altmann and Pfeifer (1969), Wisse (1972), Hruban et al. (1974), Tanuma and Ito (1978) and Tanuma, Ohata and Ito (1981) in mammalian sinusoidal cells including the human. The present study has proved that this nuclear structure of unknown nature and function is present also in bird liver sinusoidal cells.

In chicken liver Kupffer cells, relatively avid erythrophagocytosis has been revealed in the normal state; the erythrocyte adhering to the surface of the Kupffer cell may be on the one hand embraced by some pseudopods protruded from the latter and on the other penetrated by the other pseudopods deep into the cytoplasm, thus, in profile a peculiar complex interdigititation of processes of the both cell types is seen before the erythrocyte is taken up into the phagosome to be digested gradually.

The worm-like structure (micropinoctosis vermiformis) is thought to be one of the characteristic ultrastructure of the Kupffer cell, on which Tanuma (1978) recently carried out detailed observations in bat liver Kupffer cell with reference to abundant literatures. In the present study, small clusters of tubular segments with a median dense line of the worm-like structure have been demonstrated for the first time in chicken liver Kupffer cells, thus it has been established that the worm-like structure can occur also in the avian liver Kupffer cell.

The Kupffer cell is the fixed macrophage of the hepatic sinusoidal wall, and the junctional mode between the Kupffer cell and the sinusoidal endothelial cell has recently been elucidated by Ito, Tanuma and Shibasaki (1980) in normal human and bat livers. The same mode of junction by means of the junctional complex (Wisse, 1970) has been revealed not only in the organelle-rich, mature Kupffer cells but also in organelle-poor, probably immature ones, the latter of which are only rarely detected in the chicken liver.

4. Ito cell

As well known, the morphological characteristics of the Ito cell consist in: 1) it is located in the Disse's space, being separated from the sinusoid by the endothelial sheet, 2) it contains lipid vacuoles, although occasional empty one devoid of lipid vacuoles is detected, 3) it possesses well-developed RER and 4) it is provided with no basal lamina. These essential features
have been confirmed in avian Ito cells. Ito cells of avian livers contain multiple lipid droplets of small size in agreement with the result obtained by a series of light microscopic observations (Kitagawa, 1960; Tanaka, 1960; Ito, Tanaka and Nemoto, 1960 and Umahara, 1963). The RER is believed to be responsible for the fibrogenesis in the Disse's space. The collagen microfibrils found in the Disse's space are generally small in amount in bird livers examined and they seldom build compact bundles of a considerable thickness (Fig. 23, 27-29). Microfilaments and microtubules were demonstrated in the Ito cell cytoplasm in mammalian livers (Ito and Shibasaki, 1968; Wisse, 1970; Ito, 1973) and in the crucian (Tanuma and Ito, 1980). In chicken liver Ito cell, microfilaments are mainly revealed in the ectoplasmic layer, and solitary microtubules run in random directions in the cell body; both, however, converge toward the processes, in which they are oriented in parallel with their long axis to serve as supporting structures for the processes. It is known that the Ito cell sends out in the Disse's space cytoplasmic processes along the sinusoidal endothelial sheet not only in mammalian liver (Ito and Shibasaki, 1968; Muto et al. 1977) Tanuma and Ito, 1978; but also in crucian liver (Tanuma and Ito, 1980), and those closely juxtaposed to the endothelial sheet are called subendothelial processes as noted above. This is also confirmed in avian livers, and isolated segments of the subendothelial processes have been found here and there, probably also reinforcing the endothelial sheet (Ito and Shibasaki, 1968; Wisse, 1970; Muto et al., 1977; Tanuma and Ito, 1978). In the sinusoidal endothelium and Kupffer cell, the centriole is usually detected within the Golgi complex (Golgi area) (Yamagishi, 1959; Ito and Shibasaki, 1968; Wisse, 1970, 1972; Wisse et al., 1974; Naito, 1976; Tanuma and Ito, 1978; Tanuma, Ohata and Ito, 1981). Out of these authors, Wisse et al. (1974) and Tanuma and Ito (1978) demonstrated the diplosome in the rat liver Kupffer cell and bat liver endothelial cell, respectively. In the Ito cell, the diplosome was revealed by many authors also within the Golgi complex in mammals including man, and they reported that from one of the paired centrioles (distal centriole) a single cilium develops into the Disse's space (Ito and Shibasaki, 1968; Wake, 1971; Yamamoto, 1975; Yamamoto and Enzan, 1975; Tanuma and Ito, 1978; Tanuma, Ohata and Ito, 1981). These findings were confirmed further in the Ito cell of lower vertebrates (crucian) (Tanuma and Ito, 1980), and in the present study in the chicken liver. Thus, a possibility has been reinforced that the single cilium of the Ito cell may be common metaplasmic structure present in all species of the vertebrate. In the chicken liver Ito cell, it has been clarified that the single cilium develops from distal centriole into the cap-like ciliary vesicle and then into the Disse's space or into the sinusoid, and concerning the distal centriole (basal body), an unusual fact has been discovered that the distal centriole, as seen in the longitudinal section, is provided with a conical basal foot with the cross striation on the lateral wall and protrudes a cross striated rootlet-like cord from the proximal end into the cytoplasm. These structures are known to be usual accessories of the basal body of the motile cilia, but have never been revealed in that of the single cilium of the Ito cell, which is thought to be non-motile but sensory in nature. It may also an interesting finding that one of the microtubules arises from the tip of the basal foot. In capillary endothelial cells found in the interscapular brown fat of the bat, Umahara (1968) revealed the diplosome in the Golgi area, and he found
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a cross striated rootlet protruding from one of the paired centrioles, though he could not demonstrate a single cilium developed from the centriole. Sparse bristle-coated micropinocytotic caveolae and vesicles have been demonstrated along the plasma membrane in the avian liver Ito cell, but the ordinary smooth-walled ones of smaller size, have hardly been revealed in Ito cells of various mammals, and the crucian (Tanuma and Ito 1978, 1980) as well as birds except in those of the normal human liver, in which numerous smooth-walled ones were actually revealed (Ito and Shibasaki, 1968; Muto et al. 1977; Tanuma, Ito and Shibasaki, 1982). In the present study, they have been detected only in one case of the Ito cell of the chicken liver, in which glycogen \( \beta \)-particles have been demonstrated along the boundary of lipid vacuoles, suggesting their participation in lipid synthesis in the Ito cell, as recently discussed in detail by Tanuma, Ohata and Ito (1981) in the study on a kitten liver. The origin, nature and significance of vesicular or vacuolar inclusions found in nucleoli of the Ito cell nuclei of the chicken liver are unknown, but it may be expected that they would give a vesicular appearance to the nucleolus under the light microscopic observation.

