A Scanning Electron Microscopic Study on the Liver of Mice

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OIKAWA, K. *A Scanning Electron Microscopic Study on the Liver of Mice.* Tohoku J. exp. Med., 1979, 129 (4), 373-387 — The three-dimensional fine structures of several tissue components of the liver in normal mice were studied by scanning electron microscopy. The tissue components observed were as follows: hepatocytes, sinusoidal endothelial cells, the Kupffer cells, fat-storing cells, reticulin fibers and epithelial cells of the bile duct. Two types of fenestrations were found in the sinusoidal endothelial cells. One was smaller and clustered, and the other larger and scattered. Both of them were distributed equally throughout the hepatic lobule. Inter cellular gaps were found at the endothelial junction. The Kupffer cell which was localized in a large gap between the endothelial cells was characterized by numerous villous projections, and by the absence of fenestrations which were observed in the endothelial cells. Fat-storing cells were located between hepatocytes. They elongated their processes into the space of Disse, but never protruded into the sinusoidal lumen. They were clearly distinguished from the endothelial cells and the Kupffer cells by their morphological feature and location. No transitional form was seen among the endothelial cells, the Kupffer cells, and the fat-storing cells. — SEM; liver; Kupffer cell; sinusoid; mice

Transmission electron microscopy (TEM) is suitable to observe the inner structures of cells. On the other hand, scanning electron microscopy (SEM) is useful for the observation of three-dimensional features of the cell surface. SEM had formerly been used only for observation of the surface of organs, or free cells such as blood cells and cultured cells. The technical methods have recently been improved in preparing the tissue specimens for SEM (Murakami 1974; Tokunaga et al. 1974). By these methods, the observation of the interior structures of parenchymatous organs has been made possible. The purpose of the present paper is to demonstrate the three-dimensional features of the fine architecture of the liver and the cell surface in situ.

MATERIALS AND METHODS

Four male mice of the ICR strain, aged 5 weeks and weighing from 20 to 25 g, were used. Under anesthesia with ether, laparotomy was carried out. The liver was perfused with phosphate buffer solution (pH 7.4) through the portal vein under a pressure

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of 50 cmH₂O for 5 min, followed by perfusion with a 2.8% glutaraldehyde solution (pH 7.4) for 20 min. Venesectomy of the inferior vena cava was carried out in the thoracic cavity simultaneously with the starting of the perfusion. The liver was taken out of the peritoneal cavity, and cut into small blocks of about 5×5×2 mm size. The blocks were fixed further in the 2.8% glutaraldehyde solution (pH 7.4) at 4°C for 24 hr. Then they were immersed in a mixture of glycine, sucrose, sodium glutamate, and arginine hydrochloride, 2 g of each in 100 ml of distilled water, at 4°C for 16 hr. After being rinsed in distilled water, they were immersed in 2% tannic acid solution at 4°C for 24 hr. The blocks were postfixed in 2% osmium tetroxide solution at room temperature for 6 hr after the rinse in distilled water (Murakami 1974). They were rinsed for 30 min in distilled water which was changed several times, followed by dehydration with a graded series of ethanol. Then they were immersed in isoamyl acetate for 15 min, and cracked with tweezers in liquid nitrogen (Tokunaga et al. 1974). The cracked blocks were dried at the critical point in CO₂. Finally they were coated with gold by the ion sputtering method. The samples were examined and photographed in a scanning electron microscope (Hitachi, HSM-2B) at 25 kV of the accelerating voltage.

RESULTS

Hepatocyte and hepatocellular plate

Hepatocytes were found to form one-cell-thick plates, 10 to 15 μ m in thickness (Figs. 1 and 2). Each hepatocyte was polyhedral with the following surfaces; (1) the sinusoidal surface facing to the space of Disse, (2) the intercellular surface being in contact with an adjacent hepatocyte, and (3) the bile canalicular surface. On the sinusoidal surface, numerous microvilli grew thick. They were about 0.3 μ m in length and about 0.1 μ m in diameter. The intercellular surface was rather smooth, though there were some pits and protrusions (Fig. 3) which were called stud-like structures by Fawcett (1955). This fact makes the cells firmly contact with each other. The bile canalicular surface was a lengthwise groove seen at the central part of an intercellular surface of hepatocyte (Figs. 2, 3 and 4). This groove was a half part of the bile canaliculus, i.e., the canaliculus was formed by conjunction of two grooves. The bile canaliculus often branched out into a dead end (Fig. 4). The bile canaliculus was 1 μ m in width on the average. Numerous short microvilli were found on the bile canalicular surface of hepatocytes. They were from 0.2 to 0.3 μ m in length. The intracellular bile canaliculi were found open in the intercellular bile canaliculi (Fig. 4).

