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G. Macchiarelli
University of Rome

P. M. Motta
University of Rome

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THE THREE-DIMENSIONAL MICROSTRUCTURE OF THE LIVER
A Review by Scanning Electron Microscopy

G. Macchiarelli and P.M. Motta*

Department of Anatomy, University of Rome
Italy

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Abstract

The improvement in scanning electron microscopy (SEM) techniques has permitted us to describe the microstructure of the liver. By SEM,the liver peritoneal surface is composed of flat mesothelial cells possessing microvilli and cilia. Hepatic sinusoids connect the portal vessels with the terminal branches of the hepatic vein (central veins).Endothelial cells of the portal space arteries are elongated and arranged longitudinally, while those of the central and portal veins are polygonal and flattened,possessing microvilli. The sinusoidal endothelial cells show both small fenestrations (sieve plates),up to 200 nm in diameter, and large ones,up to 1 μm. Within the sinusoids are seen bridging structures,covered by fenestrated endothelium,seeming to have a fibrillar core. Kupffer cells resemble macrophages,showing microvilli, blebs, lamellipodia and filopodia. Within the Space of Disse are seen the fat-storing cells,having laminar dendritic projections. The polyhedral liver cell faces the Space of Disse (vascular pole) or faces an adjacent hepatocyte (biliary pole). Vascular facets are evenly covered by microvilli. Biliary facets show a central longitudinal depression,bordered by microvilli (bile hemicanaliculi). Canaliculoductular junction and bile duct epithelia show blebs, microvilli and cilia. Up to now,fetal liver and liver pathology have been scarcely investigated by SEM: in the future, they can be successfully approached by three-dimensional studies.

KEY WORDS: Liver, Sinusoid, Sinusoidal Cells, Hepatocyte, Biliary System, Pathology, Fetal, Scanning Electron Microscopy.

*Address for correspondence:
P. M. Motta, Department of Anatomy, University of Rome, via A. Borelli 50, 00161 Rome, Italy.
Phone No. (39-6) 49.26.23.

Introduction

The structure of the liver reflects its extremely complex function. In fact, the liver can be considered as both exocrine and, to some extent, endocrine in nature (81). In addition, it metabolizes, detoxifies and inactivates many different substances of both endogenous and exogenous origin (46). It further plays an important role in the hemodynamic regulation of the splanchnic system.

When the first microscopists began studying the histological organization of the liver, it immediately appeared clear that a visualization of its three-dimensional structure was fundamental to unravelling its complex functional mysteries. Several ingenious attempts to draw the liver's three-dimensional pattern have been made (7, 15-17). Only in the second half of this century, however, through careful studies using stereological methods and statistical analysis of transmission electron microscopic (TEM) pictures (18, 126, 145), has it been possible to confront the heterogeneous complexity of the hepatic gland. The limits of these techniques have been recently reviewed (73) and, at the present time, scanning electron microscopy (SEM) seems to be the technique of choice for three-dimensional studies. Thus, after more than 15 years since the first SEM reports of mammalian liver lobule (4, 23), so many SEM liver images have been obtained, that we feel confident in our description of the "microstructure of the liver" (73). In fact, SEM has been extensively used to obtain data from the livers of many mammalian species including humans, and other animals, such as birds and fish (see Table 1).

Most of this literature will be reviewed in this paper. Further, new SEM images of sinusoids from an unexplored rodent (Praomys Mastomys Natalensis) will be presented.

Table 1.

SOURCE OF LIVER TISSUE FOR SEM INVESTIGATIONS.
(Bibliography update 1985)

Source	Bibliography
CAT	62; 69-72; 81.
CHICK	113.
DOG	69; 70.
FISH	97.
FROG	111.
HUMANS	27; 36-40; 44; 62; 76; 81; 89; 91; 134; 136-138; 153; 160; 161.
PIG	62; 70; 71.
PRIMATES	
Baboon	57.
Monkey	87; 108; 133; 135; 139.
RODENTS	
Guinea-Pig	35; 62; 69-71.
Hamster	59; 160.
Mouse	27; 69-71; 92; 121; 130; 141.
Rabbit	27; 105; 111; 125; 158.
Rat	8; 9; 11; 14; 21; 22; 26; 27; 30; 34; 41; 43; 45; 51; 54; 55; 62; 65; 67-72; 74-79; 81; 86; 88; 90; 93; 94; 99; 101; 112; 114; 115; 123; 130-132; 142; 144; 149; 151-154; 158; 160; 162.
SHEEP	157.
FETAL	
animals	71; 72; 76; 113; 157.
humans	58; 71; 76.

Preparation of Liver Samples for SEM

The improvement in electron microscopic techniques during the past 15 years has permitted us to obtain optimal liver specimens for SEM investigation with relatively simple procedures. In order to avoid artifacts and to yield a clean surface for observation, however, it seems imperative to respect certain crucial points during the preparation of samples (27, 81).

In order to obtain good preservation of hepatic subcellular structures, fixation must be performed by vascular perfusion (148). In fact, immersion primary fixation alone does not adequately avoid cellular anoxic damage, does not preserve endothelium (150) and does not display a homogeneous fine structure in the whole sample (119).Furthermore, immersion fixation may create spatial artifacts due to the non-homogeneous hardening of the liver tissue and it may also cause the disappearance of delicate structures such as endothelial fenestrae (156, 157).

Primary fixation is usually performed with solutions of 1-3% glutaraldehyde in cacodylate or phosphate buffer. Paraformaldehyde has also been used, alone (4% in phosphate buffer), or with glutaraldehyde (1.5 - 4% in phosphate buffer). No side effects have been demonstrated when the aldehyde phase of fixation has been prolonged. This suggests that the samples may also be left in the primary fixative for a long period in order to obtain optimal tissue fixation and hardening (5).

In a blood-rich organ such as liver, it is necessary to accurately wash out all blood from the vascular bed prior to fixation (81,116). In this way, precipitation of plasma proteins which obscure vessel surfaces, can be avoided (27). Several washing solutions have been used but no data can be referred as to which is the solution of choice.

Both the addition of anticoagulants (heparin) or vasodilatants (procaine), to infusates, and the injection of either during anesthesia improves the quality of perfusion, which can be monitored by noting the change in liver colour from a dark-red to a sandy-beige (116).

Some authors suggest the maintenance of infusates at body temperature in order to mimic the physiological environment and to enhance the speed of fixative penetration (5). On the other hand, the use of lower temperatures may reduce anoxic damage.

Attention must be paid to the infusion pressure (153). In fact, the use of vasodilatant drugs and aldehyde fixation through the arterial circulation, has the effect of converting the blood vessels into a rigid pipe system, causing loss of the arteriolar "barrage" (22). Thus, the infusates may reach the postarteriolar vessels (sinusoids) with a pressure higher than physiological values, which range from 6 to 10 mmHg in the mammalian portal system. In addition, there is evidence that a high infusion pressure of infusates, whether injected via the portal vein or via the arterial system, is associated with an increase in the number of larger gaps in the sinusoidal endothelium (21). Considering certain properties of endothelium, such as filtering and sieving, these larger gaps have been also described as artifacts (155) or pathological features (21).

In animal experimentation, perfusion preferably is performed via the portal vein, but may also be done via the aorta, or directly via the left ventricle. Biopsy samples also have been perfused, through the introduction of a small needle into the parenchyma (puncture-perfusion) (38,40,86,134,137) or through cannulation of the cut vessels (58,76,81).

Osmication is not always required for SEM. In fact, it doesn't seem to offer any advantage during observation of liver samples (133). However, the metal-impregnation technique with tannin-osmium may enhance the conductivity under the electron beam of non-coated specimens (84,85).