5. Cells other than three types of the sinusoidal cells found in the hepatic parenchyma.

In the hepatic parenchyma of mammals including the human, several types of mesenchymal cells such as lymphocytes, lymphocytoid cells, plasma cells, macrophages were described (Rouiller et al., 1967; Ito and Shibasaki, 1968; Hruban et al., 1974; Tanuma and Ito, 1978; Tamaru, 1979; Tanuma, Ohata and Ito, 1981), and the migration of lymphocytoid cells and macrophages into the sinusoid were reported (Tanuma and Ito, 1978; Tanuma, Ohata and Ito, 1981). In fishes, the existence of Kupffer cells in the hepatic sinusoid had not been discerned, although the existence of macrophages in hepatic parenchyma was elucidated (Tanuma and Ito, 1980; Fujita, Tamaru and Miyagawa, 1980). Since it was proved by a series of the light microscopic observations that the avian liver possessed lymphopoietic foci both in the parenchyma and interlobular connective tissue (Kitagawa, 1960; Tanaka, 1960; Ito, Tanaka and Nemoto, 1960; Umahara, 1963), it may be natural to find in the present electron microscopic study lymphocytes and plasma cells in the interhepatic space abutting on the Disse's space of avian livers examined. It may be more interesting and important that especially in the chicken liver parenchyma, macrophages of different differentiations frequently occur in the same location as just described and that they occasionally show cytological sign of migration through endothelial lining into the sinusoid, sending out pseudopod into the latter, as recently reported by Tanuma and Ito (1978) in the bat liver and by Tanuma, Ohata and Ito (1981) in the kitten liver. Highly differentiated macrophages resemble the Kupffer cell, but less differentiated ones are characterized by numerous free polysomes, short and sparse cisternae of the RER, a small Golgi complex, diplosomes and large mitochondria. These less differentiated macrophages are occasionally found also in the sinusoid and suggest that they might transform into the organelle-poor immature Kupffer cell, being fixed by means of the junctional complex (Wisse, 1970) to the endothelial lining. The macrophages in the hepatic parenchyma of the chicken may therefore be referred to as reserved precursors of the Kupffer cell.
References


