

# Fine Structure of Hepatic Sinusoids and Sinusoidal Cells in Disease

B. LE BAIL, P. BIOULAC-SAGE, R. SENUITA, A. QUINTON, J. SARIC, AND C. BALABAUD

*Laboratoire des interactions cellulaires, Université de Bordeaux II (B.L.B., P.B.-S., R.S., J.S., C.B.); Laboratoire d'Anatomie Pathologique, Hôpital Pellegrin (B.L.B., P.B.-S.); and Service des Maladies de l'Appareil Digestif, Hôpital Saint André (A.Q., C.B.) Bordeaux, France.*

**KEY WORDS** Sinusoids, Endothelial cells, Kupffer cells, Perisinusoidal cells, Pit cells, Space of Disse, Extracellular matrix, Disease

**ABSTRACT** Liver sinusoids are special capillaries that are limited by fenestrated endothelial cells, without a genuine basement membrane, surrounded by perisinusoidal cells storing vitamin A, and harbouring Kupffer cells and pit cells, resident macrophages, and large granular lymphocytes, respectively. Each nonparenchymal cell and parenchymal cell of the liver interacts with all others and with the extracellular matrix. Therefore, the functional ability of each cell is constantly being modified by the metabolic activity of the others.

Human liver biopsies (132), needle or surgical, perfusion-fixed with glutaraldehyde and processed for transmission electron microscopy (TEM), and occasionally for scanning electron microscopy (SEM), were examined. The study included liver diseases (such as alcoholic liver diseases, benign and malignant liver tumors, cholestasis of various origins, fulminant hepatitis, acute rejection after orthotopic liver transplantation, Budd-Chiari syndrome), as well as general or extrahepatic diseases (such as diabetes, hemochromatosis, hypervitaminosis A, various hematological disorders), and normal controls.

Ultrastructural abnormalities are described and illustrated under two different headings: 1) elementary lesions of sinusoidal cells (endothelial, Kupffer, perisinusoidal and pit cells), nonsinusoidal cells (in the space of Disse and/or in the lumen), the extracellular matrix; and 2) the major pathological entities including perisinusoidal fibrosis, capillarization of sinusoids, sinusoidal dilatation, and peliosis. In the discussion, an overview of the major abnormalities reported in the literature is presented, and some specific questions regarding 1) perisinusoidal fibrosis in liver with normal histology, 2) the overload of perisinusoidal cells with lipids in non-hypervitaminosis A intoxication and 3) the etiological relationship of sinusoidal dilatation, peliosis, perisinusoidal fibrosis, or sinusoidal tumors with drugs and toxic compounds are discussed. In the event that lesions are not specific to any diagnosis, the knowledge of the ultrastructure of sinusoids is extremely useful from the perspective of the liver as an ecosystem.

## INTRODUCTION

Hepatic sinusoids are fenestrated capillaries without a genuine basement membrane that spread from the terminal portal venules to the centrilobular veins (terminal hepatic venules) between the single-lined layers of hepatocytes. The normal ultrastructure of human sinusoids has been well described, particularly when perfusion-fixed biopsies were used (Gendrault et al., 1982; Balabaud et al., 1988). Four different constitutive types of nonparenchymal cells have been identified (Brouwer et al., 1988; Jones and Summerfield, 1988; Gendrault et al., 1988; Bioulac-Sage et al., 1988c). The sinusoidal wall is lined by fenestrated endothelial cells, sometimes by intercalated Kupffer cells. Kupffer cells, which are resident macrophages of the liver, are attached to the endothelial barrier and exhibit a sentinel position in the lumen. Perisinusoidal cells (also called fat-storing or Ito cells) are mesenchymal cells implicated in fibrogenesis. Their cell body and long, thin, discontinuous processes form a network around the endothelium. Recently, a resident popula-

tion of large granular lymphocytes (with natural killer cell activity) has been described (Wisse et al., 1976; Bioulac-Sage et al., 1986c). These cells, termed *pit cells*, are located within the lumen and are often in contact with endothelial or Kupffer cells. The space of Disse is a thin zone situated between the sinusoidal wall and the sinusoidal surface of hepatocytes. It contains components of the extracellular matrix (collagens I, III, IV, V, laminin, fibronectin, proteoglycans) and the numerous microvilli of the sinusoidal membranes of hepatocytes (Rojkind, 1988).

All these elements create a morphological (McCuskey, 1988) and functional unit (Wisse and De Leeuw, 1984; Jones and Summerfield, 1985; Wisse et

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Address reprint requests to P. Bioulac-Sage, Laboratoire des Interactions Cellulaires, Université de Bordeaux II, 146, rue Léo-Saignat, 33076 Bordeaux Cedex, France.

al., 1985; Bouwens et al., 1987) implicated in metabolic processes between blood and hepatocytes (such as filtration, secretion) or in immunological (Vuitton and Seilles, 1988), antiinfectious (Nolan and Cohen, 1988) and antitumoral responses (Sherwood and Williams, 1988) of the liver. Each nonparenchymal or parenchymal cell of the liver interacts with all the others and also with the extracellular matrix (Rojkind and Greenwel, 1988). Therefore, the functional ability of each cell is constantly being modified by the metabolic activity of the others. Liver function is the result of these complex interactions that maintain homeostasis and establish a constant milieu of cells and matrical components characteristic of a normal liver. If any cell fails in its respective function, other cells may proliferate and excessive amounts of connective tissues will be produced.

Both textbooks on liver disease and specific ultrastructural studies of liver pathology give little attention to sinusoids (Johannessen, 1979; Jezequel, 1985; Pfeifer, 1983; Tanikawa, 1979; Phillips et al., 1987a,b). The aim of this study is to present briefly the fine structure of sinusoidal cells and the space of Disse in hepatic or extrahepatic diseases, limiting ourselves to data obtained from our institution.

## MATERIALS AND METHODS

The material consisted of human surgical or needle liver biopsies ( $n = 107$ ) taken from patients with:

### Liver Diseases

Alcoholic liver diseases including alcoholic fibrosis (3), steatosis (5), and cirrhosis (8); benign tumors: liver cell adenoma (4), focal nodular hyperplasia (2), polycystic disease of the liver (1); malignant tumors: hepatocellular carcinoma in cirrhotic (3) or noncirrhotic liver (4), metastasis of different origin (10), nonmetastatic liver during extrahepatic cancers (2); alcoholic hepatitis (3), chronic active hepatitis (3); fulminant hepatitis: virus (4), phalloidin (1), or drug induced (1); cholestasis of extrahepatic (6) or intrahepatic (4) origin (i.e., benign recurrent cholestasis, drug-induced cholestasis, viral cholestasis); acute rejection after orthotopic liver transplantation (7); thrombosis of a branch of the portal vein (1), Budd-Chiari syndrome (2).

### General or Extrahepatic Diseases

Diabetes of types I (3) or II (2); amyloidosis (1); hemochromatosis (1); hypervitaminosis A (1); hematological disorders: chronic leukemia (7), non-Hodgkin's lymphoma (7), thrombocytopenic purpura (10), agnogenic myeloid metaplasia (4).

This material was compared with surgical liver biopsies obtained in patients (25) without liver diseases undergoing surgery for gallbladder lithiasis, peptic ulcer, or oesophagitis. Perisinusoidal fibrosis and/or mild sinusoidal dilatation was observed in some patients taking oral contraceptives and/or drugs. Only subjects with normal liver histology on light and electron microscopy constituted the control group (15).

Part of each biopsy was prepared for routine histol-

ogy, fixed in Bouin's reagent or formaldehyde, and stained with hematoxylin/eosin and Sirius red. Another part was frozen to perform immunohistochemical localization of the components of the extracellular matrix. The rest of the biopsy was perfusion-fixed with 1.5% glutaraldehyde (Balabaud et al., 1988) and embedded in epon. Well-fixed areas were chosen on 1  $\mu\text{m}$  sections stained with toluidine blue. Ultrathin sections were double-contrasted with uranyl acetate and lead citrate, then examined on Philips EM 201-301 or CM 10 electron microscopes (Centre de Microscopie Electronique, Université de Bordeaux II). Some surgical biopsies were perfusion-fixed with 3% glutaraldehyde and processed for routine scanning electron microscopy. The tissues were examined on a Philips SEM 515 scanning electron microscope (SEM, Centre de Microscopie Electronique, Université de Bordeaux II).

## RESULTS

The primary ultrastructural aspects of sinusoids in normal control patients are presented in Figure 1. Sinusoids and sinusoidal cells have different shapes according to the plane of sectioning and from one zone of the acinus to another (Balabaud et al., 1988). For example, endothelial fenestrae are more numerous in zone 3 than in zone 1, Kupffer cells are particularly abundant in zone 1 and sinusoids are widest in zone 3.

Compared with those in the rat liver, collagen bundles are more abundant in the space of Disse, and fragments of basement membrane-like material are occasionally seen. Some perisinusoidal cells contain no lipid droplets (Sztaek et al., 1986a), and these cells are sometimes difficult to differentiate from Schwann cells. Nerve endings are occasionally seen in the space of Disse, often close to the perisinusoidal cells (Lafon et al., 1988).

In patients with hepatic or extrahepatic disease(s), sinusoidal abnormalities are extremely diversified, and variations in the intensity of the lesions are evident from one sinusoid to another within the same biopsy. In an effort to simplify the data, sinusoidal pathology is presented under two different headings: elementary lesions and major pathological entities.

### Abbreviations

A	amyloid
B	blebs
Co	collagen
D	cellular debris
E	endothelial cell
Ec	neocavities
Er	erythroblast
H	hepatocyte
K	Kupffer cell
Lb	lymphoblast
M	macrophage
My	myelocyte
PN	polymorphonuclear cell
PS	perisinusoidal cell
R	red blood cell
S	sinusoid

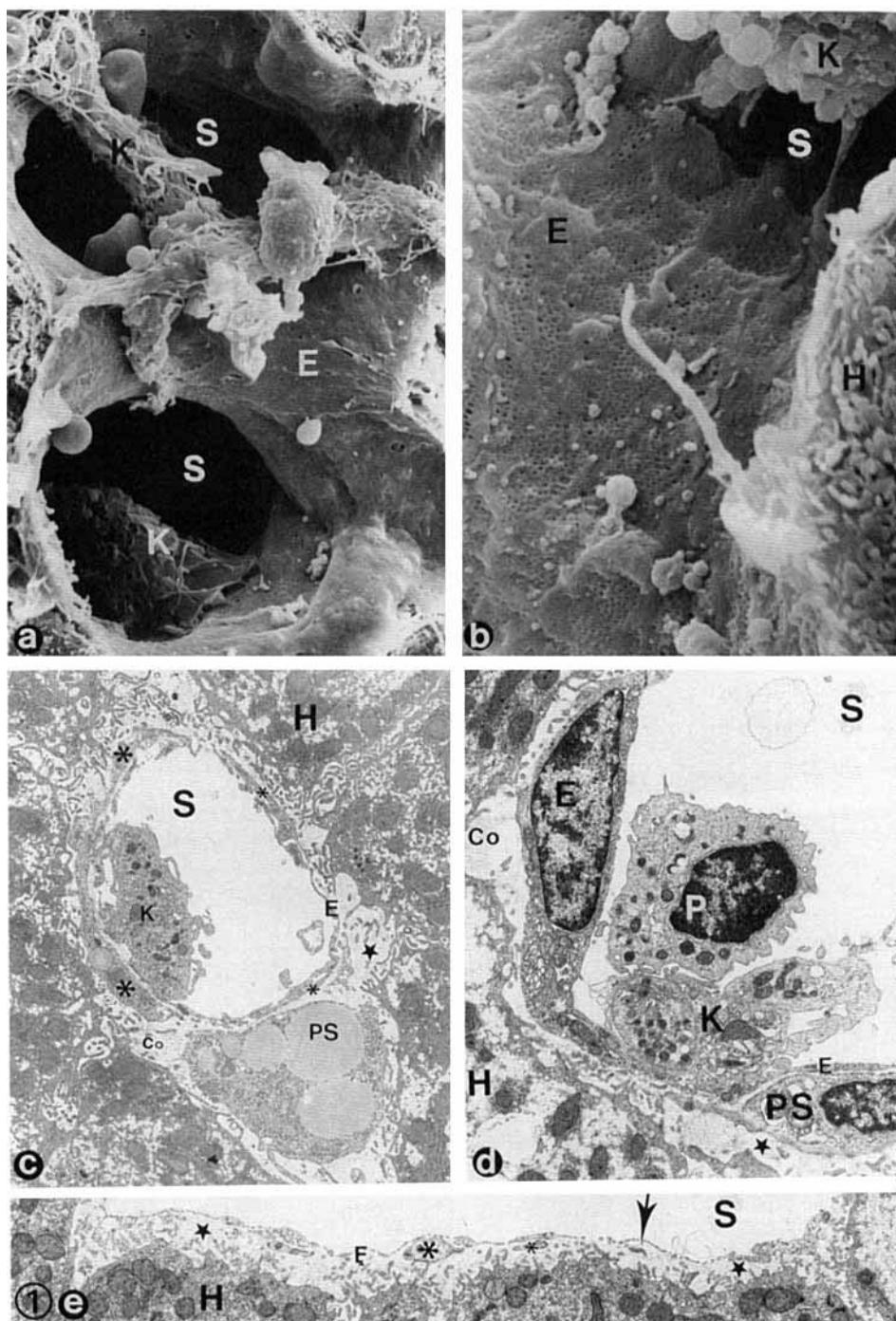


Fig. 1. Ultrastructure of sinusoids in control patients. **a:** The lumen of two sinusoids (S) is crossed by a Kupffer cell (K). Scanning electron microscopy.  $\times 4,300$ . **b:** Fenestrae of the endothelial cell (E) are organized in characteristic sieve plates. Scanning electron microscopy.  $\times 11,400$ . **c:** This small sinusoid is lined by a fenestrated endothelial cell and a Kupffer cell forming part of the sinusoidal barrier. The perisinusoidal cell (PS) contains lipids in its cell body and in its

processes (asterisk) surrounding the sinusoid.  $\times 4,900$ . **d:** The four types of sinusoidal cells are recognizable: a pit cell (P) in contact with a Kupffer cell, an endothelial cell, and a perisinusoidal cell in the space of Disse (star), nearby collagen fibers (Co).  $\times 5,800$ . **e:** The narrow space of Disse is limited by a thin fenestrated (arrow) endothelial wall and the sinusoidal membrane of hepatocyte (H).  $\times 5,100$ .