Dehydration can be carried out gradually, either with ethyl alcohol or acetone (81), followed by critical-point drying (see Howard and Postek, 1979 for further references) (28). Freeze-drying also has been performed, and is advantageous in exposing more of the surface area (8), but it seems inferior to critical-point drying in high-magnification SEM (98).

Samples are usually coated with gold or gold-palladium; in our experience, carbon-gold also gives good results (74).

The surface tissue exposure is of particular importance. Several techniques have been suggested. Methods such as mechanical tissue dissociation by digital pressure (performed in dried samples) (55,99,157), tissue dissociation with jewelry forceps after cutting with a sharp razor blade (both in wet or dried samples) (81), freeze-fracture (in alcohol-infiltrated or critical-point dried samples) with freon or liquid nitrogen (8,29,30,81,128), all have valuable applications in delineating different structures. Thus, internal hepatocytic organelles can be well visualized in freeze-fractured dried samples (81) and bile hemicanaliculi are exposed after simple cutting or fragmentation of wet tissues (75). Isolated hepatocytes (from mechanical-dissociated dried samples) can be collected on a double-face tape placed on a stub (81). Hepatocytes can also be studied after isolation by elutriation and culture (114,142). In addition, intracellular hepatocytic components have been exposed by cracking of resin-infiltrated samples (125), by fracturing of tannin-osmium treated samples (36, 39), by chemical dissociation with boric acid (130), or by the osmium-DMSO-distilled water method (31). The hepatocyte cytoskeleton has been recently studied after treatment with Triton-X 100 (112).

Liver vasculature can also be studied well by vascular corrosion cast methods (for details about this technique see Ohtani et al, 1983 and Lametschwandtner et al., 1984) (53,111) that visualize an exact reproduction of vessel disposition in space (3,83,160). In addition, the outer sinusoidal wall and adjacent intercellular spaces may be investigated in resin-cast non-corroded tissue specimens (12).

Always, all of these methods should be used in preparing liver samples, because they complement each other.

Finally, we believe that it is extremely useful to obtain stereo pictures during SEM observation, to allow a better understanding of the three-dimensional relationships of liver cells (73).

The Peritoneal Liver Surface

The external surface of the liver is almost completely covered by a monolayer of serosal cells that rest with the basal lamina on a variable amount of connective-tissue fibres, forming the fibrous capsule (73).

The fine granules and filamentous structures, intermingled with fibroblasts and collagenous fibrils of the fibrous capsule, can be easily recognized by means of SEM in chemically dissociated tissue (31) or through breaks of the peritoneal layer. Further, in vascular corrosion casts, Glisson's capsule, which follows the hepatic vessels into the liver parenchyma, has a poor capillary network supplied by arterioles from the branches of the hepatic arteries and emptying into the sinusoids 109).

It is demonstrated by SEM that the peritoneal sheath which is absent only within the area of the liver-diaphragmatic attachment (46), is flat, and is seen as such in samples fixed by the perfusion technique (Fig. 1). But this sheath appears corrugated when the samples are directly placed in the fixative (Fig. 2). This is probably due to a reactive contraction of the underlying cells, such as smooth muscle cells.

Serosal cells, which, when examined by conventional electron microscopy, appear coupled together by junctional complexes (73) and covered by a highly positive ruthenium-red coat (glycocalyx) (56,68), are flattened and polygonally shaped, often populated by abundant microvilli of 1-2 μm in length when examined by SEM (Fig. 1) (81). Observed at high magnification, microvilli show granules and filamentous structures (Fig. 3) which are thought to correspond to material derived from glycocalyx or serosal exudate (68). The pits and the blebs that are often detected on the serosal surface may be considered as signs of the surface activity of the serosal cells. In fact, mesothelial cells are involved in the processes of secretion, absorption and exchange of fluids which may act as surface lubricants (1,2, 73).

Furthermore, the serosal cells covering the liver, like those seen in other organs, possess a single cilium of variable length (Fig. 4). The cilium might represent some rudimentary structure, or it might have some chemoreceptorial or motile function (80,129). In any case, the role of these cilia is still to be clarified.

Liver Architecture and Vasculature

The Lobules and Acini.

Modern SEM techniques permit us to fully appreciate the complex microarchitecture of the liver gland (73). The liver is supplied by an extraordinary number of nutritive and functional vessels (hepatic arteries and portal veins). Such vessels contribute to create a three-dimensional labyrinthic structure (72), basically characterized by the morpho-functional tissue unit (117) called the lobule or "acinus", as Wepfer (146) and Malpighi (61) first supposed. As recently reviewed (73), the three tissue units of the hepatic architecture represent different ways of looking at the same structure (46): the classic lobule of Kiernan (50), the portal lobule of Mall (60) and the liver acinus (118) emphasize peculiar aspects revealed in different mammalian species according to the morphophysiological condition considered. The smallest hepatic functional unit seems to be the "acinus hepaticus" (117), and it offers explanations for many histopathological liver changes (46). The classic lobule that can be called, in some instances, an endocrine lobule (if the direction of the blood flow is considered) and the portal lobule that can be called an exocrine lobule (when one considers the direction of the bile flow) (81) are more easily recognized in tissue preparations by light or electron microscopy (72).

When fractured liver samples from properly perfused animal livers are examined by SEM, hepatic lobules can be identified as irregular polyhedral structures (Fig. 5) with a centrally located terminal hepatic vein or portal triad, which are surrounded by the hepatic laminae (26, 45,49,81,92,139).

Different aspects can be noted in the arrangement of the hepatic laminae. This seems to be correlated with the changes in the blood-pressure gradient along the ramifications of the hepatic veins producing a plastic deformation of the liver laminae which may temporarily alter their location in space. Thus, the observer may have the impression that the hepatic laminae radiate from the portal triad (portal lobule or acinus) or that they converge toward a central vein (classic lobule) (81).

Vessels within the Portal Space.

Arterial and venous vessels within the portal space present remarkable differences in their wall structure and endothelial surfaces (73).

In cross sections vascular components appear close to the lymphatic vessels and the bile duct, surrounded by connective tissue fibres that also delimit spaces for the interstitial fluids (Fig. 6) (26,81).

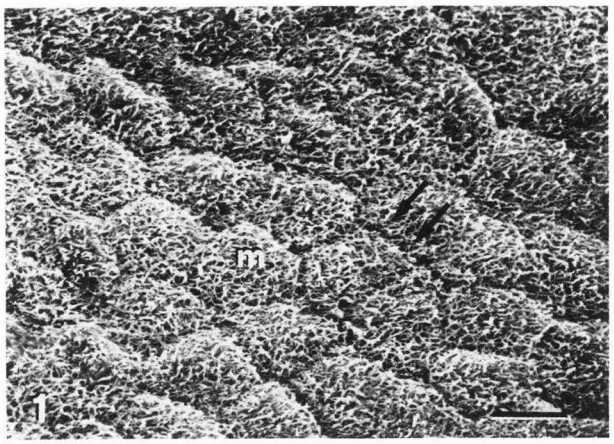
The branches of the hepatic artery show a thick wall formed by several layers of smooth muscle cells . Arterial endothelium is arranged in longitudinal columns running along the major axis of the vessels. Endothelial cells are elongated, with a slight central (nuclear) protrusion. Their surface is smooth, but, occasionally, microvillous projections and blebs can be detected. Endothelial cell margins easily can be recognized, and sometimes there is overlapping of the borders of adjacent cells (Fig. 7).