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Explanation of Figures

Plate I

Fig. 1. Cross section of a hepatic cylinder. Conical hepatocytes (H) packed by numerous large mitochondria (M) are radially arranged surrounding a central bile canalicule (BC). E erythrocyte, EC endothelial perikaryon, EL membranous endothelial lining, KU Kupffer cell, LE leucocyte, N nucleus of hepatocyte, PS Disse's space containing hepatocytic microvilli, SN sinusoid, X subendothelial process of Ito cell. Chicken liver. ×4,800

Fig. 2. Profile of a large endothelial perikaryon (EC) with complicated configuration sending out six membranous cytoplasmic extensions (EL) along three hepatic cylinders to surround three sinusoids (SN) between them. H hepatocyte, KU Kupffer cell, MP macrophage in the interhepatocytic space, PS Disse's space, X subendothelial process of Ito cell. Arrow indicates two sieve plates in a tangentially sectioned part of endothelial membranous extension. Chicken liver. ×4,800
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Plate II

Fig. 3. Supranuclear portion of a hepatocyte. About three Golgi complexes (G) are distributed at variable intervals. They are composed of a stack of flattened cisternae dilated at indefinite sites to make vacuoles and small vesicles. Some vacuoles contain small electron-lucent granules or vesicles, probably corresponding to VLDL particles (arrows) and others amorphous material. Numerous large mitochondria (M) are surrounded by elongated cisternae of RER. CL cytolysosome, D lysosome, N nucleus of hepatocyte containing a nucleolus (NL), P peroxisome. Chicken liver. ×25,000

Fig. 4. a Accumulation of vacuoles (V) in the ectoplasmic layer of the apical cytoplasm of hepatocytes bordering a bile canaliculus (BC). Majority of them contain moderately electron-dense amorphous material like those found in the Golgi region. C diplosome of hepatocyte, CL cytolysosome containing a degenerated mitochondrion, JC junctional complex between hepatocytes devoid of desmosome. M mitochondria, MV microvilli of hepatocytes. Chicken liver. ×17,000

b Emiocytotic discharge of the pericanalicular vacuoles (V) into bile canaliculus (BC), probably morphological sign of bile secretion. JC junctional complex, M mitochondria, MV microvilli of hepatocyte. Chicken liver. ×17,000
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Plate III

Fig. 5. Bristle-coated caveolae (CP) and vesicles (CV) along the plasma membrane of hepatocytes bordering the bile canalicule (BC). The pericanalicular ectoplasmic layer of hepatocytes is rich in microfilaments (MF) running in several directions, some of which enter into the axis of the hepatocytic microvilli (MV) protruding into the bile canalicule. R polysome. Pigeon liver. ×55,000

Fig. 6. Usual spindle-shaped profile of a endothelial perikaryon. Along the sinusoidal surface of the nucleus (N) a lamellar array of elongated Golgi cisternae (G) is conspicuous. In the perikaryonal cytoplasm, there are densely distributed smooth tubules, either curved or tortuous, filled with dense material. D lysosomes, E erythrocyte in sinusoid (SN), EL thinner portion of the cytoplasmic extension with “sieve plates” (arrows). H hepatocyte, M mitochondria, MV irregular hepatocytic microvilli protruding into the Disse's space (PS). Quail liver. ×12,000
Plate IV

Fig. 7. Usual spindle-shaped profiles of two sinusoidal endothelial perikaryons (EC). From both diametrical end parts of them cytoplasmic processes (EL) extend to surround respective sinusoids (SN) containing a erythrocyte (E), thrombocytes (TC) and Kupffer cells (KU). In the perikaryonal cytoplasm and sequent thicker portion of the process many coated micropinocytotic vesicles and electron dense smooth tubules are characteristic. In the cytoplasm of the perikaryon on the right two macropinocytotic vesicles and in the nucleus of the perikaryon on the left a small spheridy with an electron lucent halo are visible. FSC empty Ito cell, H hepatocyte, MP macrophage in the parenchyme, PS Disse's space; an arrow indicates a junction between the endothelial cells. Chicken liver. ×4,600