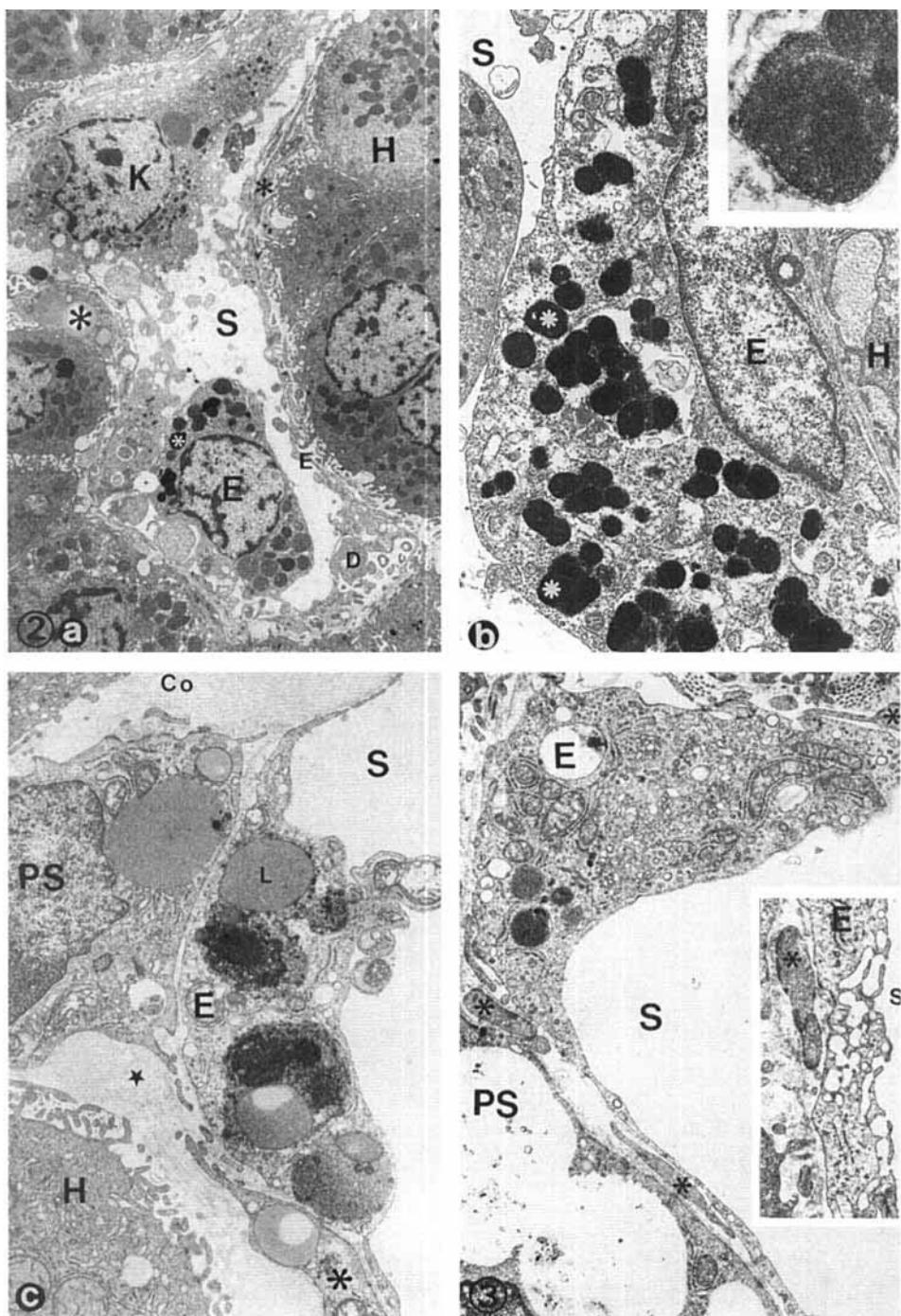


Fig. 2. Endothelial Overload. **a:** Numerous heterogeneous electron dense bodies (white asterisk) are present in an endothelial cell (E) bulging into the sinusoidal lumen (S). A Kupffer cell (K) forms part of the sinusoidal barrier. Cellular debris (D) are more or less invaginated in the endothelial wall (*benign recurrent cholestasis*).  $\times 4,000$ . **b:** Electron dense siderosomes (white asterisk) overload the cytoplasm of an endothelial cell.  $\times 7,800$ —Inset: Detail of a membrane-bound conglomerate of ferritin molecules (*hemochromatosis*).  $\times 31,200$ . **c:** Lipid vacuoles (L) next to heterogeneous lysosomes are seen in an endothelial cell. A perisinusoidal cell (PS) and processes

(asterisk) containing lipids are visible in the space of Disse (star), next to collagen fibers (Co) (*hypervitaminosis A*).  $\times 9,000$ .

Fig. 3. Endothelial hyperactivity. The thick cellular body of this endothelial cell (E) exhibits numerous pinocytotic vesicles, a prominent rough endoplasmic reticulum, and some secondary lysosomes. The endothelium is underlined by perisinusoidal cell (PS) processes (asterisk) (*hypervitaminosis A*)  $\times 9,000$ .—Inset: Fusion of vesicles forming transendothelial channels (*acute rejection after liver transplantation*).  $\times 11,100$ .

### Elementary Lesions of Sinusoids

**Sinusoidal Cells.** *Endothelial cells.* Endothelial changes were found to occur early and to be frequent and polymorphous.

1. Overload (Fig. 2): a. Dense bodies and heterogeneous compound bodies (Fig. 2a): Polymorphous compounds and dense bodies were present in the cytoplasm of endothelial cells under many circumstances, but especially in intrahepatic and extrahepatic cholestasis. Their exact composition was not clearly defined. Some inclusions appeared similar to those seen during iron overload (see below). Others, such as granular electron-dense masses, could be interpreted as copper storage, whereas various figures corresponded to biliary material at different stages of metabolism. Most of this material appeared within lysosomes, the content of which was homogeneously or heterogeneously electron dense and sometimes contained lipoidal material (phospholipid-bile salt complexes). On occasion, lamellar whorls of phospholipids, typical of Kupffer cells, were observed. The lysosomes were numerous (5–15 per section of endothelial cells) and larger than those found in Kupffer cells. Other characteristic elements of biliary material—such as granular, vesicular, or crystalline structures—usually described in hepatocytes were absent from endothelial cells. Dense and heterogeneous compound bodies also were found in most types of hepatitis, in hematologic disorders (particularly in agnogenic myeloid metaplasia), amyloidosis, and other diseases.

b. Iron Overload (Fig. 2b): Numerous iron-containing compounds were observed in the endothelial cytoplasm in severe hemochromatosis. Ferritin molecules often formed round, homogeneous conglomerates, or were included within single membrane-bound siderosomes, sometimes packed with hemosiderin particles. Some free particles of iron-poor ferritin also were found. No intranuclear ferritin or specular crystalline arrangements were observed in the conglomerates. Siderosomes were larger, more numerous, and more regular in comparison to those in hepatocytes. Less prominent aspects of iron overload were observed in cholestasis and hemolytic anemia.

c. Lipid overload (Fig. 2c): Homogeneously electron-dense fat droplets were occasionally observed within the cytoplasm of hepatic endothelial cells of subjects suffering from hypervitaminosis A. Compared with fat vacuoles of perisinusoidal cells, they were smaller and less numerous. Fat droplets were also present in the endothelial cells of patients with several diseases, including diabetes and amyloidosis.

2. Hyperactive cells (Fig. 3): Although hyperactivity is a functional parameter, certain morphological features are indicative of a high activity level in endothelial cells, such as swelling and bulging of the cell body into the sinusoidal lumen, thickening of the processes, increased number and prominence of organelles (smooth endoplasmic reticulum, Golgi apparatus), increased number and size of pinocytotic vesicles (coated or uncoated), micropinocytotic vesicles (bristle coated ones), dense bodies and phagolysosomes. Such active cells were found in amyloidosis, hepatocel-

lular carcinoma (peritumoral liver), cholestasis, and acute rejection after orthotopic liver transplantation. In idiopathic thrombocytopenic purpura and in hypervitaminosis A, micropinocytotic activity was greatly increased. Enhanced activity was also represented by the presence of transendothelial channels (Milici et al., 1986). These structures are thought to permit the exchange of water and macromolecules across the cell on the basis of size and charge. They are formed of fused plasmalemmal vesicles, 600–650 Å in diameter, arranged like a string of pearls, and fitted with two diaphragms, the luminal one being uncharged. This system of transendothelial channels could be an intermediary system of exchange, i.e., between the system of gaps (used by large structures) and the system of fenestrations and pinocytosis (used by smaller molecules). Similar transendothelial channels were not observed in control livers.

3. Increased number of fenestrae and gaps (Fig. 4): Variation in the number of fenestrae was difficult to define by transmission electron microscopy (TEM) and was better appreciated by SEM (Fig. 4a). This number appeared to increase in some individuals, including one patient with steatosis. The presence of gaps was assessed indirectly by the passage of cells (Fig. 4b) or cellular debris (Fig. 4c) through enlarged pores. Hepatocytic debris, corresponding to lesions of clasmatisis, were frequently observed to reach the sinusoidal lumen in patients with cholestasis (extrahepatic or intrahepatic), tumors (hepatocellular carcinoma or peritoneal metastatic liver), alcoholic hepatitis, phalloidin hepatitis, and acute rejection. Erythrocytes were observed to cross endothelial gaps from the lumen to the space of Disse, creating pseudopeliotic cavities in the perisinusoidal space of centrilobular zones in Budd-Chiari syndrome.

4. Endothelial suffering (Fig. 5): Regardless of the cause, endothelial suffering was very monomorphous, and damage was either limited or diffuse. Dying cells presented the appearance of a “bubbling cell.” The organelle lesions consisted of swollen mitochondria devoid of crests and dilatation of the endoplasmic reticulum. Damaged organelles were frequently expelled from the cells. Dilated vesicles accumulated under the plasmalemmal membrane, fused with it, and exocytosed into the sinusoidal lumen. Glycogen particles and pinocytotic vesicles gradually disappeared from the cells, and focal necroses were seen within the cytoplasm. The plasmalemma exhibited focal interruptions, detachments, and projections into the sinusoidal lumen. Endothelial damage was obvious in fulminant hepatitis and frequently observed in different types of hepatitis, severe cholestasis, and acute rejection, although less severe forms were seen in other disease states. Whether or not such pathological changes, e.g., increased number of gaps or evidence of enhanced transendothelial passage, are representative of specific cellular insults remains unresolved.

5. Decreased porosity (Fig. 6a,b): Endothelial porosity appears to be diminished under certain circumstances, e.g., when cellular processes are thick, piled up, or overlapping (as in hepatocellular carcinoma) when the number of fenestrae are decreased (as in

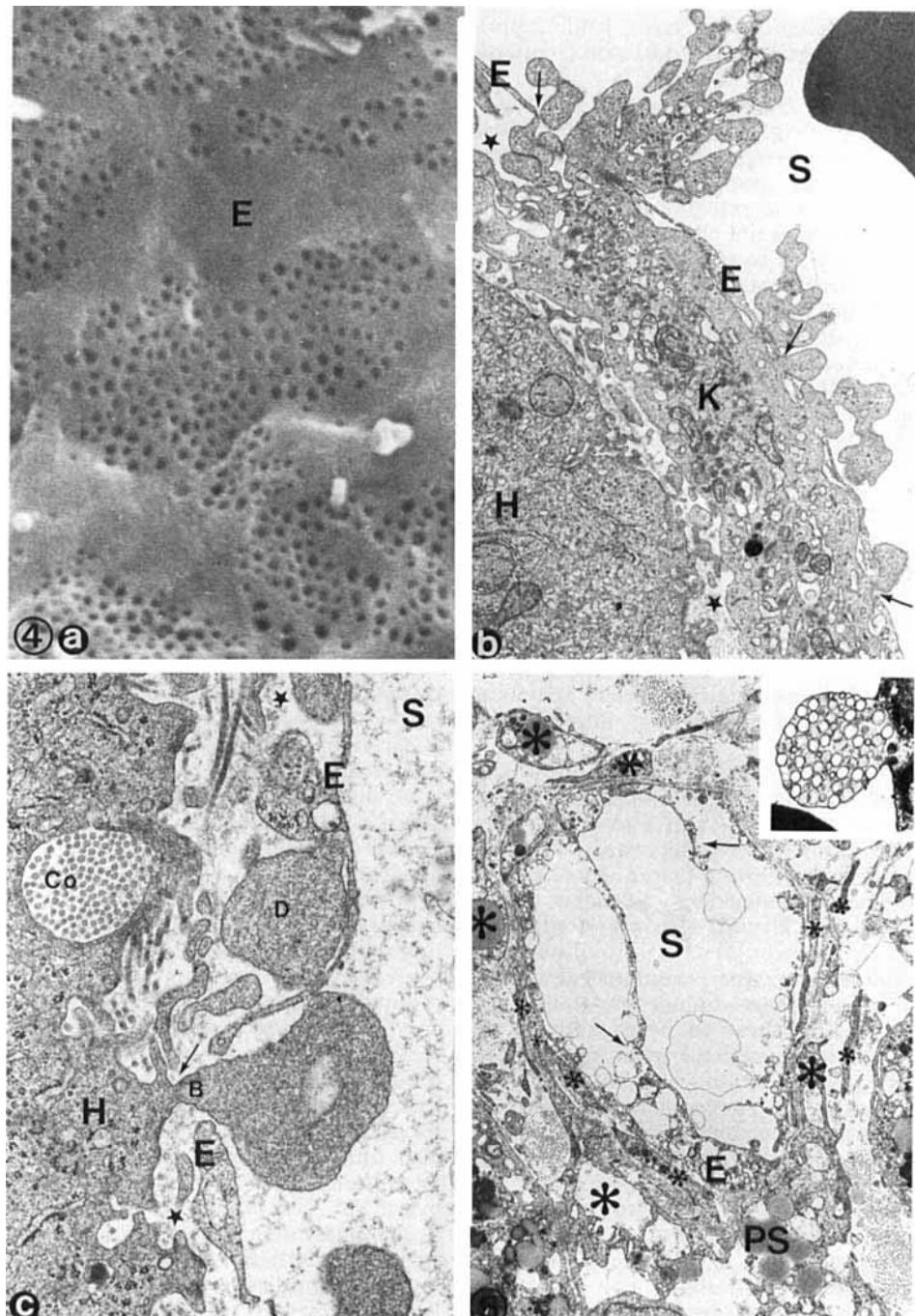


Fig. 4. Increased number of fenestrae and gaps. **a:** In this area of an endothelial cell (E), the number and size of fenestrae forming sieve plates are increased. Scanning electron microscopy (*gallbladder lithiasis*).  $\times 30,200$ . **b:** A Kupffer cell (K) infiltrated in the space of Disse (star) sends pseudopodia and filopodia through enlarged endothelial gaps (arrow) toward the sinusoidal lumen (S) (*acute rejection after liver transplantation*).  $\times 7,200$ . **c:** Cellular debris (D) and blebs (B) detaching from hepatocyte (H), representing clasmotosis, reach the sinusoidal lumen through large endothelial gaps (*extrahepatic cholestasis*).  $\times 13,500$ .