Sinusoidal endothelial cell and the Kupffer cell

The sinusoidal wall consisted of endothelial cells. The endothelial cells were bulged into the sinusoidal lumen at the nuclear portion. The bulges became lower like a ridge of a range of mountains. The pits, measuring 0.1 μ m in the diameter and corresponding to the so-called pinocytotic invaginations, were scattered on the ridges. In the hollow between the ridges, numerous small fenestrations measuring about 0.1 μ m in the diameter were observed (Fig. 5). They formed a "sieve plate" as described by Wisse (1970). Occasionally, microvilli of hepatocytes protruded into the sinusoidal lumen through the fenestrations. A few large fenestrations, measuring 0.3 to 1.5 μ m in the diameter, were also scattered in the

endothelial cell (Fig. 6). Hepatocytes could be observed through the large fenestrations. The smaller and the larger fenestrations were distributed equally throughout the lobules. The endothelial cell usually overlapped the adjacent one at the junction (Fig. 7), or partial gaps were observed between adjacent endothelial cells (Fig. 8).

The Kupffer cells were situated in a large gap at the junctional part between two endothelial cells. They bulged remarkably in the sinusoidal lumen. Their surface was covered with numerous villous projections (Fig. 9). The Kupffer cells came into close contact with endothelial cells by their leaf- or thread-like projections (Fig. 10).

Fat-storing cell

The fat-storing cells were generally seen between hepatocytes and in the space of Disse. Their surface was rather smooth without any fenestration or villous projection. They branched out several flat and long processes (subendothelial processes) like tongues into the space of Disse, where the processes came into contact with the endothelial cells and the Kupffer cells. Ruptured cavities or casts of fat droplets were occasionally found in the processes of the fat-storing cell (Fig. 11). Furthermore, reticulin fibers were seen occasionally around the processes of the fat-storing cell (Fig. 12).

Reticulin fiber

Reticulin fibers were distributed in the space of Disse (Fig. 13) and between hepatocytes (Fig. 14). A mark, pressed by the fiber, could be occasionally observed on the intercellular and sinusoidal surface of the hepatocytes (Fig. 14). As stated already, the processes of the fat-storing cell ran with reticulin fibers (Fig. 12).

Interlobular bile duct

Numerous microvilli were seen on the luminal surface of the ductal epithelial cells. They were about $0.3\ \mu\text{m}$ in length and $0.1\ \mu\text{m}$ in diameter. The number of the microvilli, however, varied from cell to cell. It was easy to recognize the cell boundary because of a difference in distribution density of microvilli at the cell margin (Fig. 15). Besides microvilli, each cell had a central flagellum. The flagellum was about $5\ \mu\text{m}$ in length and $0.2\ \mu\text{m}$ in diameter.

DISCUSSION

Fawcett (1955) first described by TEM that the endothelial lining of the hepatic sinusoid is not continuous. Further studies have been published on the discontinuity of the endothelial lining. Concerning this discontinuity, there have been three different interpretations as follows; (1) the intercellular gaps at the junction between the endothelial cells (Bennett et al. 1959; Tanikawa et al. 1965), (2) the fenestrations of the endothelial cells (Ito and Shibasaki 1968; Wisse 1970;

Ogawa et al. 1973), and (3) both of the gaps and the fenestrations (Papadimitriou and Walters 1968; Orci et al. 1971). These interpretations were given on the basis of TEM investigation.

The present observation has elucidated both of the fenestrations and the junctional gaps of the endothelial cells of the sinusoids. The fenestrations have been recognized in most of the recent SEM studies using various species of animals; rats (Brooks and Haggis 1973; Motta and Porter 1974; Muto 1975; Grisham et al. 1975), guinea pigs (Itoshima et al. 1974), mice (Nopanitaya and Grisham 1975), monkeys (Vonnahme 1977), and humans (Muto et al. 1977). The gaps at the endothelial junction were also reported by Muto et al. (1975, 1977). The fenestrations could be divided into two types of the larger fenestrations and the smaller. Two investigators reported on the distribution of the larger fenestrations in the lobules in rats and guinea pigs. One reported that the larger ones are more numerous in the central area of the lobules in guinea pigs (Itoshima et al. 1974), and the other reported that they are more in the peripheral area of the lobules in rats (Grisham et al. 1975). The present observation could not detect such difference in the distribution of the larger fenestrations in mice. Both the larger and the smaller fenestrations were distributed equally throughout the lobules. The number of the fenestrations, however, seemed to be a little different according to the species of animal. For example, the number is smaller in mice than in rats (Nopanitaya and Grisham 1975).