Fig. 8. A profile of the endothelial perikaryon (EC) which lies between two sinusoids (SN) as if to bridge the reciprocally confronting sinusoidal walls. Along the confronting sinusoidal walls, cytoplasmic processes (EL) extend in two directions to invest the two sinusoids (SN) on both sides of the perikaryon. In the perikaryonal cytoplasm and sequent thicker portion of the processes, smooth tubules filled with electron dense material are distributed, and along the plasma membrane, are seen coated caveolae and vesicles. D lysosome, E erythrocyte, G Golgi complex, M mitochondria, MV microvilli of hepatocytes, PS Disse's space. Arrow indicates "sieve plate". Quail liver. ×9,200

Fig. 9. A profile of the sinusoidal endothelial perikaryon (EC) intervening between two hepatic cylinders (H). Two free surfaces of the perikaryon border two sinusoids (SN) on opposite sides and two cytoplasmic extensions (EL) are protruded from there along the hepatic cylinders to surround the two sinusoids. D lysosome, E erythrocyte, G Golgi complex, M mitochondria, MP macrophages, MV hepatocytic microvilli, PS Disse's space, SP spheridy. Arrows indicate "sieve plates". Chicken liver. ×6,200
Plate V

Fig. 10. A profile of the sinusoidal endothelial perikaryon (EC) and tangentially cut “sieve plates” (arrows) of the cytoplasmic process. D lysosomes, FSC Ito cell, H hepatocyte, M mitochondria, PS Disse’s space, cisternae of RER, R free polysomes, SP spheridy. Chicken liver. ×12,000

Fig. 11. The nucleated portion of a Kupffer cell (KU), sending out numerous irregular pseudopods into the sinusoid (SN). Sinusoidal surface of the cell body and pseudopods is covered by unsatisfactorily preserved fuzzy coat. On the right side of the nucleus, a small cluster of tubular segments with a median dense line of the worm-like structure, and at the top left corner, a single segment are seen. Arrow indicates a cross section of the tubular segment. D lysosomes, H hepatocyte, M mitochondria, PS Disse’s space, cisterna of RER. Chicken liver. ×14,000
Plate VI

Fig. 12. A part of Kupffer cell body containing a cluster of tubular segments with a median dense line of the worm-like body and projecting long filopodia, one of which penetrates a fenestrum of the endothelial lining (EL) of the sinusoid (SN). Sinusoidal surface of both cell body and filopodia are covered with unsatisfactorily preserved fuzzy coat. Endothelial processes (EL) are attached to the diametrical marginal part of the perisinusoidal surface of the Kupffer cell body (arrows). E erythrocyte, EC endothelial perikaryon, H hepatocyte, MV microvilli of hepatocyte, PS Disse's space. Chicken liver. ×10,000

Fig. 13. Undifferentiated, immature Kupffer cell (IK) with a relatively large nucleus (N) containing three small spheridies (SP). Cytoplasm contains a few short cisterna of RER, many free ribosomes and relatively large mitochondria (M), and it sends out a few pseudopods. Endothelial cell consists of perikaryon (EC) and cytoplasmic process (EL) connected with perisinusoidal surface of the Kupffer cell at two points (arrows). E erythrocyte, H hepatocytes, KU a portion of differentiated Kupffer cell, PS Disse's space, SN sinusoid, TC thrombocyte, X subendothelial process of Ito cell. Arrowhead points to a junctional complex between endothelial cells. Chicken liver. ×8,000
Plate VII

Fig. 14. Kupffer cell with many pseudopodia, a large Golgi apparatus (G), well-developed RER and numerous relatively large mitochondria (M). Cell coat is poorly preserved. D lysosome, E erythrocyte, EL endothelial process, H hepatocyte, PS Disse’s space, SN sinusoid, X subendothelial process of Ito cell. Chicken liver. ×10,000