Fig. 5. Endothelial suffering. This endothelial cell (E) is severely damaged (arrow): mitochondrial ballooning, reticulum dilatation, membrane rupture (....) underlined by perisinusoidal cell (PS) processes (asterisk) with lipid droplets and dilated reticulum (*fulminant phalloidin hepatitis*)  $\times 4,850$ . Inset: Protrusion of a portion of a "bubbling" endothelial cell (*acute rejection after liver transplantation*).  $\times 13,800$ .

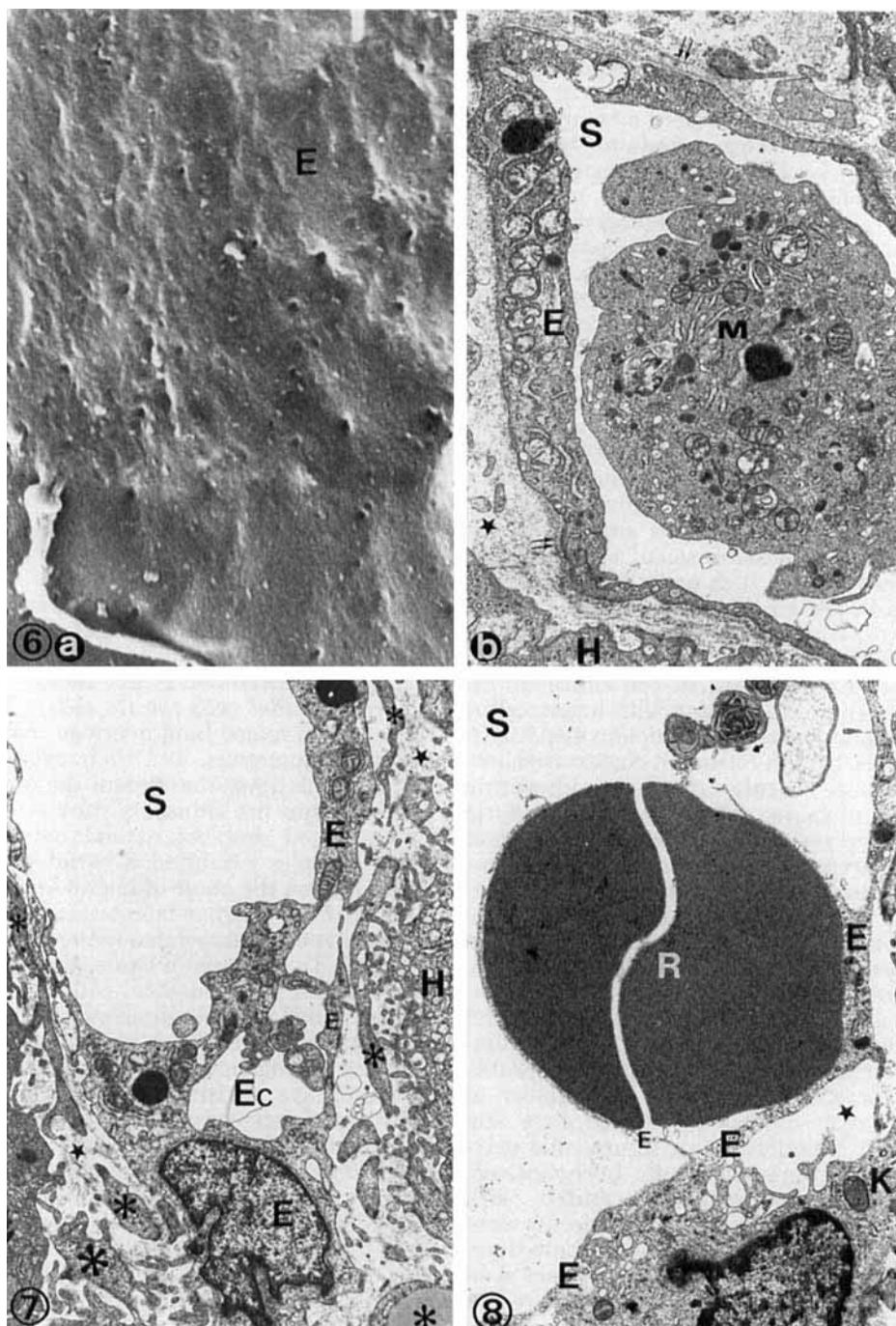


Fig. 6. Decreased porosity. **a:** This endothelial cell (E) is poorly fenestrated. Scanning electron microscopy (*fulminant toxic hepatitis*).  $\times 25,900$ . **b:** This thick, nonfenestrated endothelial cell is underlined by an almost continuous basement membrane (double arrow). The space of Disse (star) is enlarged, and the sinusoidal membrane of hepatocyte (H) is flattened. Macrophage (M) in the sinusoidal lumen (S) (*acute rejection after liver transplantation*).  $\times 8,850$ .

Fig. 7. Neoangiogenesis. Endothelial cell (E) sprouting forms

neocavities (Ec), lined by perisinusoidal cell processes (asterisk) in the space of Disse (star) (*acute rejection after liver transplantation*).  $\times 3,800$ .

Fig. 8. Transendothelial passage. Red blood cells (R) are packed within an endothelial pocket (E) bulging into the sinusoidal lumen (S). A Kupffer cell (K) is infiltrated in the space of Disse (star) (*Budd-Chiari syndrome*).  $\times 7,400$ .

alcoholic cirrhosis, hepatocellular carcinoma, liver cell adenoma, amyloidosis, cholestasis, acute rejection), when the diameter of fenestrae is reduced or when the fenestrae are closed with diaphragms (diaphragms were always absent in control liver) as seen in benign liver cell adenoma, and when basement membrane-like material is increased or forms a genuine basement membrane as in hepatocellular carcinoma, adenoma, or at the periphery of cirrhotic nodules.

6. Endothelial regeneration–Neoangiogenesis (Fig. 7): Although mitotic figures were rarely seen, endothelial sprouting was frequently observed, for example, in Budd-Chiari syndrome, focal compression of hepatic veins by tumoral tissue, or in acute rejection. Cellular sprouts into the space of Disse formed complete or incomplete neolumens of various sizes, and these cavities occasionally contained red blood cells.

7. Transendothelial passage: In cholestasis, hepatocellular debris invaginates into the endothelial wall and ultimately extrudes into the sinusoidal lumen. Depending on the plane of sectioning, debris appears to be either imprisoned in the wall or surrounded by endothelial processes. In postsinusoidal hypertension, red blood cells are found in such endothelial outpocketings as well as in the sinusoid lumen and space of Disse (Fig. 8).

*Kupffer cells.* 1. Hyperplasia and hyperactivity (Fig. 9): The number of Kupffer cells in the sinusoids increased conspicuously in association with hepatocellular necrosis (alcoholic hepatitis, phalloidin hepatitis), cholestasis (lithiasis, benign recurrent cholestasis), immunological response (acute rejection, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia), antitumoral response (peritumoral liver next to hepatocellular carcinoma or metastasis, chronic leukemia, non-Hodgkin's malignant lymphoma), and fibrosis (agnogenic myeloid metaplasia). Hyperactivity was always associated with hyperplasia of Kupffer cells and was present in similar types of diseases. Morphological features of active cells included an increased number and lengthening of pseudopodia and filipodia (Fig. 9c), sometimes resembling a great number of wormlike structures (Fig. 9d), and considerable evidence of phagocytosis, e.g., increased number of lysosomes and phagolysosomes of various sizes and contents (Fig. 9a,b). Pseudomyelinic figures and polymorphous dense bodies were nonspecific. In cholestasis, lysosomes exhibited a heterogeneous matrix with electron-dense ferritin-like granules. Lipofuscins were frequently observed in Kupffer cells of patients diagnosed with diabetes, whereas lipid droplets were seen only in hypervitaminosis A. Viral particles were not found in the cytoplasm of human Kupffer cells during viral hepatitis.

2. Phagocytosis of cells (Fig. 10): Complete cells or identifiable fragments are sometimes seen within the cytoplasm of Kupffer cells. Some of these images represent true phagocytosis. For example, hepatocytic debris is frequently found in Kupffer cells in cytolytic diseases (hepatitis); red blood cells in hepatocellular carcinoma, benign liver cell adenoma, hemolysis, Budd-Chiari syndrome, phalloidin hepatitis; and plate-

lets in idiopathic thrombocytopenic purpura and debris of tumoral cells in liver cancer.

3. Infiltration of Kupffer cells into the space of Disse (Fig. 11): In certain pathological conditions, the number of Kupffer cells (or macrophages) increase, and some partly (Fig. 11a) or totally (Fig. 11b) infiltrate the space of Disse. They often form the sinusoidal barrier. As a result of this infiltration, Kupffer cells achieve close proximity to perisinusoidal cells, hepatocyte microvilli, and all the components of the extracellular matrix.

4. Decreased number and activity: Kupffer cells are absent in hepatocellular carcinoma and their number is decreased in benign liver cell adenoma, as well as in many cirrhotic nodules. In severe cases of hepatic amyloidosis, sinusoids contain few or no Kupffer cells. Those that are observed are flat, partially or totally devoid of pseudopodia, contain few lysosomes and appear to be hypoactive cells.

5. Death and regeneration (Fig. 12): In acute rejection (5 to 7 days after liver transplantation), suffering Kupffer cells with retracted, dark cytoplasm and pyknotic nuclei were frequently observed (Fig. 12a). Dead Kupffer cells were difficult to identify, although active and healthy-looking Kupffer cells (or macrophages) also were observed (Fig. 12b). Mitotic figures were absent. The altered and normal cell populations originate from the liver donor and the host bone marrow, respectively (Steinhoff et al., 1987).

*Perisinusoidal cells (or Ito cells).* 1. Lipid overload (Fig. 13): In major lipid overload, perisinusoidal cells are more numerous, and their cytoplasm is literally stocked with lipids that indent the nucleus. Cells often protrude into the sinusoidal lumen, or processes containing lipid droplets extend between hepatocytes. Lipid vacuoles exhibited a variable electron density, depending on the stage of metabolism, were of various sizes, and were either membrane bound or free. Lipid overload is often associated with perisinusoidal fibrosis (Fig. 13). In less severe cases, the rough endoplasmic reticulum of perisinusoidal cells is obvious and often dilated. Until recently, lipid overload was synonymous with hypervitaminosis A and characterized by extensive and homogeneous lipid deposition. However, mild to impressive perisinusoidal cell lipid overload was observed in patients without obvious hypervitaminosis A intoxication. Several of these patients were diagnosed with diabetes or malignant tumors (hepatocellular carcinoma, metastasis).

2. Fibroblastic/myofibroblastic changes (Fig. 14): In liver disease, perisinusoidal cells may exhibit marked variability related to differentiation: a) transitional cells may be characterized by the progressive loss of lipids and an increase in the rough endoplasmic reticulum (Fig. 14a); b) fibroblast-like cells contain no lipids, but do contain a conspicuous rough endoplasmic reticulum, a prominent Golgi apparatus, and have an elongated shape (Fig. 14b); c) myofibroblast-like cells exhibit an indented nucleus and thick processes that are often underlined by fragments of basement membrane-like material. The cytoskeleton is greatly developed, and microfilaments are often condensed below the plasma membrane. Lipid vacuoles are rare or

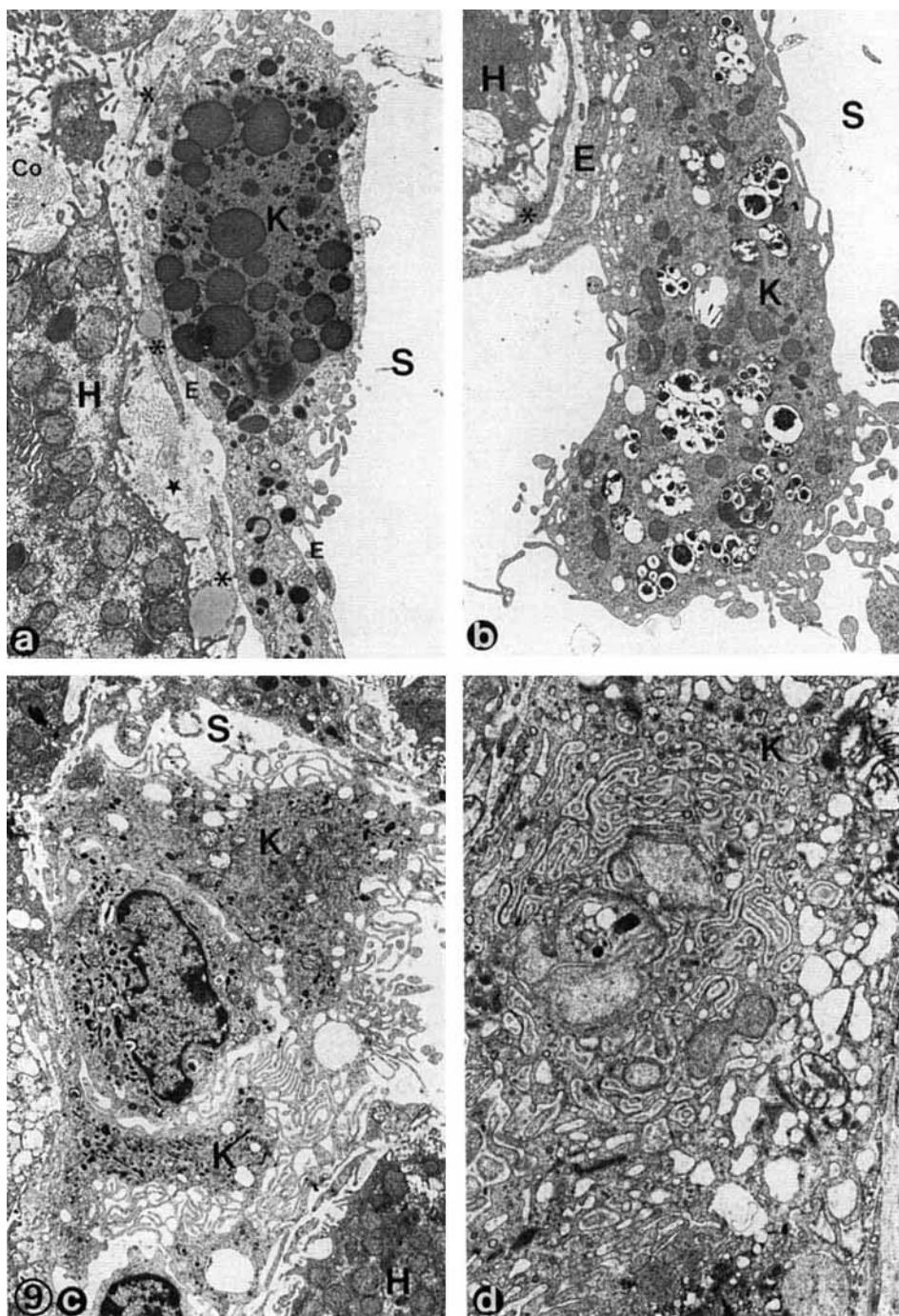


Fig. 9. Kupffer cell hyperactivity. **a:** This Kupffer cell (K) containing many phagolysosomes with lipidal structures sends a large process below the endothelial wall (E). The enlarged space of Disse (star) contains perisinusoidal cell processes (asterisk) near collagen fibers (Co) depressing the sinusoidal membrane of the hepatocyte (H) (*type II diabetes*).  $\times 5,250$ . **b:** This active Kupffer cell bulging into the sinusoidal lumen (S) contains many heterogeneous lysosomes with