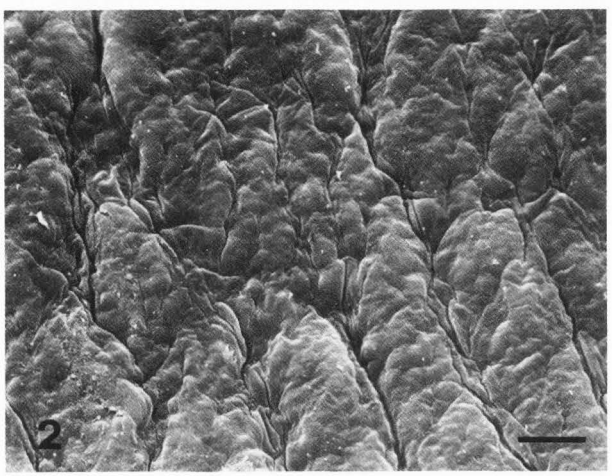
The portal veins, as a rule, are larger than arterial vessels, but their walls are thinner, presenting one or two layers of smooth muscle cells. Venous endothelial cells are polygonally shaped or elongated. On their flat surface microvillous projections and pits are often observed. Occasionally, outlets of smaller vessels and sinusoids can be seen (Fig. 8).

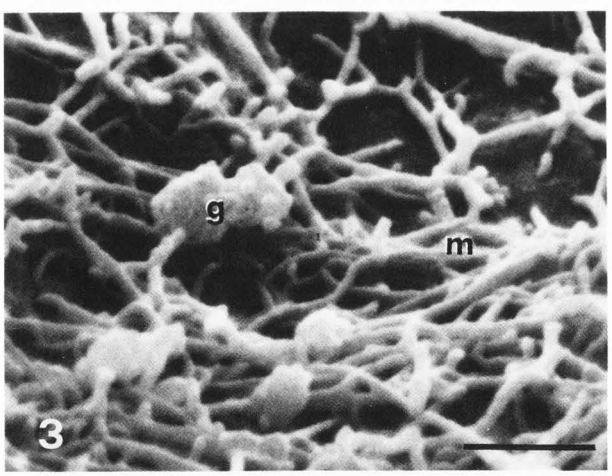
By means of vascular corrosion cast methods, SEM has revealed a peribiliary capillary plexus supplied by the branches of the hepatic arteries of the portal space (87,108). These vessels anastomose sinusoidal channels or portal veins (96, 109). These two kinds of efferent branches of the plexus have been described in human and rabbits with the same frequency. On the other hand, the peribiliary plexus is drained mostly by portal veins in the rat, and by sinusoids in the monkey (87,109). This type of "portal" vascular system, considering its close connection with the bile ducts, could play a role in the intralobular feedback control of bile production (81,87,105, 110).

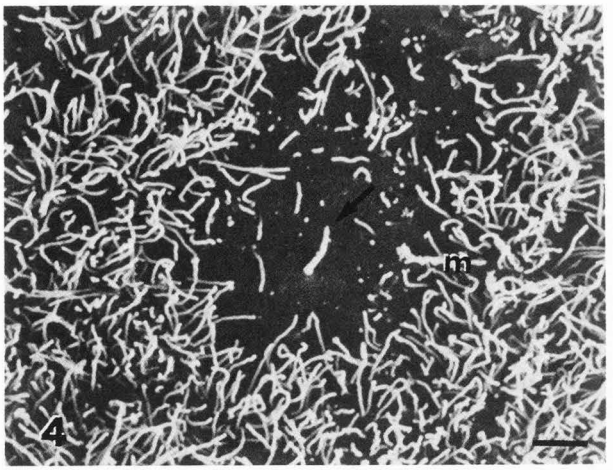
Terminal Hepatic Veins.

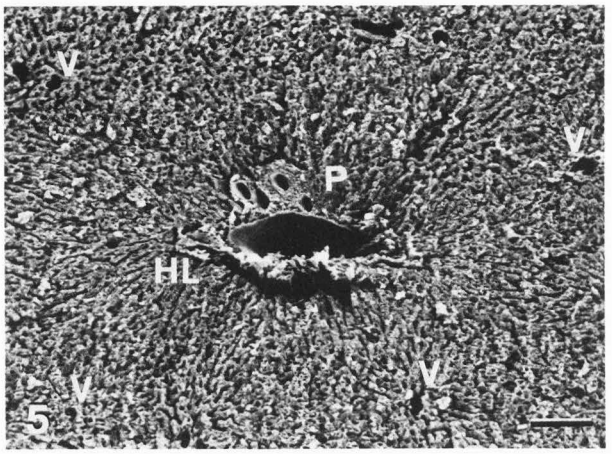
Terminal hepatic veins characteristically may be observed, in cross sections, at the center of converging hepatic laminae of the classic lobule (central veins) (Fig. 9). They present a wall mostly composed of the intimal lining, resting on a very thin muscular layer. The vascular lumen shows numerous sinusoidal openings often crossed by bridging structures (151). These bridges, sometimes seen trapping blood cells (Fig. 10), are formed by a collagen core covered by fenestrated endothelium (151). The fenestrated endothelium even may be recognized around the sinusoidal outlets for a small area (Fig. 10). Endothelial cells have a polygonal shape. The rounded nucleus is clearly seen to bulge in the central area of the cells, whose borders are easily detectable (Fig. 11). Leukocytes and Kupffer cells are frequently adherent to venous endothelium (Fig. 11). The different hemodynamic and structural patterns of these vessels (high pressure and thick wall in arteries; low pressure and thin wall in veins) may be responsible for the above-mentioned ultrastructural endothelial











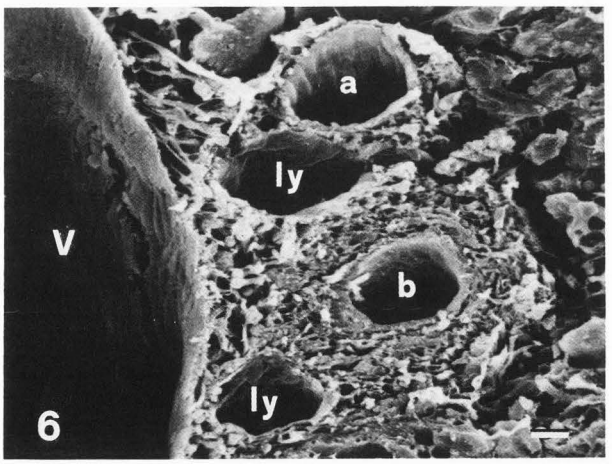


Fig. 1 The serosal cells covering the surface of the liver are populated by numerous microvilli(m) Arrows delineate cell borders (Bar = 10 μm). Rat.

Fig. 2 Liver surface appears corrugated in samples fixed by immersion (Bar = 10μm). Hummingbird.

Fig. 3 High magnification reveals granules (g) adhering to microvilli (m) of mesothelial cells (Bar = 1 μm). Mouse.

Fig. 4 An isolated cilium (arrow) arising from the serosal surface of the liver. (m, microvilli) (Bar = 1 μm). Cat.

Fig. 5 Mammalian portal lobule.(P, portal space; V, terminal hepatic vein; HL, hepatic laminae) (Bar = 100 μm). Rat.

Fig. 6 Portal area. (V, branch of portal vein; a, artery; b, bile duct; ly, lymphatics) (Bar = 10 μm). Rat.

differences. The deforming transmural pressure indeed may stimulate vessels with a highly reactive musculature (such as arteries) that respond with a functional contraction, creating the longitudinal folding of the arterial endothelium.

Sinusoids, Sinusoidal Cells and Space of Disse.

As seen by SEM, in fractured samples, as well as in corroded vascular casts, the liver "sinusoidal" network anastomoses the portal vessels with the terminal branches of hepatic veins (26,48,68,73,106,107).