Fig. 15. Kupffer cell (KU) and an adhering erythrocyte (E). From the Kupffer cell pseudopods are protruded to embrace and penetrate the erythrocyte. C centriole, G Golgi complex, H hepatocyte, M mitochondria, PS Disse’s space, cisternae of RER, SN sinusoid, X subendothelial process of Ito cell. Arrow points to a junctional complex between Kupffer cell and endothelial process and arrowhead indicates a tubular segment of the worm-like body. Chicken liver. ×10,000

Fig. 16. Elongated Kupffer cell (KU) fixed to the endothelial lining (EL) by means of two junctional complexes (arrows). One of the erythrocytes (E) in the sinusoid (SN) adheres to the Kupffer cell. D lysosomes, G Golgi complex, H hepatocytes, LD lipid droplet, M mitochondria, PS Disse’s space, cisternae of RER. Arrowhead indicates tubular segment of the worm-like body. Chicken liver. ×8,000
Plate VIII

Fig. 17. A portion of Kupffer cell cytoplasm containing tubular segments of the worm-like body with a median dense line (WL) and many large lysosomes (D). Along the limiting membrane of the worm-like body, many bristle-coated caveolae (CP) are found. In the cytoplasm, there are many microtubules (MT), and in the pseudopods, microfilaments are seen. CV bristle-coated vesicle, E erythrocyte, H hepatocyte, PS Disse's space, SN sinusoid, X subendothelial process of Ito cell. Arrow indicates an opening of the worm-like body to the cell surface. Chicken liver. ×25,000

Fig. 18. Kupffer cell (KU) ingesting an erythrocyte (E). Cytoplasmic processes of the erythrocyte interdigitate with pseudopodia of Kupffer cell. In the sinusoid, a macrophage (MP) of low differentiation exists, being fixed with a cytoplasmic process to the endothelial perikaryon (EC), which has probably come from the hepatic parenchyme. C centriole, D lysosomes, EL endothelial lining of the sinusoid, M mitochondria, MV microvilli of hepatocyte (H), PS Disse’s space, V vacuoles corresponding to macropinocytotic vesicles of endothelial cell. Chicken liver. ×10,000
Plate IX

Fig. 19. Kupffer cell (KU) elongated along the hepatic cylinder (H). Kupffer cell ingests two erythrocytes in phagosomes which are in different digestive stages. BC bile canalicule, E erythrocytes in sinusoid (SN), EL endothelial lining of the sinusoid connected with the Kupffer cell at its end part (arrow), EP solitary bile-ductal epithelial cell between hepatocytes, MP macrophage within the hepatic parenchyme, N hepatocytic nucleus, PS Disse’s space. Chicken liver. × 4,600

Fig. 20. Fat-storing cell (FSC, Ito cell) with two cytoplasmic processes in the Disse’s space (PS). It is separated from the sinusoid (SN) by fenestrated endothelial lining (EL). E erythrocyte, H hepatocytes, KU Kupffer cell, MP macrophage, LD small lipid droplets, cisternae of RER. Arrows indicate “sieve plates”. Chicken liver. × 9,000
Plate X

Fig. 21. Ito cell (FSC) with a long cytoplasmic process in Disse's space (PS) and adjacent endothelial perikaryon (EC) protruding a cytoplasmic extension (EL), the end part of which is connected with a Kupffer cell (KU) by junctional complex (arrow). Endothelial perikaryon contains, besides mitochondria (M) as small as those of Ito cell and electron dense smooth tubules, many macropinocytotic vesicles (V) and abundant coated micropinocytotic vesicles as well as coated caveolae along the plasma membrane. G Golgi complex, H hepatocytes, LD small lipid droplets in FSC, LE leucocyte in sinusoid (SN), cisternae of RER, TC thrombocyte. Chicken liver. ×9,600

Fig. 22. A centriole (C) in the Golgi complex (G) and well-developed cisternae of the RER of the Ito cell (FSC). Sparse small mitochondria (M) are randomly distributed and microfilaments and microtubules (arrow) are concentrated along the ectoplasmic layer. EL endothelial lining of the sinusoid, H hepatocyte, KU Kupffer cell, LD lipid droplets, PS Disse's space. Arrowhead indicates coated micropinocytotic pit. Chicken liver. ×15,900
Plate XI