pseudomyelinic structures suggesting bile pigments (*extrahepatic cholestasis*).  $\times 6,750$ . **c:** Several Kupffer cells exhibiting numerous filopodia and primary lysosomes almost occlude the sinusoidal lumen (*alcoholic liver disease*).  $\times 9,450$ . **d:** Invaginated pseudopodes form numerous "worm-like structures" in the cytoplasm of a Kupffer cell (*non-Hodgkin's malignant lymphoma*).  $\times 12,740$ .

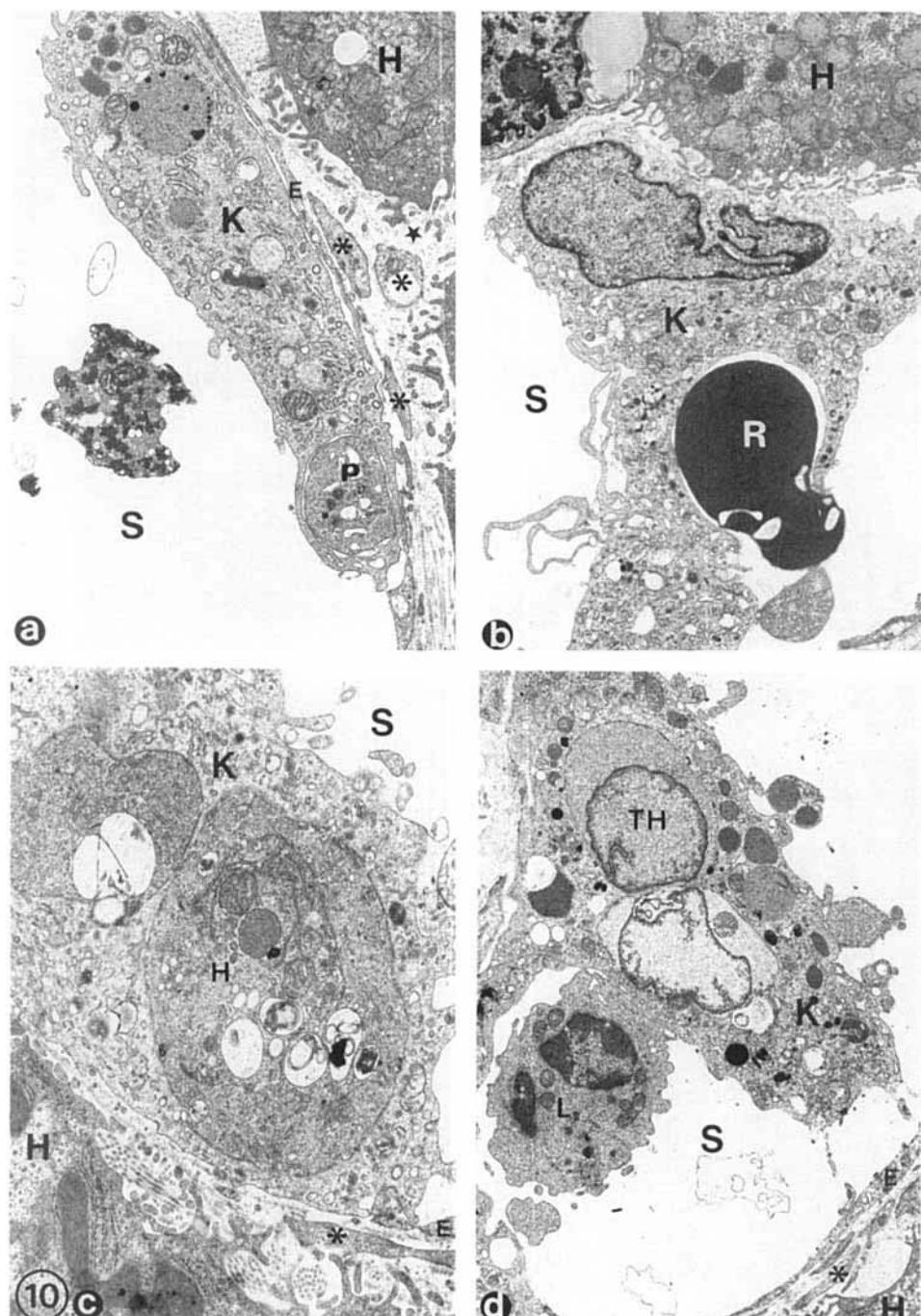


Fig. 10. Phagocytosis of cells by Kupffer cells. **a:** A platelet (p) is engulfed in a Kupffer cell (K) (*chronic myeloid leukemia*).  $\times 8,320$ . **b:** A red blood cell (R) is being invaginated in a large Kupffer cell bulging into the sinusoidal lumen (S) (*normal liver; cholecystectomy*).  $\times 4,940$ . **c:** Fragments of hepatocytes (H) are phagocytized by a

Kupffer cell (*alcoholic hepatitis*).  $\times 12,600$ . **d:** This Kupffer cell containing two tumoral hepatocytes (TH) is in close contact with a lymphocyte (L) (*tissue in the vicinity of an hepatocellular carcinoma*). E, endothelial cell; asterisk, perisinusoidal cell process; star, space of Disse.  $\times 5,320$ .

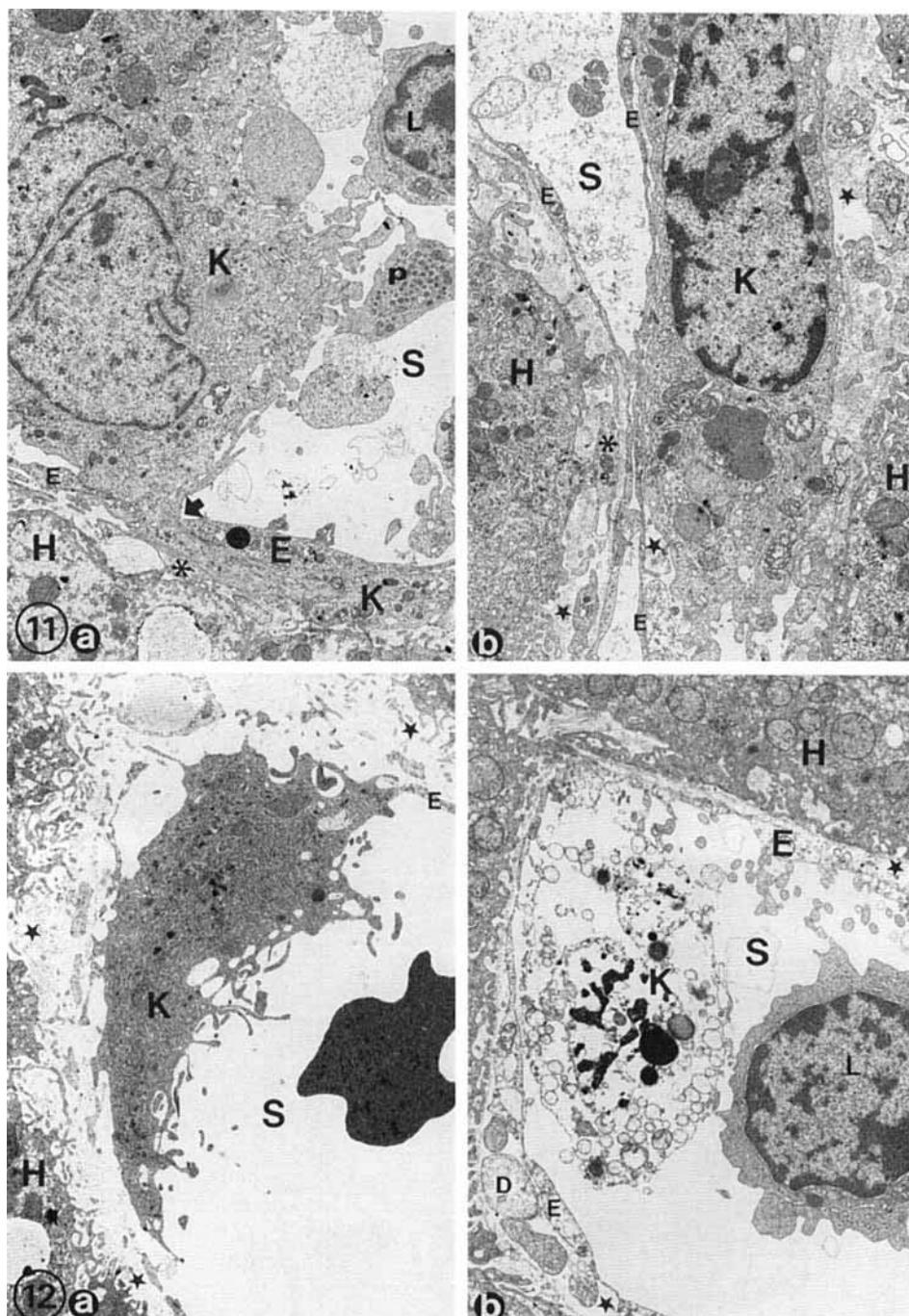


Fig. 11. Infiltration of Kupffer cells in the space of Disse. **a:** A large Kupffer cell (K) is anchored on the sinusoidal barrier by a "cytoplasmic foot" passing through a large gap (arrow), below the endothelial cell (E). It is in contact with a lymphocyte (L) and a platelet (p) in the sinusoidal lumen (S). (*non-Hodgkin's malignant lymphoma*).  $\times 4,000$ . **b:** The whole Kupffer cell is infiltrated in the space of Disse (star) behind a thin endothelial wall and nearby the hepatocyte (H). Asterisk: perisinusoidal processes. (*benign recurrent cholestasis*).  $\times 5,200$ .

Fig. 12. Suffering Kupffer cells. **a:** This dark and retracted Kupffer cell (K) forms part of the barrier of a damaged sinusoid (S) (*acute rejection after liver transplantation*).  $\times 5,250$ . **b:** Debris of a necrotized Kupffer cell, still recognizable by some lysosomes, near a healthy lymphocyte (L) in the sinusoidal lumen (*acute rejection after liver transplantation*). Star, space of Disse.  $\times 6,300$ .

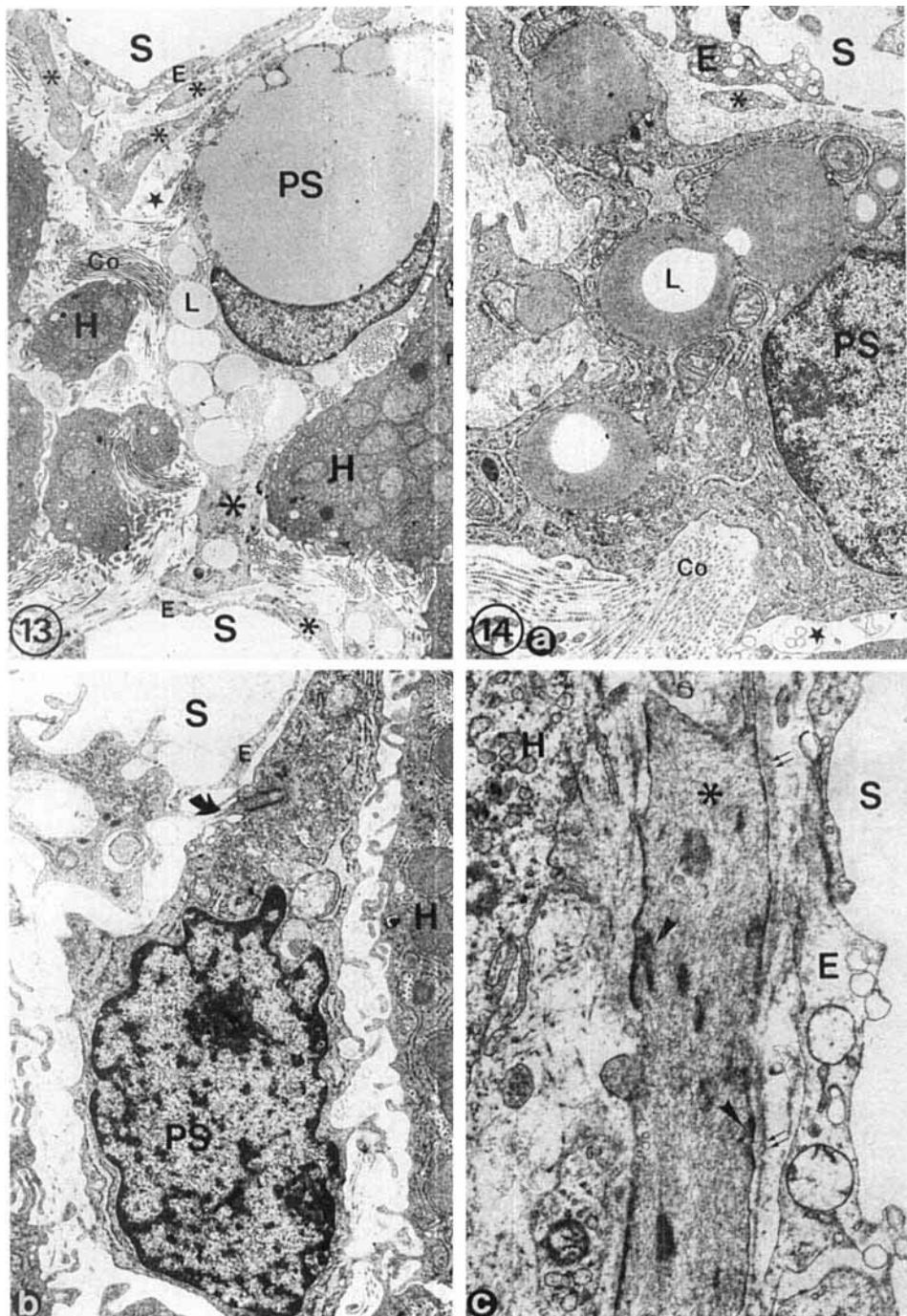


Fig. 13. Lipid overload of perisinusoidal cells. This intercalating perisinusoidal cell (PS) between two sinusoids (S) is filled with numerous and large lipid vacuoles (L) indenting the nucleus; its processes (asterisk) are thick, surrounded by collagen bundles (Co) in an enlarged space of Disse (star) (*Hypervitaminosis A*).  $\times 4,800$ .