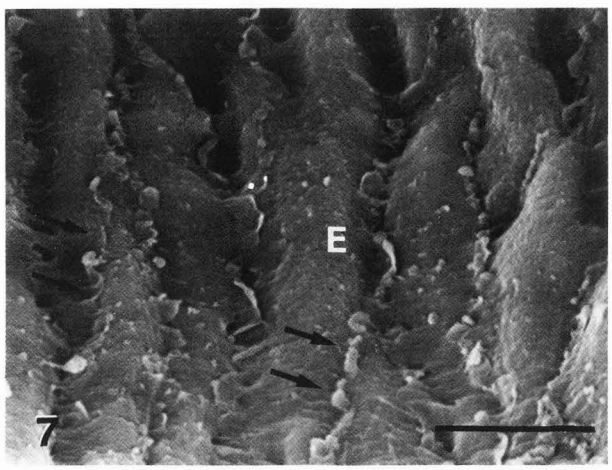
A flat fenestrated endothelium, forming the sinusoidal wall, separates the capillary lumen from the perivascular interstitial spaces (perisinusoidal space of Disse) (Fig. 12) (72,88, 148,). Sinusoidal cells are the cellular elements populating the sinusoids and the Space of Disse (6,73,103,104).

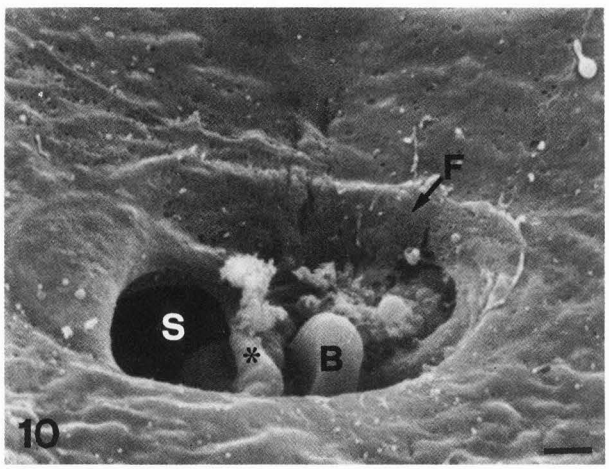
Three principal sinusoidal cell-types have been morphologically identified by SEM-TEM studies: the endothelial cell , the Kupffer cell and the perisinusoidal stellate cell. The latter is also called "Ito cell" or "fat-storing cell" (32, 140). Others types of perisinusoidal cells have been reported, such as "pit cells" (6,47,149) and "pericytes" (97), but through employment of SEM, it is difficult to adequately detect their surface features.

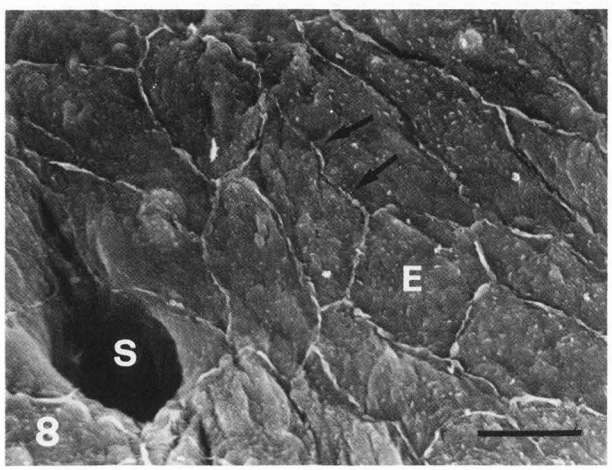
In perfusion-fixed samples, endothelial cells are flattened. Their only protrusion is seen over the nuclear region. Sinusoidal endothelial cells are of an extremely variable size, and they possess several cytoplasmic openings evenly distributed over their extensive cytoplasmic processes (Fig. 12) (71,78,79). These openings, or "fenestrae", permit a communication between Disse's space and the sinusoidal lumen. Dimensions and distribution of fenestrae have been extensively studied (26,27,35,66,79,89,127, 132,152-155,157,158). Two principal types of openings are usually described (35,73): small fenestrae (100-200 nm), often clustered, forming the so-called "sieve plate" (148); and large fenestrae (up to 1 μm) (Fig. 12).Larger gaps generally are considered as artifacts or as a result of some toxic agent (21,22,57,81,94,150). This is supported by the increased number of large fenestrations under particular experimental and/or pathological conditions (21,22,72,79). On the other hand, endothelium should not be considered as a rigid structure, but as a dynamic one (67, 81,139). Its morphology changes from organ to organ (120) and even within the same organ, adapting its ultrastructural features to several different pathological and physiological conditions. According to such considerations, larger fenestrae may be an expression of temporary injuries that may involve both endothelial and Kupffer cells, whose long cellular projections can also be seen penetrating and enlarging the endothelial fenestrations (70,81). Endothelial dynamism also can be proven by our recent SEM observations on liver of the rodent, Praomys Mastomys Natalensis. In this animal, intrasinusoidal bridges arising from endothelium are often noted (Fig. 13). By SEM, these bridges (up to 4 μm in length) appear to be covered by fenestrated endothelium, and they probably possess a collagen core (Fig. 14). Considering their intraluminal location, their possible role may be related to intrasinusoidal regulation of the blood flow, a role similar to that which the long cytoplasmic projections of the Kupffer cells are thought to play (73).

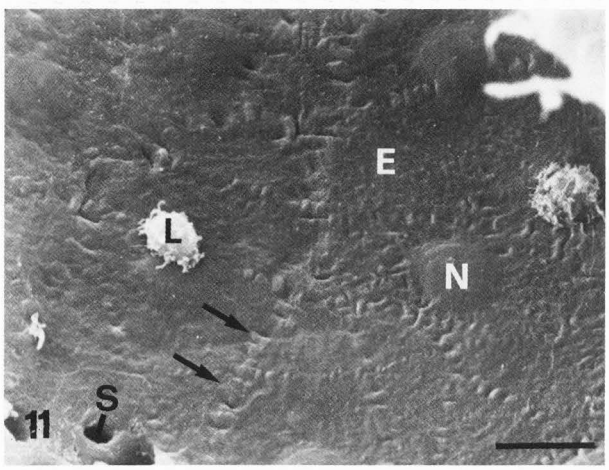
The three-dimensional aspect and the location of the Kupffer cells are best revealed in stereo SEM pictures (Fig. 15) (70,81). These cells have an irregular cell body, which, depending upon the activation of the cell, appears to be provided with a variable number of blebs, microvilli, holes, lamellipodia and filopodia (26, 35,51,69,70,79,89) that are characteristic features of macrophages (70,121). Kupffer cells, through their cell bodies and/or cytoplasmic processes, are in contact with endothelial cells (Fig. 16) (42,69,124), with which they may form junctional complexes (33). Blood cells are also frequently seen associated with the Kupffer cells (Fig. 15), possibly as an expression of the immunological role of liver macrophages . Further, Kupffer cell projections may, in some instances, create a kind of intrasinusoidal micro-labyrinth that may play a role in the regulation of blood flow (70).