Some substances have been thought to be able to pass through the fenestrations into the space of Disse or oppositely into the lumen of the sinusoid (Wisse 1970). For example, chylomicrons could be observed in the space of Disse and also just in the fenestrations (Ogawa et al. 1973; Naito and Wisse 1977). Consequently, the fenestrations have been generally assumed to function as a sieve or a filter (Wisse 1970; Motta and Porter 1974). In addition, the microvilli of hepatocytes protruded into the sinusoidal lumen through the fenestrations. Then, hepatocytes come into contact with blood stream in the sinusoid.

The Kupffer cells were formerly thought to be transformed from the endothelial cells (Tanikawa 1965; Imai et al. 1967; Ito and Shibasaki 1968; Carr 1968). However, the Kupffer cells revealed two characteristic SEM features. One is numerous villous projections which play an important role in phagocytic activity (Satodate et al. 1977). The other is that the Kupffer cells have no fenestrations which function as a filter. The Kupffer cells could be clearly distinguished from the endothelial cells by these two points. Furthermore, no transitional form between the two cells has been recognized in the recent TEM (Wisse 1972; Naito 1975) or SEM (Motta 1975; Muto 1975; Muto et al. 1977) studies.

The fat-storing cell was first described by Ito and Nemoto (1952). Argument once heated on the identification of the fat-storing cell; whether the fat-storing cell actually exists or not. However, the function of the fat-storing cells was known to be different from that of the Kupffer cells (Nakane 1963; Wake 1971). They are rich in fat droplets and relate to metabolism of vitamin A (Bronfenmajer et al.

1966; Ito and Shibasaki 1968; Kusumoto and Fujita 1977). As already mentioned in the findings of the present paper, the fat-storing cells were found between hepatocytes, and branched out the processes into the space of Disse. They never appeared in the sinusoid or on the endothelial lining. In addition, cavities were found occasionally in the fat-storing cell. These cavities were thought to be made after the intracellular fat droplets were ruptured and removed in the procedure preparing the specimen for SEM. It was another characteristic finding that reticulin fibers were often observed closely to the fat-storing cells. These characteristics were also recognized by other investigators (Bronfenmajer et al. 1966; Wake 1971). Furthermore, it was reported that the fat-storing cells were closely related to the intralobular fibrosis (Ito and Shibasaki 1968; Yamoto and Enzan 1975; Kent et al. 1977). Kusumoto and Fujita (1977) and Kent et al. (1977) showed that cells similar to the fat-storing cells of the liver were also in the mesenchymal space in other organs.

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References

- 1) Bennett, H.S., Luft, J.H. & Hampton, J.C. (1959) Morphological classifications of vertebrate blood capillaries. *Amer. J. Physiol.*, **196**, 381-390.
- 2) Bronfenmajer, S., Schaffner, F. & Popper, H. (1966) Fat-storing cells (lipocytes) in human liver. *Arch. Path.*, **82**, 447-453.
- 3) Brooks, S.E.H., & Haggis, G.H. (1973) Scanning electron microscopy of rat's liver: application of freeze-fracture and freeze-drying techniques. *Lab. Invest.*, **29**, 60-64.
- 4) Carr, I. (1968) Some aspects of the fine structure of the reticulo-endothelial system; the cells which clear colloids from the blood stream. *Z. Zellforsch.*, **89**, 355-370.
- 5) Fawcett, D.W. (1955) Observations on the cytology and electron microscopy of hepatic cells. *J. nat. Cancer Inst.*, **15**, 1475-1502.
- 6) Grisham, J.W., Nopanitaya, W., Compagno, J. & Nägel, A.E.H. (1975) Scanning electron microscopy of normal rat liver; the surface structure of its cells and tissue components. *Amer. J. Anat.*, **144**, 295-322.
- 7) Imai, Y., Sue, A. & Yamaguchi, A. (1967) Observation on the cytology and electron-microscopy of the hepatic sinusoidal cells. *Proc. Jap. Soc. RES*, **7**, 87-114.
- 8) Ito, T. & Nemoto, M. (1952) Über die Kupfferschen Sternzellen und die "Fettspeicherungszellen" ("fat storing cells") in der Blutkapillarenwand der menschlichen Leber. *Folia anat. jap.*, **24**, 243-258.
- 9) Ito, T. & Shibasaki, S. (1968) Electron microscopic study on the hepatic sinusoidal wall and the fat-storing cells in the normal human liver. *Arch. histol. jap.*, **29**, 137-192.
- 10) Itoshima, T., Kobayashi, T., Shimada, Y. & Murakami, T. (1974) Fenestrated endothelium of the liver sinusoids of the guinea pig as revealed by scanning electron microscopy. *Arch. histol. jap.*, **37**, 15-24.
- 11) Kent, G., Inouye, T., Minick, O.T. & Bahu, R.M. (1977) Role of lipocytes (perisinusoidal cells) in fibrogenesis. In: *Kupffer Cells and Other Liver Sinusoidal Cells*, edited by E. Wisse & D.L. Knook, Elsevier/North-Holland Biomedical, Amsterdam, pp. 73-82.