Fig. 14. Fibroblastic/myofibroblastic changes of perisinusoidal cells. **a:** This transitional perisinusoidal cell (PS) still contains some lipids (L), but its rough endoplasmic reticulum is greatly increased with some dilated cisternae. Collagen fibers (Co) are seen in the space of Disse (star) (*extrahepatic cholestasis*).  $\times 9,700$ . **b:** This perisin-

soidal cell looks like a fibroblast with a conspicuous rough endoplasmic reticulum and a prominent Golgi apparatus in an elongated cytoplasm. Note also a ciliary structure (curved arrow) (*alcoholic fibrosis*).  $\times 9,000$ . **c:** This thick process (asterisk) of a perisinusoidal cell exhibits a myofibroblastic aspect with numerous filaments often condensed in the center of the cytoplasm or below the plasma membrane (arrow head). A well-defined basement membrane (double arrow) almost completely underlines the process on the endothelial (E) side (*liver cell adenoma*).  $\times 16,500$ .

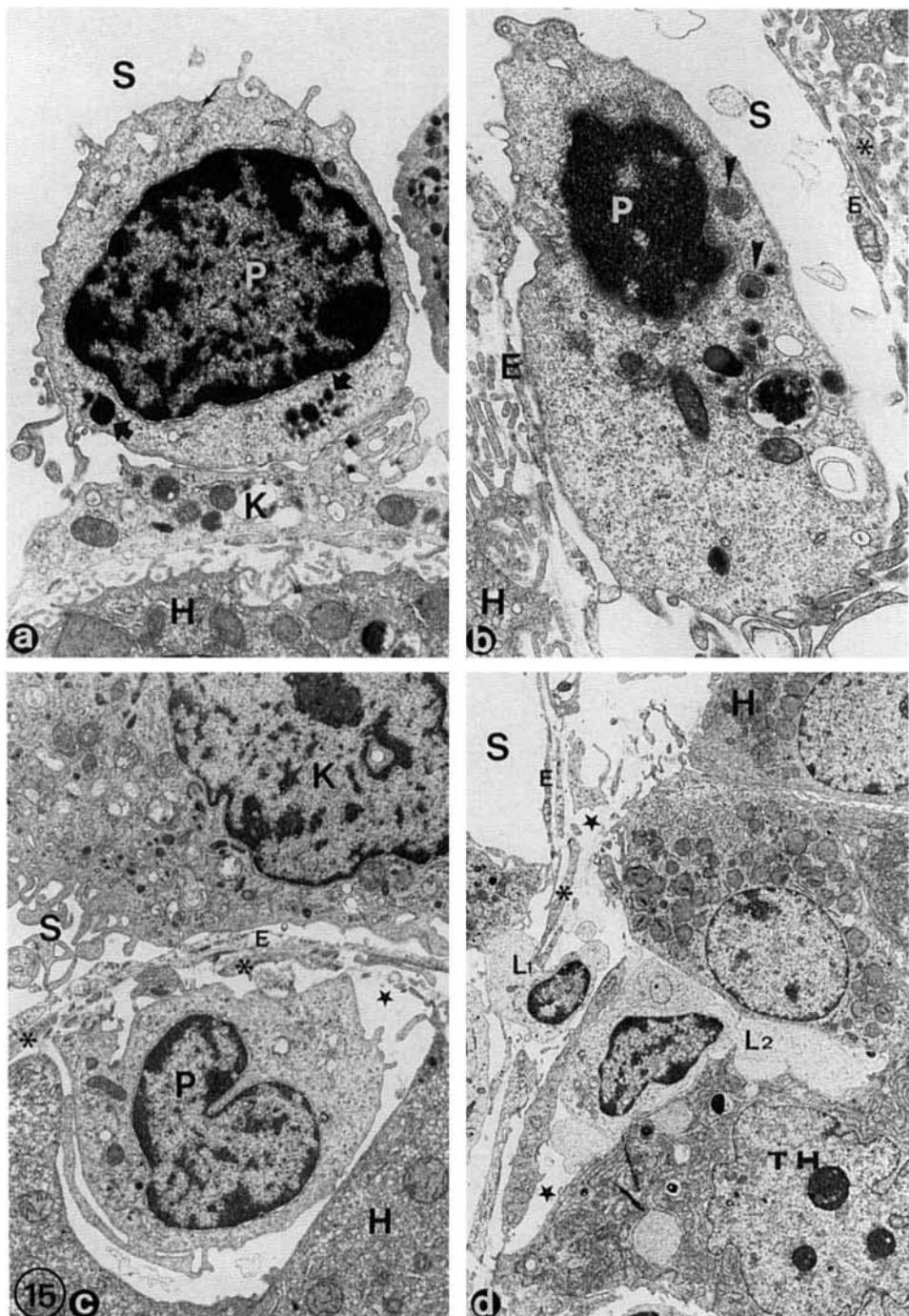


Fig. 15. Pit cells. **a:** This typical pit cell (P) in the sinusoidal lumen (S) is in close contact with a Kupffer cell (K); it is easily recognizable by its characteristic dense granules (large arrow) and rod-cored vesicles (small arrow) in a clear cytoplasm (*control patient*).  $\times 8,200$ . **b:** This pit cell, anchored on the endothelial wall (E), presents several dense granules, some heterogeneous structures looking like lysosomes, and two multivesicular bodies (arrowhead) (*extrahepatic*

*cancer without liver metastasis*).  $\times 14,000$ . **c:** This cell, possibly a pit cell, is infiltrated in the space of Disse, close to hepatocytes (H) (*alcoholic patient with subnormal liver histology*).  $\times 6,300$ . **d:** Lymphocytes passing through the endothelium (L1) or infiltrated (L2) in the space of Disse (star), are located nearby a tumoral hepatocyte (TH) (*hepatocellular carcinoma*). Asterisk: perisinusoidal processes.  $\times 4,350$ .

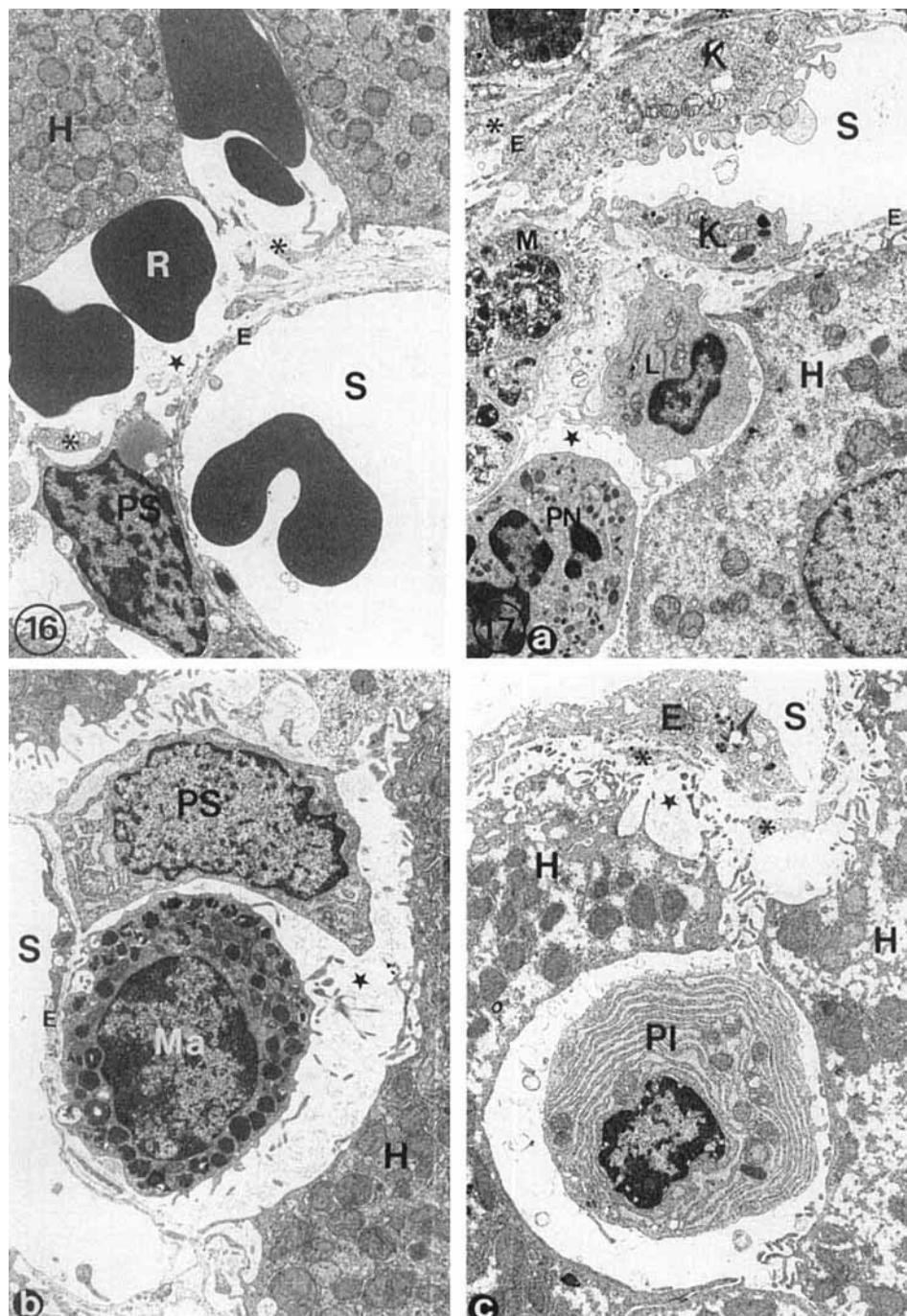


Fig. 16. Infiltration of the space of Disse by red blood cells. Some red blood cells (R) are infiltrated in the space of Disse (star) and in a recessus between two hepatocytes (H) (*alcoholic hepatitis*). Asterisk: perisinusoidal cell processes.  $\times 4,200$ .

Fig. 17. Infiltration of the space of Disse by normal non sinusoidal cells (other than red blood cells). a: A lymphocyte (L), a polymorphonuclear cell (PN) and a macrophage (M) are infiltrated in the space of Disse (star) under the sinusoidal barrier formed by endothelial (E)

and Kupffer cells (K) and close to the flattened lateral membrane of an hepatocyte (H) (*alcoholic cirrhosis*).  $\times 7,500$ . b: A mast cell (Ma) easily recognizable with its typical granulations is seen near a perisinusoidal cell (PS) in the space of Disse (*acute rejection after liver transplantation*).  $\times 7,500$ . c: A plasma cell (Pl) is thrust deeply into a recess between hepatocytes (*noncirrhotic liver at distance from an hepatocellular carcinoma*). Asterisk: perisinusoidal processes.  $\times 6,250$ .

absent (Fig. 14c). In fact, the characteristics of fibroblast-like and myofibroblast-like cells were often mixed and the cells had a "fibroblastic/myofibroblastic" appearance. Such a transformation, often associated with perisinusoidal fibrosis, was obvious in cirrhotic nodules, hepatocellular carcinoma, benign liver cell adenoma, diabetes, and idiopathic thrombocytopenic purpura. In focal nodular hyperplasia, perisinusoidal cells often appeared as myofibroblasts.

**Pit cells** (Fig. 15). Cells with large numbers of rod-cored vesicles and/or granules were not seen. The number of pit cells, however, was increased in the nontumoral tissue of some patients with hepatocellular carcinoma or metastasis, as well as in some patients with severe type 1 diabetes and high-grade non-Hodgkin's lymphoma. In all of these cases, pit cells were also observed in the space of Disse.

### Nonsinusoidal Cells

**In the space of Disse.** 1. Red blood cells (Fig. 16): The presence of one or a few red blood cells in the space of Disse without obvious rupture of the endothelial lining was occasionally observed in conditions such as hypervitaminosis A, alcoholic fibrosis, cirrhotic nodules, intrahepatic cholestasis, uncomplicated gallbladder lithiasis, and liver cell adenoma. Except for the latter, it was associated with perisinusoidal fibrosis. On rare occasions in patients with hepatic tumors, numerous red blood cells were seen in the nontumoral part of the liver, without evidence of postsinusoidal hypertension. Red blood cells were packed either in the space of Disse, compressing atrophic hepatocytes or distributed in poorly defined neocavities (peliosis).

2. Macrophages (Fig. 11b): In many cases, the space of Disse was infiltrated with macrophages, particularly in conditions with hepatocellular damage (cholestasis, acute rejection).

3. Neutrophils, mast cells, plasma cells (Fig. 17): These cells were easy to identify. Neutrophils were seen primarily in acute rejection and alcoholic hepatitis; mast cells in amyloidosis and acute rejection (Fig. 17b); plasma cells in acute rejection, chronic myeloid leukemia, and agnogenic myeloid metaplasia (Fig. 17c).