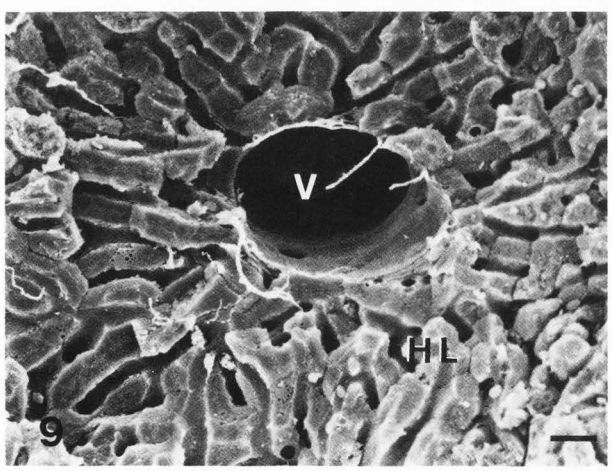
Ito cells, fibroblasts and fibrocytes are the elements populating the space of Disse. These cells, which easily can be differentiated in TEM sections, are intermingled within a delicate network of fibrils and collagen fibers supporting the endothelial lining (73). The fat-storing cells (perisinusoidal stellate cells or Ito cells) may be considered as vitamin A-storing lipocytes (140). In SEM they can be identified through the larger gaps of sinusoidal endothelium, possessing numerous laminar dendritic processes, and rarely, microvillous projections (Fig. 17) (34,44,81,136). Occasionally, a single cilium floating in Disse's space, or emerging through a sinusoidal gap into the vascular lumen, can be recognized (81,104). The function of the fat-storing cells appears related to the metabolism and storage of vitamin A, as documented by the large increase of these elements in Vitamin A-treated animals (30, 52,140), and also by the features they have in common with similar stellate vitamin A-storing cells found in other











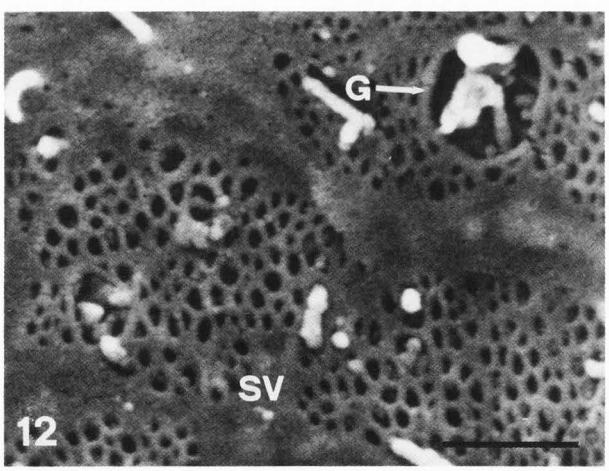


Fig. 7 Endothelium of the hepatic artery in the portal space. (E, endothelial cell; arrows, cell limits) (Bar = 10 μm). Rat.

Fig. 8 Endothelium of the portal vein of the portal space. (E, endothelial cell; S, sinusoidal opening) (Bar = 10 μm). Rat.

Fig. 9 Central vein (V) surrounded by hepatic laminae (HL) (Bar = 10 μm). Rat.

Fig. 10 A sinusoidal opening in the central vein, endothelial fenestrations (F) can be recognized. (*,bridge; B, red blood cell). (Bar = 10 μm).Rat.

Fig.11 Endothelium of the terminal hepatic vein.(N, nucleus; E, endothelial cell; L, lymphocyte; arrow, cell limits; S, small sinusoidal opening). (Bar = 10 μm). Rat.

Fig.12 Fenestrated endothelium.Sieve plates (SV) and large fenestrae (G) of sinusoidal endothelium (*,microvilli of the hepatocyte).(Bar= 1 μm).Rat.

organs (73,140). These data demonstrate that sinusoidal cells should be considered morphologically as three distinct cell types whose respective roles seem to be somewhat integrated. In fact, sinusoidal cells have an intimate spatial relationship, and this seems to further represent coordination of certain activities carried out within the sinusoidal spaces, including filtration and discernment of blood substances. Thus, the mechanical sieving activity of the endothelial sieve plates (148) may be enhanced by the anatomical barrier created within the sinusoids by the Kupffer cell prolongations. In this way the Kupffer cells contact blood elements and substances, exerting a phagocytic, and/or immunological role. Such functional complementarity of sinusoidal cells also may be suggested by experimental studies involving treatment of cholesterol remnants that reach hepatic sinusoids. Indeed, endothelial cells and Kupffer cells may work together in recycling or removing cholesterol remnants that, when too large, cannot pass through the endothelial fenestrae (14,20,21,158) or are phagocytized by Kupffer cells (147).

Liver Parenchymal Cells and Intrahepatic Biliary Tree.

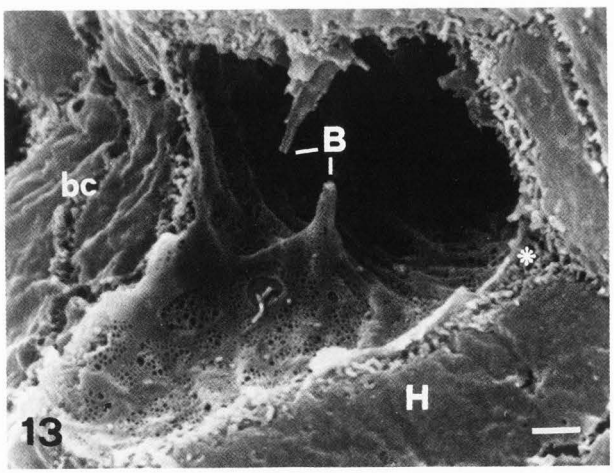
The hepatic parenchymal cell is able to carry on its various functional activities simultaneously due to the close spatial relationship that this cell shares with both the vascular compartment and biliary system. Such morphological relations can be successfully studied by SEM (73). In fact, SEM clearly demonstrated that the physiological polarity of the hepatocytes corresponds to an anatomical polarity (46,81). By SEM, the hepatocyte appears as a polyhedral cell with 6 or more facets, possessing vascular poles facing the perivascular, interstitial spaces and biliary poles delimiting the bile canaliculi (Fig. 18) (75,82). Hepatocytic facets may have various morphological appearances in relation to their different functional polarity (19). The vascular facets, observable in isolated hepatocytes or through the larger sinusoidal gaps, are richly covered by short microvilli (Fig. 19) (77) which considerably increase the membrane surface area destined for the absorption and treatment of the interstitial fluids (73). Biliary facets, having a different functional polarity, have different features. In properly fractured samples, biliary facets present a centrally located longitudinal depression (width: 0.5 μm in the central areas of the lobule; up to 2.5 μm, near the periphereal area) (73) that runs along the entire cell surface (75,135). These channels (bile hemicanaliculi) are bordered by short, thick microvilli (Fig.20). Bile canaliculi may possess branches and lateral sacculations (75). In stereo views, the canalicular bed appears to be provided with numerous microvilli, large holes and small diverticulae that may be the intracellular sacculations observed in TEM sections (73,75).

Both surfaces at the canalicular sides are relatively smooth. Such smooth bands (0.1-0.4 μm width) are the areas of adjacent hepatocytes' junctional attachments; the latter represent the anatomical barrier between vascular and biliary compartments. It is believed that where these barriers are very narrow, bile regurgitation may occur under experimental or pathological conditions (73,90). Protrusions and holes also can be distinguished on these bands. The former together may serve as an additional system of attachment for adjacent hepatocytes, as well as for sealing bile canaliculi (73,81).

Bile canaliculi become wider (1-2.5 μm) near the portal zones, where they may be seen emptying into the intralobular ductules of Hering (62,38). Two kinds of connections of bile canaliculi with bile ductules (canalicular-ductular junctions) are described (41). Bile canaliculi usually empty into bile ductules, forming an ampulla just before the junction. Sometimes, bile canaliculi may also be connected directly with the bile ductules without any sacculation or dilatation. The ductule's wall is made of a few epithelial cells joined to converging hepatocytes (37,62,81). Numerous microvilli and, rarely, long cilia arising from epithelial cells, are also described (27,74,).

Ductules empty bile directly into the biliary ducts located in the portal space (Figs. 5-6). The epithelial cells forming the bile duct wall possess microvilli and cilia (37). Microvilli are characteristically disposed in longitudinal rows (62,81) that, in the larger ducts, seem to delimit hexagonal profiles corresponding to the cell borders (Fig. 21). Almost all epithelial cells of the larger ducts show a centrally located cilium on their surface (Fig. 21). Luminal holes (0.1 μm diameter) are present in the smallest bile ducts. These openings represent the inlets of bile canaliculi that occasionally may empty directly into biliary ducts without ductular passage (62).