- 12) Kusumoto, Y. & Fujita, T. (1977) Vitamin A uptake cells distributed in the liver and other organs of the rat. *Arch. histol. jap.*, **40**, 121-136.
- 13) Motta, P. (1975) A scanning electron microscopic study of the rat liver sinusoid; endothelial and Kupffer cells. *Cell Tissue Res.*, **164**, 371-385.
- 14) Motta, P. & Porter, K.R. (1974) Structure of rat liver sinusoids and associated tissue spaces as revealed by scanning electron microscopy. *Cell Tissue Res.*, **148**, 111-125.
- 15) Murakami, T. (1974) A revised tannin-osmium method for non-coated scanning electron microscope specimens. *Arch. histol. jap.*, **36**, 189-193.
- 16) Muto, M. (1975) A scanning electron microscopic study on endothelial cells and Kupffer cells in rat liver sinusoids. *Arch. histol. jap.*, **37**, 369-386.
- 17) Muto, M., Nishi, M. & Fujita, T. (1977) Scanning electron microscopy of human liver sinusoids. *Arch. histol. jap.*, **40**, 137-151.
- 18) Naito, M. (1975) Electron microscopical study on the origin of Kupffer cells. *Proc. Jap. Soc. RES*, **15**, 76-82.
- 19) Naito, M. & Wisse, E. (1977) Observations on the fine structure and cytochemistry of sinusoidal cells in fetal and neonatal rat liver. In: *Kupffer Cells and Other Liver Sinusoidal Cells*, edited by E. Wisse & D.L. Knook, Elsevier/North-Holland Biomedical, Amsterdam, pp. 497-505.
- 20) Nakane, P.K. (1963) Ito's "fat-storing cell" of the mouse liver. *Anat. Rec.*, **145**, 265-266.
- 21) Nopanitaya, W. & Grisham, J.W. (1975) Scanning electron microscopy of mouse intrahepatic structures. *Exp. mol. Path.*, **23**, 441-458.
- 22) Ogawa, K., Minase, T., Enomoto, K. & Onoé, T. (1973) Ultrastructure of fenestrated cells in the sinusoidal wall of liver after perfusion fixation. *Tohoku J. exp. Med.*, **110**, 89-101.
- 23) Orci, L., Matter, A. & Rouiller, C. (1971) A comparative study of freeze-etch replicas and thin sections of rat liver. *J. Ultrastruct. Res.*, **35**, 1-19.
- 24) Papadimitriou, J.M. & Walters, M. N-I. (1968) Fluid flow in the liver demonstrated with horse radish peroxidase. *Amer. J. Anat.*, **123**, 475-488.
- 25) Satodate, R., Sasou, S., Oikawa, K., Hatakeyama, N. & Katsura, S. (1977) Scanning electron microscopical studies on the Kupffer cell in phagocytic activity. In: *Kupffer Cells and Other Liver Sinusoidal Cells*, edited by E. Wisse & D.L. Knook, Elsevier/North-Holland Biomedical, Amsterdam, pp. 121-129.
- 26) Tanikawa, K., Yoshimura, K. & Gohara, S. (1965) Fine structure of the reticulo-endothelial cells in the normal rat liver; morphological classification. *Kurume med. J.*, **12**, 139-147.
- 27) Tokunaga, J., Edanaga, M., Fujita, T. & Adachi, K. (1974) Freeze cracking of scanning electron microscope specimens. A study of the kidney and spleen. *Arch. histol. jap.*, **37**, 165-182.
- 28) Vonnahme, F.J. (1977) A scanning electron microscopic study of Kupffer cells in the monkey liver. In: *Kupffer Cells and Other Liver Sinusoidal Cells*, edited by E. Wisse & D.L. Knook, Elsevier/North-Holland Biomedical, Amsterdam, pp. 103-108.
- 29) Wake, K. (1971) "Sternzellen" in the liver; perisinusoidal cells with special reference to storage of vitamin A. *Amer. J. Anat.*, **132**, 429-462.
- 30) Wisse, E. (1970) An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. *J. Ultrastruct. Res.*, **31**, 125-150.
- 31) Wisse, E. (1972) An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. *J. Ultrastruct. Res.*, **38**, 528-562.
- 32) Yamamoto, M. & Enzan, H. (1975) Morphology and function of Ito cell (fat-storing cell) in the liver. *Proc. Jap. Soc. RES*, **15**, 54-75.