4. Lymphocytes (Fig. 17a): These were frequently observed in acute and chronic hepatitis, as well as in acute rejection.

**In the lumen and the space of Disse.** 1. Hemopoietic cells (Fig. 18): Erythroid, lymphoid, myeloid cells, and megakaryocytes were seen at different stages of maturation in agnogenic myeloid metaplasia. Cells were observed more frequently in the lumen than in the space of Disse and often in contact with Kupffer cells.

2. Tumoral cells of blood origin (Fig. 19): Immature and tumoral blood cells of different lineages, depending on the hematological disorder, were observed, either in the sinusoidal lumen in contact with large Kupffer cells or pit cells or, more rarely, in the space of Disse. In hairy-cell leukemia, numerous hairy cells (and lymphocytes) were seen in the sinusoidal lumen. They

occasionally formed the sinusoidal barrier, replacing the disrupted endothelial wall.

3. Epithelial malignant cells: Malignant cells were not observed at great distance from malignant tumors (primary or secondary). On the periphery of tumors (primary or secondary), malignant cells were identified between nontumoral atrophic hepatocytes (Fig. 15d).

### Extracellular Matrix

**Increase in the Extracellular Matrix** (Fig. 20, 21). 1. Concomitant increase of collagenous and noncollagenous material (Figs. 20a,b, 21): Global increase of the extracellular matrix was defined ultrastructurally by the widening of the space of Disse, with evidence of thick striated bundles of collagen (Figs. 20a, 21), thin fibrillar or granular deposits (Fig. 20b,c), with or without fragments of basement membrane-like material (Fig. 20b,d). Such abnormalities were observed: a) at distance from hepatocellular carcinoma (in noncirrhotic liver), where types I, III, IV collagens, laminin, and fibronectin were increased; b) in cirrhotic nodules, where all components of the extracellular matrix were apparent; c) in idiopathic thrombocytopenic purpura, where all components were increased, with prominence of type I, type IV collagens and laminin; d) in agnogenic myeloid metaplasia, where the perisinusoidal space contained many thick collagen bundles and fibrils; e) in non-Hodgkin's lymphoma, chronic myeloid, and chronic lymphoid leukemia, where perisinusoidal fibrosis was always present and consisted of thick collagen bundles, identified principally as type I collagen by immunohistochemistry; f) in diabetes (types I and II), where the space of Disse contained accumulations of collagen and basement membrane-like material (immunohistochemical analysis demonstrated types I, III, IV collagens, laminin, and fibronectin, and such lesions were found regardless of the severity of the disease); and g) in thrombosis of a branch of the portal vein, where thick collagen bundles depressed the sinusoidal membrane of hepatocytes and infiltrated lateral recesses of the space of Disse. In rare cases, giant fibers of collagen resembling "flower-fibers" described in inherited connective tissue diseases could be observed; whether or not they represent collagen degradation remains unsolved (Fig. 2, inset).

2. Selective increase in glycoproteins and proteoglycans: The increase of nonfibrillar proteins—such as fibronectin, laminin, or glycosaminoglycans—in the space of Disse was morphologically represented by deposits of finely granular or filamentous material. Concomitantly, the amount of fibrillar collagen was normal or decreased. For example, within hepatic tumors such as liver cell adenoma or hepatocellular carcinoma (whether developed on cirrhotic or noncirrhotic liver), fibronectin, laminin, and type IV collagen were increased, whereas there was a paucity of collagen fibers in the perisinusoidal space.

3. Increase in basement membrane-like material (Fig. 20 b,d,e): An increase in basement membrane-like material was apparent in certain diseases. It was generally deposited between endothelial cells and a)

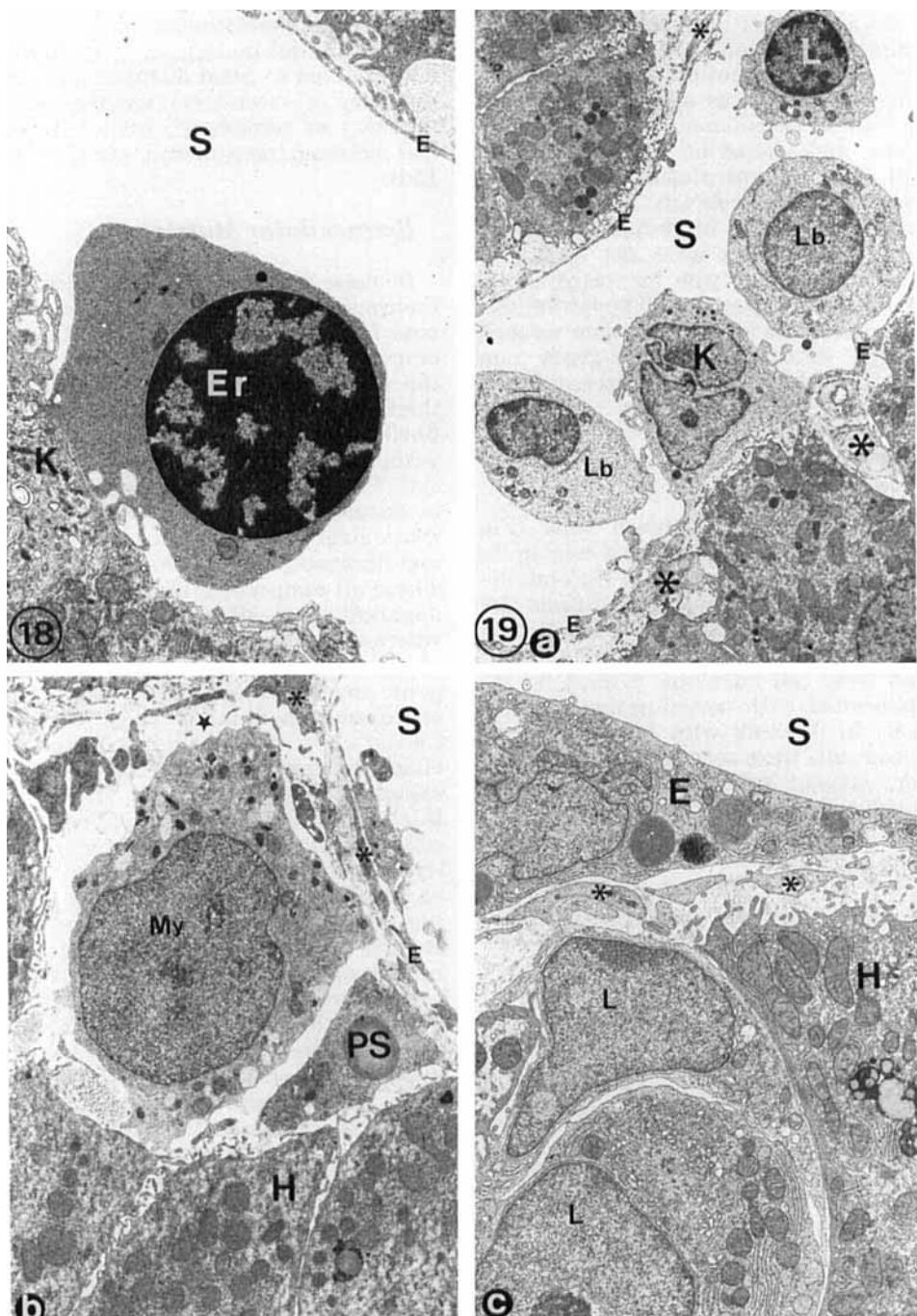


Fig. 18. Presence of hemopoietic cells in the sinusoids. A typical erythroblast (Er) is in close contact with a Kupffer cell (K) in the sinusoidal lumen (S) (*agnogenic myeloid metaplasia*).  $\times 9,200$ .

Fig. 19. Abnormal blood cells in sinusoids. **a:** Two lymphoblasts (Lb) next to a Kupffer cell (K) and a lymphocyte (L) are seen in the sinusoidal lumen (S). A mild perisinusoidal fibrosis and perisinusoidal processes (asterisk) surround the endothelial wall (E) (*lym-*

*phocytic malignant lymphoma*).  $\times 2,800$ . **b:** An immature myelocyte (My) is infiltrated in the space of Disse (star) next to a portion of a perisinusoidal cell (PS) (*agnogenic myeloid metaplasia*).  $\times 4,500$ . **c:** Tumoral lymphoid cells are infiltrated under the endothelial cell, close to the flattened lateral membrane of hepatocyte (H) (*non-Hodgkin's malignant lymphoma*).  $\times 6,500$ .

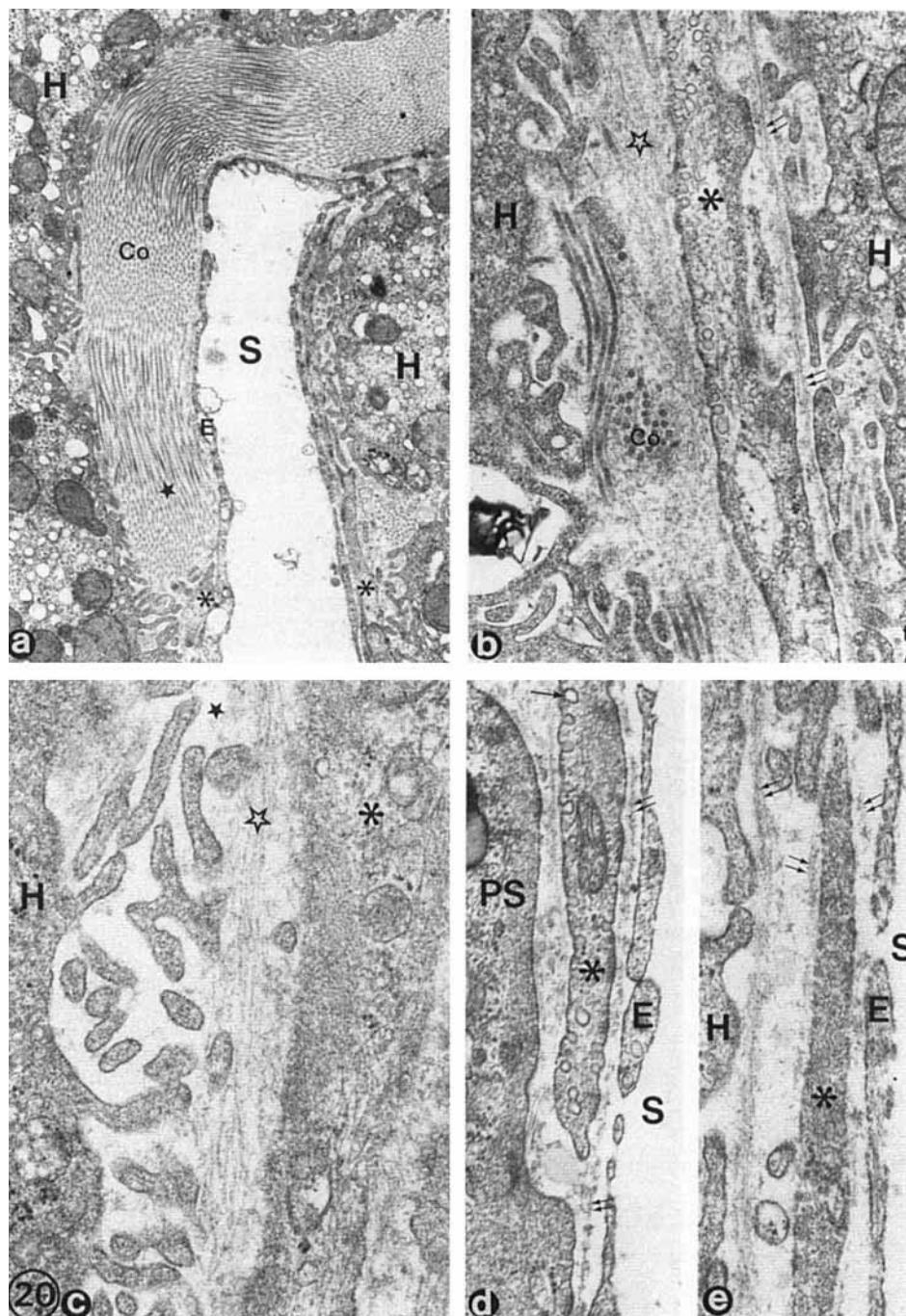


Fig. 20. Extension of the extracellular matrix. **a:** Thick collagen bundles (Co) fully occupy the enlarged space of Disse (star) between the endothelium (E) and the sinusoidal membrane of hepatocyte (H) (*idiopathic thrombocytopenic purpura*).  $\times 9,450$ . **b:** Some collagen bundles, granular and fibrillar material (empty star), and fragments of basement membrane (double arrow) surround a thick perisinusoidal process (asterisk) in a recess of the space of Disse (*idiopathic thrombocytopenic purpura*).  $\times 3,000$ . **c:** Unorganized fibrillar extra-

cellular material is deposited between a perisinusoidal process (asterisk) and the hepatocyte microvilli (*idiopathic thrombocytopenic purpura*).  $\times 42,900$ . **d:** A perisinusoidal cell (PS) process with numerous micropinocytic vesicles (arrow) is surrounded by fragments of basement membrane (double arrow) under the endothelium (*hypervitaminosis A*).  $\times 30,000$ . **e:** A continuous basement membrane is close to the sinusoidal membrane of an hepatocyte (H) and around the perisinusoidal cell process (*type II diabetes*).  $\times 45,000$ .