Intracellular components of the liver parenchymal cells are poorly studied by SEM (36,81, 112,125). In fractured hepatocytes, the nucleus appears spherical and centrally located (81) (Fig. 22). Nuclear pores have been demonstrated in chemically dissociated tissues (36). When examined three-dimensionally, other cytoplasmic organelles and inclusions appear evenly dispersed throughout a network of fibrous tubular



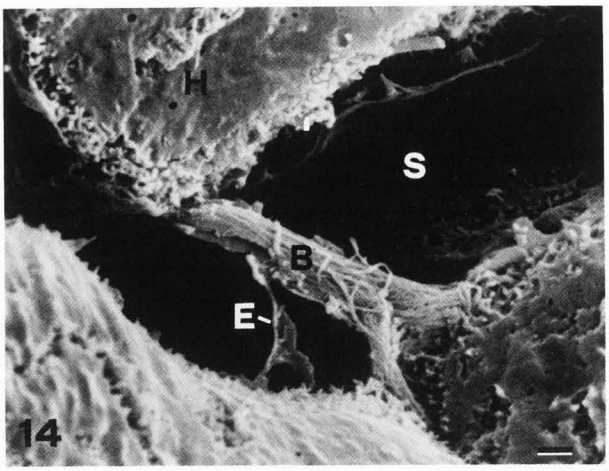
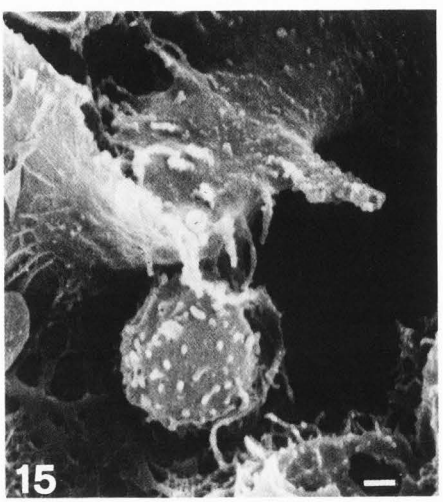
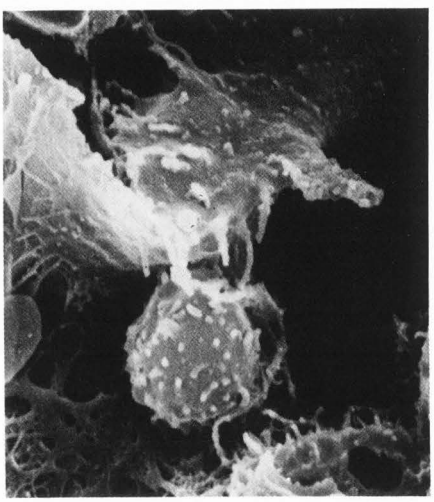
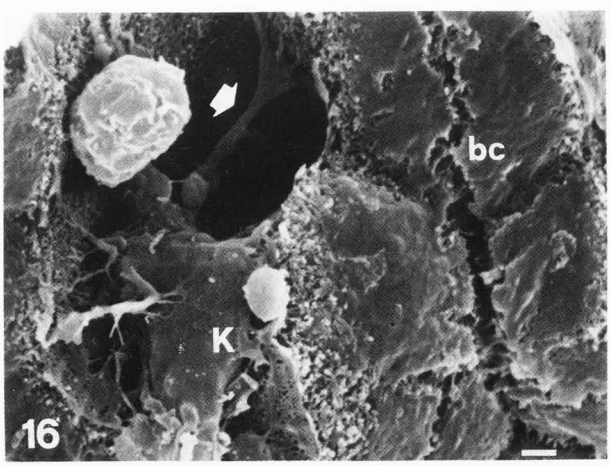


Fig. 13 An endothelial bridge (B) covered by fenestrated endothelium,within a sinusoid.(*,space of Disse; H,hepatocyte; bc,bile canaliculus. Fig. 14 A bridge where endothelium (E) has been removed.Note the fibrillar composition of this structure.(S,sinusoid; H,hepatocyte). (Bars = 1 µm). Praomys.

Fig.15 A stereo pair showing the intimate relationship between a Kupffer cell and a lymphocyte. (Bar = 1 µm). Rat.







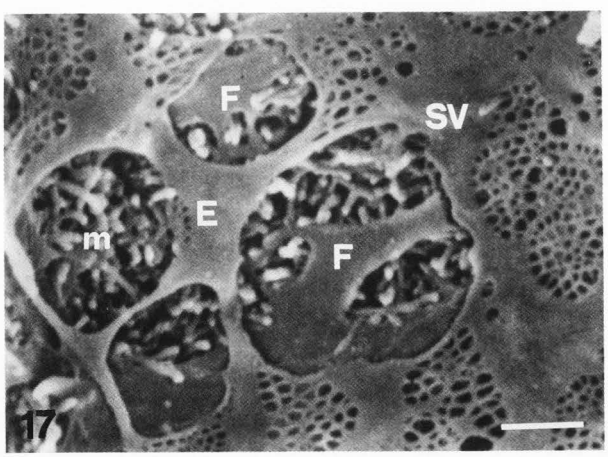


Fig. 16 Within a sinusoid is present a Kupffer cell (K) having a long cytoplasmic extension (arrow). (bc, bile canaliculus). Fig. 17 Through an artifactual gap of the sinusoidal endothelium it is possible to see the laminar dendritic processes of a fat-storing cell (F) located in the space of Disse. (SV, sieve plate; m, microvilli of the hepatocyte). (Bars = 1 µm). Rat.

components (in cracked resin-infiltrated samples) (125) or filamentous structures (as recently evidenced in liver perfused with Triton-X100) (112). In any case, identification and classification of intracellular structures and organelles, along with their three-dimensional relationships, will be better clarified in SEM-TEM (freeze-etching) correlated studies.

Future Applications of SEM in Hepatology

The actual three-dimensional arrangement of the liver's cells, as well as of the cells of many other organs, has been revealed by SEM (24,49, 80,81). Normal liver structure easily can be studied by comparing SEM data with data collected through employment of other morphological techniques, including morphometrical analysis (73). The achievement of this primary step will be extremely useful for the development of other biological fields. Unfortunately, hepatic physiopathology and organogenesis are important areas of biological research that are still scarcely investigated by means of SEM.

Morphological studies, whether in experimental animal models or in human pathology, are one of the interesting topics in biological investigations, today. SEM may offer valuable information toward a better understanding of many physio-pathological processes occurring in all organs (10), especially in liver. Up to the present, however, the liver has not been extensively studied by SEM pathologists, hence, very few papers describing human pathological samples are available.