[*Illustrations follow*]

- Fig. 1. Hepatocellular plates and sinusoids in a centrilobular area. CV, central vein; HCP, hepatocellular plate; Sin, sinusoid. $\times 500$.
- Fig. 2. High magnification of the one-cell-thick hepatocellular plates. BC, bile canaliculus; HCP, hepatocellular plate; Sin, sinusoid. $\times 2,400$.
- Fig. 3. Intercellular surface of a hepatocyte. A bile canaliculus (BC), and pits (short arrows) and protrusions (long arrows) as the stud-like structures. Sin, Sinusoid. $\times 5,200$.
- Fig. 4. High magnification of a bifurcating bile canaliculus (BC). Numerous microvilli grow thick on the canalicular surface. Openings (arrows) of the intracellular bile canaliculi are seen. A branch of the canaliculus, seen in the left side of the picture, ends blindly. HC, hepatocyte. $\times 10,000$.

- Fig. 5. Endothelial surface of the sinusoid. Sinusoidal endothelial cells possessing numerous small fenestrations, and a few microvilli of hepatocytes protruding through the fenestrations (short arrows) are seen. Several pits (long arrows) are scattered on the cytoplasmic ridges. D, the space of Disse. $\times 9,000$.
- Fig. 6. Larger fenestrations (arrows) and numerous smaller ones in an endothelial cell. D, the space of Disse, HC, hepatocyte. $\times 7,300$.
- Fig. 7. An endothelial cells seen in the lower part of the picture overlaps its adjacent endothelial cell in the upper part. $\times 13,000$.
- Fig. 8. Two gaps (arrows) at a junction of the endothelial cells. The junctional overlapping is seen. However, the overlapping is not the same on the left side and the right side. The cell seen in the upper part of the picture overlaps the cell seen in the lower on the left side. On the right side, the cell in the lower overlaps the cell in the upper. $\times 14,000$.

- Fig. 9. The Kupffer cell (KC) is bulged in the sinusoidal lumen (Sin). Numerous villous projections are seen on its surface. HC, hepatocyte. $\times 5,500$.
- Fig. 10. The Kupffer cell (KC) contacting with an endothelial cell (EC) by leaf and thread-like projections. The Kupffer cell is situated in the gap revealed at the endothelial junction. No endothelial lining is seen under the Kupffer cell. $\times 14,000$.
- Fig. 11. Fat-storing cell (FSC), the surface of which is rather smooth, shows ruptured cavities of fat droplets (arrows). HC, hepatocyte; Sin, sinusoid. $\times 4,000$.
- Fig. 12. Fat-storing cell (FSC) between hepatocyte (HC) and endothelial cell (EC). It branches out processes (P). A bundle of reticulin fibers (RF) and some smaller bundles are seen beside the fat-storing cell. $\times 5,500$.

- Fig. 13. A bundle of reticulin fibers (RF) running in the space of Disse. HC, hepatocyte; Sin, sinusoid. $\times 2,000$.
- Fig. 14. A mark (arrow) of a bundle of reticulin fibers on the intercellular surface of hepatocyte (HC). The bundle is ripped away. Sin, sinusoid. $\times 2,000$.
- Fig. 15. Luminal surface of the interlobular bile duct. Numerous short microvilli and a central flagellum are seen on each ductal epithelial cell. Each cell or the cell boundary can be recognized by the different distribution of the microvilli at the cellular margin. $\times 5,500$.