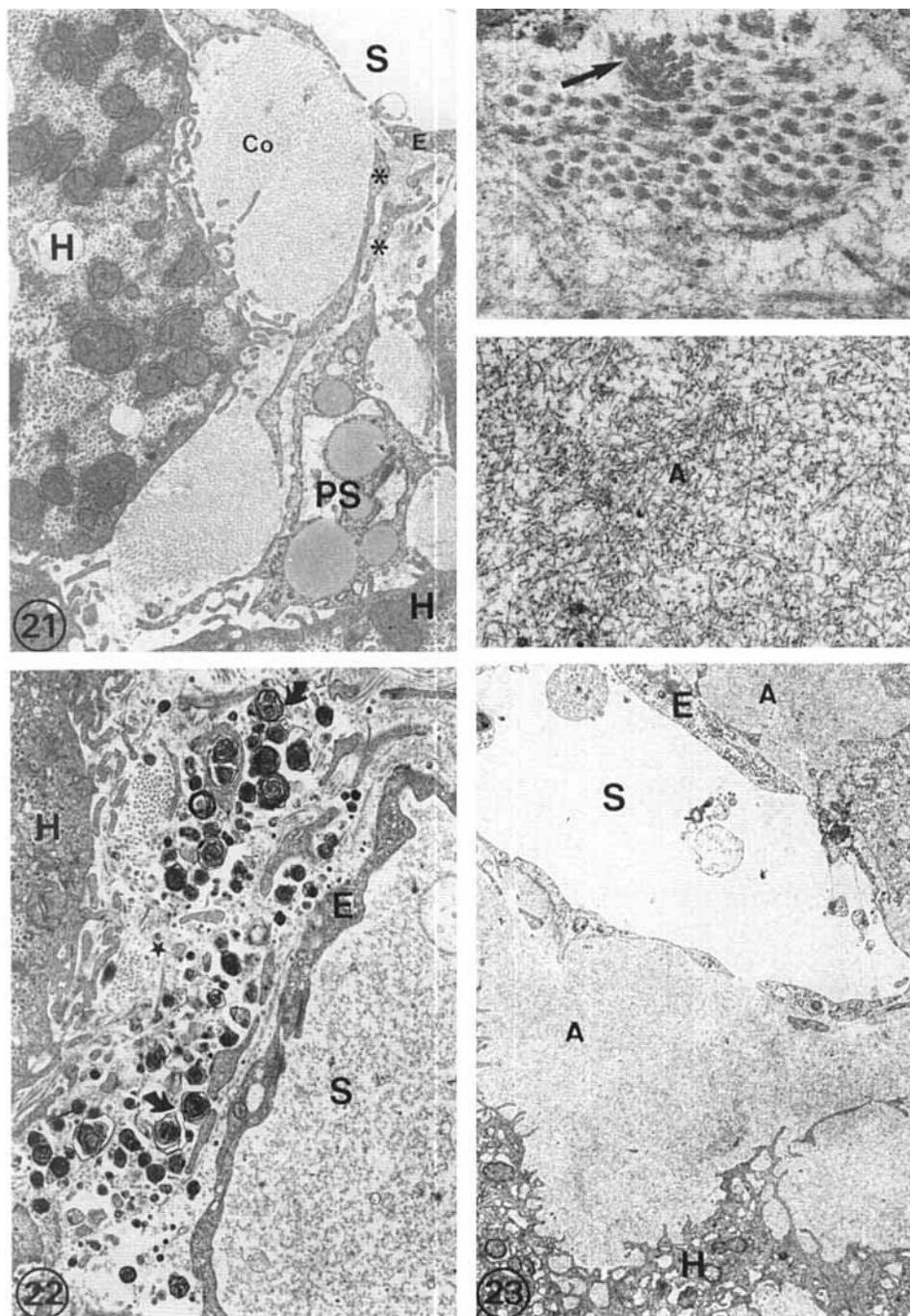


Fig. 21. Perisinusoidal fibrosis. Thick collagen bundles (Co) close to perisinusoidal cell (PS) processes (asterisk) in between two hepatocytes (H).  $\times 8,500$  (*Idiopathic thrombocytopenic purpura*). Inset: This giant collagen fiber (arrow) has the aspect of a "flower-fiber" (alcoholic fibrosis).  $\times 51,300$ .

Fig. 22. Deposit of biliary material in the space of Disse (curved arrow) (*extrahepatic cholestasis*).  $\times 9,450$ .

Fig. 23. Deposit of amyloid in the space of Disse. Amyloid (A), easily recognizable by its typical fibrillar structure (inset) fills the space of Disse between endothelium (E) and hepatocyte (H) microvilli (*amyloidosis*).  $\times 4,000$ .

perisinusoidal cells, b) perisinusoidal cell processes or, more rarely, c) the sinusoidal membrane of hepatocytes. Numerous fragments were observed in all of the fibrotic diseases mentioned; especially in diabetes, the extracellular matrix exhibited a significant increase. The basal membrane was almost continuous in liver cell adenoma (capillarization of the sinusoids), whereas several layers of discontinuous basement membrane-like material were observed in hepatocellular carcinoma (Fig. 24a).

*Reduction of the extracellular matrix.* Types I and III collagens were practically absent in sinusoids of hepatic tumors (hepatocellular carcinoma, liver cell adenoma).

*Deposits in the space of Disse.* 1. Hepatocellular debris (clasmatisis): They were frequently observed in cytolytic hepatitis and hepatocellular damage (i.e., cholestasis) (Figs. 2a, 4c).

2. Biliary material (Fig. 22): In severe cholestasis, bile was observed to be free in the space of Disse, as well as inside the sinusoidal cells.

3. Amyloidosis (Fig. 23): The space of Disse appeared to be enlarged and filled with moderately electron-dense fibrillar structures characteristic of amyloid. Amyloid compressed the sinusoidal membrane of hepatocytes, which appeared flattened or with thin, long, and often perpendicular microvilli. Normal extracellular matrix was almost completely absent.

### Major Pathological Entities.

**Perisinusoidal Fibrosis.** Mild perisinusoidal fibrosis was often not detected by routine histology. After special staining, i.e., Sirius red, perisinusoidal fibrosis was apparent and indicative of an increased perisinusoidal network. On rare occasions, a more definitive diagnosis required electron microscopic examination. The increase in thick collagen bundles was often associated with an increase in non-collagenous proteins forming fibrils and amorphous deposits. Perisinusoidal fibrosis led to the widening of the space of Disse and the depression of hepatocyte sinusoidal membranes. The other major abnormalities often associated with perisinusoidal fibrosis were: 1) an increase in basement membrane-like material, often between the endothelial and the perisinusoidal cells, 2) the presence of hyperactive Kupffer cells and 3) a fibroblastic/myofibroblastic change in perisinusoidal cells. Perisinusoidal fibrosis of various intensities was observed in many circumstances, including alcoholic fibrosis, diabetes type I and type II, nontumoral (and noncirrhotic) tissue of patients with adenoma, focal nodular hyperplasia, hepatocellular carcinoma, and metastasis, extrahepatic cancer without hepatic metastasis, polycystic liver disease, asymptomatic gallbladder lithiasis, hypervitaminosis A, thrombocytopenic purpura (idiopathic or not), agnogenic myeloid metaplasia, chronic lymphoid, myeloid or hairy cell leukemia, non-Hodgkin's lymphoma (whatever the type and grade), and chronic haemodynamic disturbances (thrombosis of a branch of the portal vein, tumoral compression of the hepatic veins with or without Budd-Chiari syndrome).

**Capillarization of Sinusoids (Fig. 24).** Capillarization of sinusoids corresponds to the differentia-

tion of hepatic sinusoids into ordinary capillaries. Ultrastructural definition of capillarized sinusoids implies the absence of open fenestrae in endothelial cells, presence of a continuous basement membrane, transformation of perisinusoidal cells into pericytes, and loss of Kupffer cells from the lumen. In fact, true capillarization is a rare event, and usually incomplete forms are encountered.

In cirrhosis, complete capillarization was rare within the nodules, but capillarized sinusoids were seen at their periphery and were a constant phenomenon in the scar tissue surrounding cirrhotic nodules. In adenoma (Fig. 24c,d), endothelial cells express a few fenestrae, some of which have diaphragms. Kupffer cells were generally few or absent, except in the presence of intense hepatocyte necrosis. There was an obvious increase in basement membrane-like material, occasionally forming double layers. In hepatocellular carcinoma, sinusoids within the tumor were more or less capillarized. Endothelial cells were active and poorly fenestrated, with occasional gaps; perisinusoidal cells presented features of myofibroblasts; the amount of collagen was reduced, but numerous short fragments of basement membrane-like material were often laid down in several layers (Fig. 24a).

**Sinusoidal Dilatation.** Although significant sinusoidal dilatation is obvious, moderate or small changes are difficult to demonstrate. In order to exclude artifacts linked to perfusion-fixation, we referred to routinely fixed biopsy material. Furthermore, care must be exercised to avoid intralobular differences, because periportal sinusoids are narrower than those of centrilobular zones. In addition to dilatation, sinusoids present abnormalities, depending on the specific etiology of the disease. For example, in postsinusoidal hypertension (i.e., Budd-Chiari syndrome), endothelial cells have few fenestrae, possess gaps, contain numerous dense bodies and vacuoles, and erythrocytes are present in the space of Disse (Fig. 25). In hairy-cell leukemia with hepatic infiltration, generalized sinusoidal dilatation is conspicuous and associated with occasional angiomatous lesions mimicking peliosis hepatitis. Numerous tumoral cells with indented nuclei and typical hairy-like cytoplasmic projections are found in the sinusoids, either lined up, packed in small aggregates, or in contact with endothelial cells and progressively replacing them as the sinusoidal barrier and in direct contact with the extracellular matrix of the space of Disse.

Abnormalities are mild in other etiologies of sinusoidal dilatation. However, endothelial cells are often "active," the perisinusoidal collagen network is increased, and perisinusoidal cells present dilated rough endoplasmic reticulum. Dilatation is conspicuous in zone 3 of the lobule in three types of hemodynamic disturbances: a) thrombosis of a branch of the portal vein, b) compression of the hepatic veins by hepatocellular carcinoma, and c) thrombotic endothelialitis of the centrilobular veins occurring in hyperacute rejection after liver transplantation. Moderate sinusoidal dilation was also noticed in some cases of acute rejection.

Sinusoidal dilatation occurs in nontumoral liver at distance from metastases of various types (oat cell

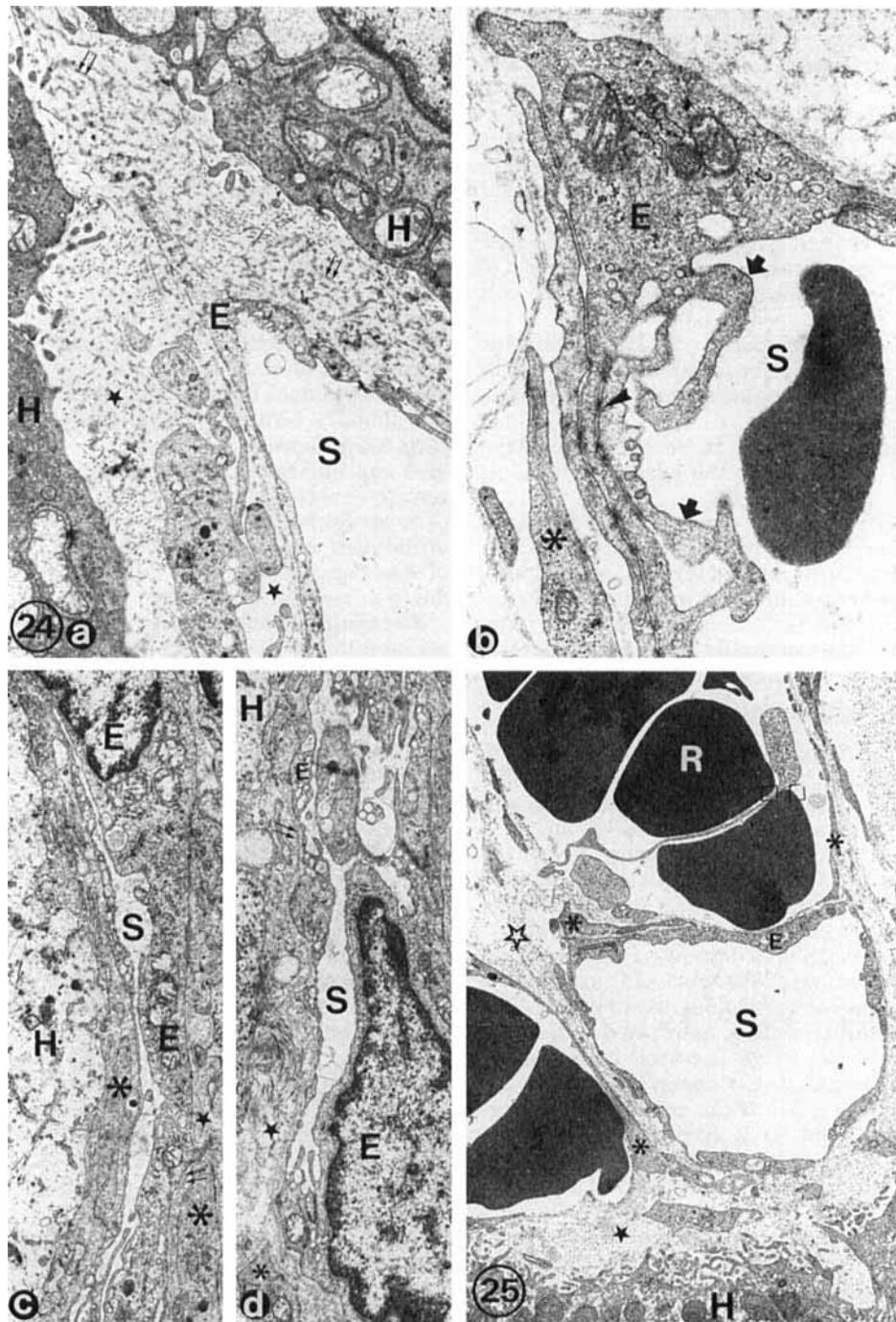


Fig. 24. Capillarization of sinusoids. **a:** Numerous fragments of basement membrane-like material (double arrow) are deposited in several layers in an enlarged space of Disse (star) between nonfenestrated endothelium (E) and flattened sinusoidal membrane of hepatocyte (H) (*hepatocellular carcinoma*).  $\times 9,500$ . **b:** Endothelial cell sprouts in the sinusoidal lumen (S) forming digitations (arrow) joined by tight junctions (arrowhead) (*hepatocellular carcinoma*).  $\times 33,000$ . **c, d:** Thick and nonfenestrated endothelial cells surrounding a narrow

sinusoidal lumen are underlined by basement membrane-like material and thick perisinusoidal processes (asterisk) (*liver cell adenoma*).  $\times 7,700$ .

Fig. 25. Peliosis. Red blood cells (R) surrounded by thin perisinusoidal processes (asterisk) are accumulated in a large space of Disse (star) containing loose and granular extracellular matrix (empty star) between endothelium (E) and hepatocytes (H) (*liver at distance from an hepatocellular carcinoma*).  $\times 5,000$ .

carcinoma of the lung, malignant melanoma, colonic adenocarcinoma) or hepatocellular carcinoma. Dilatation of sinusoids is often associated with the presence of red blood cells or inflammatory cells in the lumen or in the space of Disse. Moderate sinusoidal dilatation was observed in many kinds of leukemia and lymphoma: a) low-grade non-Hodgkin's lymphoma (1/3), high-grade non-Hodgkin's lymphoma (1/2) (dilatation was associated to  $Ki_1$  phenotype), lymphoid chronic leukemia (3/5), myeloid chronic leukemia (dilatation was mild in one of two cases). Mild sinusoidal dilatation was also present in hypervitaminosis A and in some patients taking oral contraceptives.