Fig. 23. Well-developed Golgi complex (G) and abundant cisternae of RER which are dilated at random sites in an Ito cell cytoplasm. Besides, sparse small mitochondria (M) and lysosomes (D) are seen. F collagen fibrils, H hepatocytes, PS Disse's space and hepatocytic microvilli. Chicken liver. ×14,000

Fig. 24. A portion of the Ito cell. In the Golgi area (G), paired centrioles (C) of the diplosome are seen. One of them (probably the distal centriole) is provided on the lateral wall, with the basal foot (BF) showing cross striations, and close to the distal end, with a cap-like ciliary vesicle (FV). One of many microtubules (MT) running in random directions seems to arise from the tip of the basal foot. D lysosomes, E erythrocyte in the sinusoid (SN), EL endothelial lining, M mitochondria, N nucleus of the Ito cell, PS Disse's space. R free ribosomes, cisternae of RER. Microfilaments are concentrated in ectoplasmic layer (arrows). Chicken liver. ×25,000
Fig. 25. A portion of the Ito cell. In the close proximity to the distal end of the distal centriole (C), a cap-like ciliary vesicle (FV) is present and from the proximal end a cross-striated rootlet (RL) is sent out in the cytoplasm. D lysosomes, EL endothelial lining of the sinusoid (SN), H hepatocyte, M mitochondria, PS Disse's space, RER cisternae of the rough ER accompanied with polysomes, X subendothelial process of Ito cell. Chicken liver. ×25,000

Fig. 26. Development of a single cilium from the distal centriole of the Ito cells. a Single cilium developing into the cap-like ciliary vesicle. LD lipid droplet, PS Disse's space. ×21,000, b Single cilium protruding from the ciliary vesicle into the Disse's space (PS). EL endothelial lining of sinusoid (SN), LD lipid droplet, N nucleus of the Ito cell, ×21,000 and c Single cilium protruding into the sinusoid (SN) through a fenestrum of the endothelial lining (EL). The proximal centriole is visible. LD lipid droplet, N nucleus of the Ito cell. Chicken liver. ×21,000

Fig. 27. A portion of the Ito cell (FSC) containing glycogen β-particles mainly along the border of lipid droplets (LD). Along the plasma membrane appear, besides a coated micropinocytotic pit (arrows), small smooth-walled micropinocytotic caveolae (arrows). F collagen fibril bundle, M mitochondria, PS Disse's space, R many free polysomes, cisternae of RER. This Ito cell possesses exceptionally discontinuous thin basal lamina. Chicken liver. ×17,000
Plate XIII

Fig. 28. Nuclei of Ito cells (FSC) from chicken liver.  a One of the nucleoli (NL) contains a membrane-bounded vacuole (V) in which a vesicle is seen.  ×12,000
b Membrane-bounded vacuole is filled with many vesicles.  ×12,000 In both cases vesicles hold moderately electron-dense amorphous material.  EL endothelial lining of the sinusoid (SN), F bundle of collagen fibrils in the Disse’s space (PS), H hepatocyte, KU Kupffer cell, SP spheridy surrounded by an electron lucent halo.

Fig. 29. Plasma cell in the interhepatocytic space adjacent to the Disse’s space (PS) containing microvilli of hepatocytes (H) and collagen fibrils of high electron density.  C centriole of the diplosome within the Golgi area, M large mitochondria, SN sinusoid.  Chicken liver.  ×8,000

Fig. 30. Undifferentiated macrophage characterized by abundant ribosomes and sparse short cisternae of RER in the interhepatocytic space adjacent to Disse’s space (PS), which is filled with hepatocytic microvilli and separated from sinusoid by endothelial lining (EL) with fenestrae.  BC bile canalicule, C diplosome, G small Golgi complex, H hepatocyte, M large mitochondria, SN sinusoid.  Chicken liver.  ×10,000

Fig. 31. Differentiated macrophage with well-developed Golgi complex (G), cisternae of RER, large mitochondria (M) and lysosomes (D) in the interhepatocytic space adjacent to Disse’s space (PS).  It shows morphological sign of migration into the sinusoid (SN) through sinusoidal endothelium, sending pseudopod into the latter.  BC bile canalicule, E erythrocye, EC endothelial perikaryon, EL endothelial process, H hepatocytes.  Quail liver.  ×7,600
Plate XIII

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