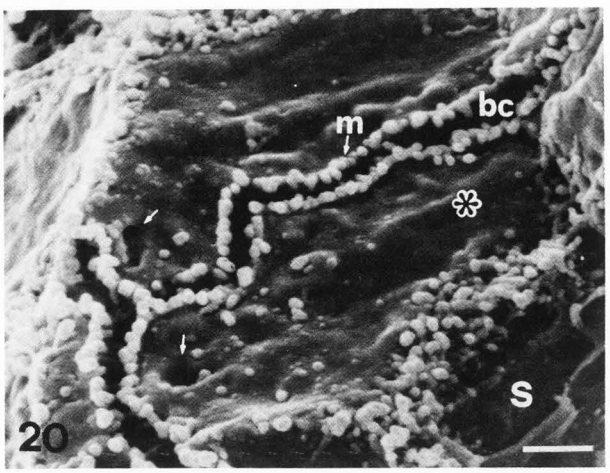
Hepatobiliary lesions produced by bile duct ligation (9,11,13,81,90,131) or treatment with cholestatic agents (54,55,65,162) in animals, as well as those biopsied in humans (137), have been studied. In intra-extra-hepatic cholestasis, SEM easily revealed diffuse dilatation of bile canaliculi that had lost their bordering microvilli and emitted numerous ramifications (81,90). In addition, increased microvillosity of newly formed side-branches of the bile canaliculi has been observed in humans (137). Diffuse proliferation of the intrahepatic biliary tree, enlarged canaliculo-ductular junctions and numerous endo-exocytotic formations of the hepatocytic surfaces also have been reported (11). These changes may help to explain the mechanism of bile regurgitation into the vascular compartment, i.e., jaundice formation. This would seem to suggest that bile regurgitation is related to increased ductular reabsorption of bile and trans-hepatocytic transport, leading to release of bile into the space of Disse (11). On the other hand, areas with minimal distance between the bile canaliculi and the space of Disse have been revealed by SEM (75). These areas, which correspond to the narrowest smooth bands present at the canalicular sides, may be considered "loci minoris resistentiae" for their supposed tendency to rupture and leak bile in response to increases in intrabiliary pressure (73,90,122). However, SEM data on the occurrence of free communications between perivascular spaces and bile canaliculi are contradictory (11,63,90), so further studies must be done to clarify this problem. SEM also has been successfully employed in studies of hepatic vascular diseases (138), including vascular tumors (59,137,159). In addition, parenchymal disorders, such as hepatic necrosis, cirrhosis and tumors, both in animals (43,81,93,99-102,115,141, 143,144,) and in humans (39,91,137,161), have been investigated three-dimensionally. All of these reports clearly have shown that SEM is a helpful technique for the elucidation of the pathogenesis of hepatic alterations (73), as well as for the collection of data for differential diagnosis in human pathology (95,137), especially when compared with other morphological and immuno-histochemical studies (10,64).

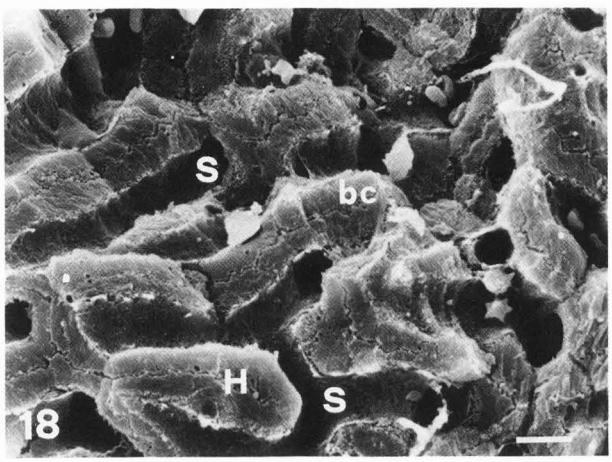
The few SEM papers concerning liver development that are presently available, lend support to the assertion that the study of the dynamic evolution of cell and tissue during hepatogenesis may be approached through a three-dimensional evaluation (58,76,113). Nevertheless, various developmental stages of the liver, and hemopoiesis, have been extensively investigated by TEM (25, 124,163). The architecture of the fetal liver is characterized by the presence of extensive vascular areas and by a greater number of sinusoids than is found in the adult liver (58,76), allowing increased blood filtration and absorption (113). Sinusoidal endothelial cells possess numerous gaps, and they seem morphologically different from Kupffer cells, even in early developmental stages (71). Hepatoblasts, which may be classified best through correlated SEM-TEM studies, show surfaces extensively covered by short microvilli (71). These microvilli, which increase as the liver matures (113), are sometimes seen to be arranged in longitudinal parallel rows. They are probably related to the formation of the bile canaliculi (73).

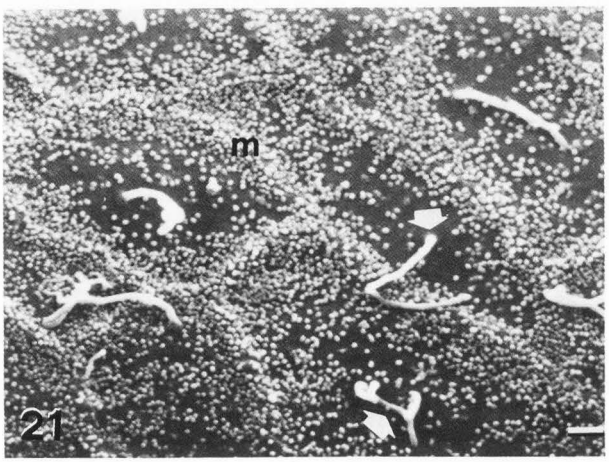
As demonstrated in this review, the "world of the liver" offers many other suitable topics for future three-dimensional studies. The exact function of endothelial fenestrae and their changes during pathological processes, the morpho-functional relationships of endothelium with other sinusoidal cells, the origin and the transformation of hepatic cells during the fetal period or during regeneration and, finally, the

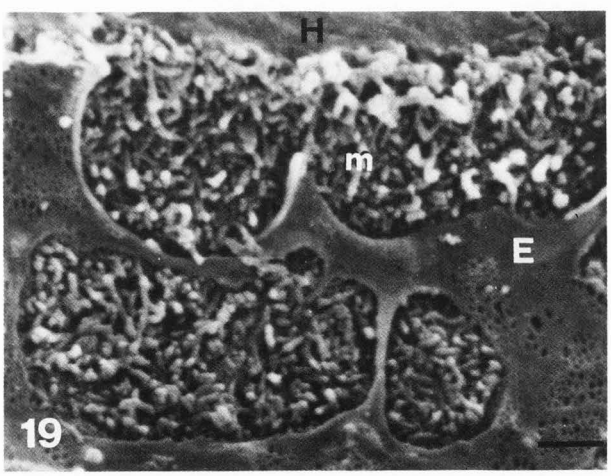
three-dimensional microstructure of liver pathological processes, a subject which is virtually unexplored and unresolved, are all fascinating problems. Indeed, we have demonstrated that many of the liver cell's components can be easily well identified only by means of SEM alone.

We therefore conclude that SEM investigations will surely help to elucidate many of the problems regarding physiological and pathological processes occurring in the liver (27,73). It is our hope, then, that scanning electron microscopists will continue in their efforts to unravel the mysteries entwined within this complex organ, especially so that clinicians and surgeons might find new approaches to treatment of the several hepatic disorders still known to be incurable.









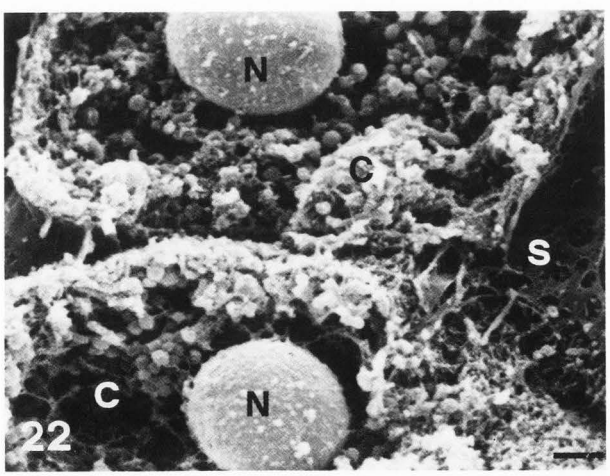


Fig. 18 Hepatic laminae. (H, hepatocytes; S, sinusoids; bc, bile canaliculi).(Bar = 10 μm). Rat.

Fig. 19 The vascular pole of a hepatocyte toward the space of Disse is seen through a large artefactual endothelial rupture. (H, hepatocyte; m, microvilli; E, endothelium). (Bar = 1 μm). Rat.

Fig. 20 Bile facet of a hepatocyte showing the bile groove (bc) bordered by microvilli (m). The arrows show holes which are part of the junctional complex (*, Space of Disse; S, sinusoid). (Bar = 1 μm). Guinea Pig.

Fig. 21 Epithelial surface of a bile duct of the portal area. The arrows evidence cilia. (m, microvilli). (Bar = 1 μm). Rat.

Fig. 22 Fractured hepatocytes. (N, nucleus; C, cytoplasm; S, sinusoid). (Bar = 1 μm). Rat.

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Discussion with Reviewers

E. Wisse: In your introduction you mention that new SEM images on the liver sinusoids of the animal Praomys will be shown. May we know your reason for looking at the sinusoids of these animals? What were the results?
Authors: Praomys Mastomys Natalensis (PMN) is a rodent native to South Africa which easily develops neoplastic processes in certain organs. Further, it has a female prostate that often develops cancer. Up to now, its ultrastructural microanatomy has not been extensively studied. Our group, in collaboration with Dr. DiDio's group at the Medical College of Ohio, is reviewing the microanatomy of the liver as well as that of other organs (ovary, uterus, heart), in order to elucidate the basis of the pathological processes. As far as the results are concerned, PMN liver, in healthy animals, appeared similar to that of other rodents, except for the sinusoidal bridging structures described in this paper. We reported these structures, in the present paper, because it is a new finding and it is of interest in understanding the hemodynamics of the liver microcirculation.

E. Wisse: You are summing up quite a number of data and considerations concerning fixation and tissue preparation. In a review like this, we might expect a point of view or a conclusion with regard to this matter. Would you please formulate your opinion on how we should judge the quality and correctness of a good SEM liver preparation?
Authors: It is not easy to define in a few words what the standard of quality is for a good SEM liver preparation. Actually, as demonstrated in this paper, one singular mode of preparation of liver samples does not exist. In fact, due to the complicated structure of the liver, different techniques are needed to evidence different structures: most of the time, it is not possible to obtain a good conservation of sinusoids and of bile canaliculi in the same sample. As to how we should judge the SEM liver preparations,our meter is represented by the pictures offered in this paper as well as those presented in other publications of our group (text ref. 73,81,82).

E. Wisse: You state that the morphology of endothelial cells differs from organ to organ, even within one organ. Do you think there only exists one type of endothelial cell with different morphological expression (adapted to local circumstances), instead of a multitude of differently programmed endothelial cell types?
Authors: Both hypotheses are likely. According to morphological data we cannot express any further comment. However "local circumstances" easily may induce changes of the endothelium.

E. Wisse: Have you seen junctional complexes between endothelial and Kupffer cells?
Authors: No, personally, we have not seen them, but they have been reported (text reference: 33).

T. Itoshima: The bridge in Fig.14 seems likely but broken.We would like to see an unbroken bridge in the whole.
Authors: See Fig. 23.

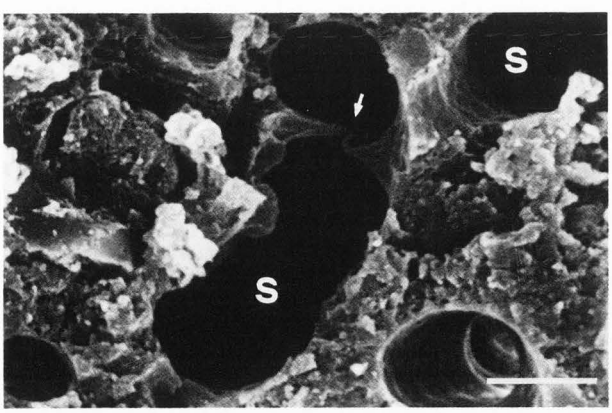

Fig. 23 Praomys liver. An entire intrasinusoidal bridge (arrow). (S = sinusoid). Bar is 10 μm.

T. Itoshima: The arrangement of the hepatic laminae: you describe that changes in the blood pressure gradient produce a deformation of the liver laminae, which may temporarily alter their location in space. I cannot understand what you say. Do you mean to say that hepatic laminae change their interrelationships dynamically? As you know hepatic labyrinth is interconnected three-dimensionally and their relationships are rigidly restricted by hepatocyte continuity.
Authors: We agree that the interrelations of the hepatic labyrinth are rigidly restricted by hepatocytic continuity. Actually, when we state that deformation of the liver laminae may occur, we mean to say that the aspect of the liver architecture is dependent upon the filling state of the sinusoids with blood. In fact, as you know, not all the liver sinusoids are filled with blood at the same time, but distribution of blood flow changes according to the momentary functional state of the liver. Thus, we can find both empty and filled sinusoids. This causes variations in the sinusoidal caliber. It is precisely due to the close relationship existing between sinusoids and hepatic laminae, that the latter changes their location in the space. The same phenomena happen in all soft, highly vascularized tissues, including the liver.

R.D. Soloway: What is known about the SEM structure of the sinusoidal valves that control the microcirculation within the lobule? It is known that kinetic studies of the hepatic microvasculature show that blood flows intermittently through many of the sinusoids.
Authors: Up to now, we have no SEM data to confirm the existence of sinusoidal valves. We believe that the regulation of the "intermittent" blood flow through the sinusoids is controlled mostly by the smooth muscle cell contraction of the intrahepatic vessels, and partially by structures present inside the sinusoids themselves. These latter structures may include the intrasinusoidal bridges or the prolongations of the Kupffer cells.

F. Low: Regarding the question of the basement membrane in the liver could you make some comments about what is revealed by SEM along the lines of Burkel and Low, Am J Anat 118:769-784 (1966)?
Authors: Unfortunately, SEM gives very little information on those structures that are not exposed during preparation. Particular techniques are needed in order to expose basement membrane surface, and it is poorly studied by SEM methods. However, SEM seems to confirm the fact that the basement membrane is not continuous in the liver, but correlated SEM-TEM studies are needed to better elucidate this problem.

S.G. McClugage: Endothelial bridges are described in figures 10, 13 and 14. Have these structures been described in other parts of the vascular system such as the cavernous sinus in the cranial vault? Does their incidence vary from one end of the sinusoid (peri-portal) to the other (central)? Since such endothelial-covered structures would increase the potential surface area for thrombosis could they not affect nutritional blood flow through the lobule under certain pathologic conditions?
Authors: We have no data concerning the presence of similar structures in organs other than liver. - The endothelial bridges were found uniformly throughout the entire sinusoid. - Any structure that changes the regular laminar flow through a vessel creates turbulence. This happens at the level of any vascular branch or at the site of an endothelial lesion (disendothelization). When turbulent blood flow occurs, platelets may be activated, with consequent thrombus formation at the level of an endothelial lesion. Partial or total vascular occlusion occurs as a consequence of thrombotic process. (Spaet TH, Gaynor E, Stemermann MB; Thrombosis, atherosclerosis and endothelium. Am Heart J 87: 661-667, 1974 - Ross R, Faggiotto A, Bowen-Pope D, Raines E; The role of endothelial injury and platelet and macrophage interactions in atherosclerosis. Circulation 70(suppl III):77-82, 1984). Thus, structures such as the intrasinusoidal bridges may actually create a risk factor for vascular occlusion, but only when disendothelization occurs.

F.J. Vonnahme: Is there any evidence that the pits which you describe on the serosal surface of the liver may play a role in the formation of ascites?
Authors: No. But it is likely that they may.

F.J. Vonnahme: You did not mention the functional role of "fat-storing cells" in fibrogenesis. Would you please comment?
Authors: Often, fat-storing cells are seen closely associated with collagen fibrils only in those areas fronting the hepatocytes (text reference 73). Therefore, it has been suggested that they may have a role in secreting collagen and reinforcing the reticular network in the Space of Disse. This may support the hypothesis that these cells have some common origin with fibroblasts; but further evidence is needed (text references 73, 126, 140). Up to now, fat-storing cells are surely recognized as lipocytes,storing vitamin A.