**Peliosis (Fig. 25).** The presence of one or a few red blood cells in the space of Disse in some patients with hypervitaminosis A or alcoholic hepatitis (see above) could represent minor forms of peliosis. On rare occasions, it was possible to identify cavities between hepatocytes, probably representing neosinusoids that were poorly limited by endothelial cells and Kupffer cells. In peliosis, such neocavities may be limited by hepatocytes or perisinusoidal cell processes with an incomplete inner layer possibly of endothelial origin. Fragments of basement membrane-like and flocculent material surround these cavities. True neosinusoids were also observed under these conditions.

## DISCUSSION

Sinusoids have received little attention in the literature devoted to the ultrastructural pathology of the liver. A comprehensive survey on the ultrastructure of sinusoids in liver disease is premature for the following reasons: 1) too few pathologists have worked on this subject; 2) our own experience is limited to a relatively small number of cases, and a limited number of sinusoids were examined in each case; 3) the criteria for differentiating a poorly fixed cell (artifact) from a damaged cell have not been clearly defined; 4) the definition of a control has not yet been established; and 5) last, but not least, the use of TEM as the major tool of exploration is insufficient to provide definitive identities of all cell types or to characterize their activities. The study of human liver sinusoids will remain difficult and incomplete, because it is dependent on the use of needle or small surgical biopsies. The great developments in hepatic surgery (resection, transplantation) provide opportunities to obtain larger pieces of normal and pathologic liver suitable for perfusion via the portal vein, the hepatic artery or the hepatic vein for fixation, ex vivo perfusion, or cell isolation. Most of our current knowledge concerning the physiology of human liver sinusoids is derived from data obtained in animal models, primarily the rat. Morphologically, murine and human livers share many similarities, but they are not identical. For example, in humans the extracellular matrix is more developed, the perisinusoidal cell processes are thicker, and nerve endings are more frequent.

A thorough analysis of all of the ultrastructural pathologies reported in the literature is beyond the scope of this paper. Nevertheless, the major abnormalities are listed in Table 1, and the most important review articles summarizing many original investigations are in-

cluded. Hypothetical mechanisms responsible for sinusoidal cell damage and the putative consequences on the liver have been briefly summarized in Table 2.

TABLE 1. Human liver sinusoids: Major ultrastructural abnormalities<sup>1</sup>

### Drugs and Toxic Effects<sup>2</sup>

. Extensive hepatocellular injury, necrosis, collapse of the framework: Hypertrophied K (admixed with L, eosinophils, neutrophils, and plasma cells) with increased phagocytic activity (bile pigment, lipofuscins, hepatocellular remnants, hemosiderin, cholesterol clefts), sometimes occluding S (microgranulomas). Hyperplastic changes and storage of diverse materials in E. In DS: K, L, and cellular debris, often increased size and number of PS.

. Granulomatous reaction related to phagocytosis and storage of foreign substances (talc, silicone, thorotrast, lipid-derived emulsions, silica and silicates, polyvinylpyrrolidone): Enlarged M with evidence of active phagocytosis of foreign substances admixed with E, inflammatory cells, PS, acidophilic bodies, fibrin and Co fibrils.

. Granulomatous hepatic response related to drugs: As above, but absence of infectious agents or foreign bodies.

. Neoplastic transformation (see Table 5).

. Sinusoidal dilatation, peliosis, perisinusoidal fibrosis (see Table 5).

. Hypertrophy of PS, overloaded with lipids: (i.e., vitamin A, methotrexate, anabolic steroids, corticosteroids, chenodeoxycholic acid, intravenous fat emulsions, vinyl chloride).

In hypervitaminosis A: PS with large lipid vacuoles indenting the nucleus, long and thick processes with lipids, transformation in F/MF; extension of BM<sub>lm</sub>; numerous Co bundles; K and E occasionally containing lipids<sup>3</sup>.

In Amanita phalloides poisoning: Congestion of S in zone 3, with pseudopeliosis; H clasmatisis in DS and sinusoidal lumen; hyperplasia of ceroid loaded K; E occasionally swollen, with vacuoles and lipids in the cytoplasm<sup>4</sup>.

### Viral Hepatitis

\*Common features<sup>5</sup>

. Acute hepatitis: clasmatisis, apoptosis, acidophilic bodies in DS; inflammatory granuloma in the lumen or/and in DS (M, K, L, polymorphonuclear and plasma cells); hyperplastic and activated K; activated E with siderosomes; transendothelial passage of debris.

. Chronic hepatitis: capillarization of S—Perisinusoidal fibrosis—L and M in DS—Newly formed capillaries in the necrotic areas.

\*Specific features<sup>2</sup>

. Virus A: Clusters of HAV particles may be found in K.

. Virus B: Absence of viral particles in human sinusoidal cells; core, surface and Dane particles can be observed in DS.

. Virus NANB: Tubuloreticular inclusions sometimes in E.

. Virus Delta: No viral inclusions in sinusoidal cells.

. Virus CMV: Cytopathic effect on E, K, and fibroblasts: swelling of the cell, dispersion of the organelles, cell destruction; viral inclusions (aggregates of round or rod-shaped core embedded in a granular material, with radial, filamentous capsomeres) may be found in sinusoidal cells.

. HIV: Tubuloreticular inclusions may be found in E and K; T lymphocyte sinusoidal infiltration; peliosis and sinusoidal dilatation and possible perisinusoidal fibrosis.

. EBV: Sinusoidal infiltration by immunoblasts and lymphocytes.

### Cholestasis<sup>6</sup>

Large, active K and M containing lysosomes located in S and DS; active E overloaded with dense bodies and poorly fenestrated; apoptosis, blebbing, and shedding of the vascular pole of H; biliary deposit in DS and S (granular, fibrillar or lamellar, and concentric electron-dense material).

### Alcoholic Liver Disease (except cirrhosis)

. Steatosis without fibrosis: In some cases, PS filled with numerous lipid droplets.<sup>7</sup>

. Alcoholic liver injury (zone 3): Significant reduction in the number of fenestrae and porosity of E, even in the absence of perisinusoidal fibrosis; thick F/MF appearance of PS; perisinusoidal fibrosis with numerous Co bundles and fibrils; extension of BM<sub>lm</sub>.<sup>8</sup>

(continued)

**TABLE 1. Human liver sinusoids: Major ultrastructural abnormalities (continued)**

- . **Alcoholic hepatitis (zone 3):** K loaded with phagolysosomes (sometimes containing Mallory bodies), often forming the barrier; E often thin, with few fenestrae and large blebs (passage of large H blebs), containing some dense bodies; DS with numerous cellular debris often invaginated in E and occasional Mallory bodies; PS with dilated RER and often numerous and small lipid droplets. Extension of thin fragments of BMIm; inflammatory infiltrate (neutrophils, occasional L, M, monocytes), damaged H, cellular debris often close to H containing Mallory bodies; Numerous neutrophils in S.<sup>9</sup>
- Cirrhosis (nodules)**  
A wide range of abnormalities ranking from S grossly normal to true capillaries, particularly at the periphery of the nodules; E with few or no fenestrae; few K-F/MF aspects of PS; enlarged DS containing numerous Co bundles and fibrils, amorphous material and several more or less continuous fragments of BMIm; rarefaction of Hmv.<sup>10</sup>
- Tumors**
  - \***Benign tumors**
    - . **Liver cell adenoma:** S with a slit-like shape, with occasionally blind lumen; no or few K (except if necrosis); straight and few fenestrated E, underlined by obvious fragments of BMIm; widened DS containing few Co, abundant flocculent material, some RBC; rarefaction of Hmv; MF aspect of PS.<sup>11</sup>
    - . **Focal nodular hyperplasia:** Transformation of PS into transitional cells, F, and most of all, MF, particularly in contact with proliferating bile ductules; perisinusoidal fibrosis with numerous Co bundles.<sup>12</sup>
  - \***Malignant tumors**
    - . **Hepatocellular carcinoma (common trabecular type):** Few or no K; E with few fenestrations and large gaps, sprouting (digitations in the lumen) and overlapping of E processes over long distance with junctional complexes; enlarged DS containing thick and numerous fragments of BMIm often in several layers, fibrillar material, but few Co bundles, some RBC; F/MF aspect of PS.<sup>13</sup>
    - . **Close vicinity of tumors (hepatocellular carcinoma, metastasis):** S and DS infiltrated by K, M, L, and occasional tumoral cells; enlarged DS with fibrillar material; some PS filled with numerous lipid droplets; H damage.
    - . **At distance from malignant tumors:** occasional perisinusoidal fibrosis; active K; numerous P; lipid loaded PS.
- Hematological Disorders<sup>14</sup>**
  - . **Acute leukemia:** Frequent and massive infiltration of the sinusoidal lumen and the DS by malignant cells; sinusoidal dilatation.
  - . **Non-Hodgkin's lymphoma:** Sinusoidal involvement is frequent in a) peripheral aggressive T-cell lymphoma: tumoral infiltration of the sinusoid, sinusoidal dilatation, perisinusoidal fibrosis, hyperactivity of Kupffer cells; b) angiocentric immunoproliferative lesions and lymphoma: sinusoidal infiltration and congestion, hyperplasia of K, erythrophagocytosis; sinusoidal involvement is much more rare in B-cell lymphoma of low-grade and occurs only in leukemic phase.
  - . **Chronic myeloid leukemia:** Malignant infiltration (myeloblasts through to granulocytes, megakaryocytes) mainly in S.
  - . **Chronic monocytic leukemia:** Sinusoidal infiltration by malignant monocytes, in addition to portal infiltration.
  - . **Malignant histiocytosis:** Sinusoids and portal tracts are frequently involved: infiltration of tumoral cells; frequent erythrophagocytosis; hyperplasia of K.
  - . **Agnogenic myeloid metaplasia:** Dilatation of S and infiltration by hemopoietic cells (erythroid, megakaryocytic, lymphoid, myeloid) in the lumen and DS; hyperplasia and hyperactivity of K; perisinusoidal fibrosis; possible intrasinusoidal hematopoiesis.
  - . **Idiopathic thrombocytopenic purpura:** Megakaryocytic cells in DS; perisinusoidal fibrosis; hyperplasia and hyperactivity of K; MF change of PS; hyperactivity of E.
  - . **Hairy-cell leukemia:** Frequent infiltration of the sinusoidal lumen and DS by typical hairy cells and L; attachment of hairy cells to the sinusoidal wall, extension of their processes into DS through endothelial pores; replacement of E by hairy cells; disruption of the sinusoidal wall; angiomatous lesions mimicking peliosis.
- . **Chronic lymphoid leukemia:** Sinusoidal localization is rare except in B-cell origin leukemia; lymphoid sinusoidal infiltration; sinusoidal wall alteration; marked dilatation.
- . **Thrombocytopenia:** Megakaryocytic cells in DS.
- . **Severe hemolytic anemia:** Hematopoietic cells in DS.
- . **Hodgkin's lymphoma:** Occasional infiltration of periportal sinusoids by Sternberg cells and other nonspecific cells (L, eosinophils).
- . **Felty's syndrome:** Sinusoidal lymphocytosis.
- Acute Rejection After Liver Transplantation**  
Inflammatory cells in S and DS (L, plasma cells, M); active M and K phagocytizing necrotic cell fragments; sinusoidal endothelialitis with swollen, focally interrupted E; activated PS transformed into F/MF.<sup>15</sup>
- Vascular Disorders<sup>16</sup>**
  - . **Thrombosis of a branch of the portal vein:** Major perisinusoidal fibrosis with thick Co bundles; presence of some M, few mast cells and H remnants in DS; E of irregular thickness with few fenestrae and some gaps, containing numerous heterogenous dense bodies and vacuoles.
  - . **Postsinusoidal hypertension:**
    - Severe: RBC invading DS, replacing E and displacing H (hemorrhagic dissection); in chronic stages: DS filled with Co fibers, remnants of H and portions of E; presence of F and PS.
    - Mild: Enlarged S; E processes of irregular thickness with few fenestrae and some large gaps, allowing the passage of RBC; occasionally, pouches formed by E and containing RBC; in DS: RBC often surrounded by PS processes; numerous cellular debris in DS and S; flattening of sinusoidal membrane of atrophic H.
- Congenital Hepatic Fibrosis**  
Perisinusoidal fibrosis; extension of BMIm; conspicuous PS<sup>2</sup>
- Metabolic Diseases**
  - 1. **Diabetes (Type I and II):** perisinusoidal fibrosis. Increase of BMIm-F/MF aspect of PS with thick processes and frequent lipid overload<sup>17</sup>
  - 2. **Major inherited storage disorders<sup>2</sup>**
    - **Glycogenosis:** Glycogen particles in K; perisinusoidal fibrosis.
    - **Galactosemia:** Enlarged K with numerous lysosomes containing phagocytosed material; perisinusoidal fibrosis and extension of BMIm in DS.
    - **Abetalipoproteinemia:** K with hemosiderin granules and erythrophagocytosis.
    - **Tangier disease:** Markedly enlarged K showing pleiomorphic lysosomal inclusions with characteristic tubular structures and/or granular material; sometimes PS with few lipid droplets.
    - **Nieman-Pick disease:** Large, pleiomorphic, membrane-bound inclusions distending the cytoplasm of K; inclusions also in E, PS, and fibroblasts.
    - **Cholesterol storage disease and Wolman's disease:** Enlarged K filled with lipid droplets, cholesterol clefts, and pleiomorphic lysosomes; same lesions in PS; some fat droplets in E.
    - **Mucopolysaccharidoses:** Hyperplasia of PS with a) lysosomal hypertrophy corresponding to the stored material and b) decrease in the normal fat content.<sup>18</sup>
    - **Mucolipidosis:** Vacuolar inclusions in K and E.
    - **Oligosaccharidoses:** K with multivacuolated, distended cytoplasm; similar vacuoles in E and PS.
    - **Farber's disease:** K and E with lysosomes containing typical curvilinear or tubular structures.
    - **Fabry's disease:** K and E with lysosomes containing very pleiomorphic osmophilic and lamellar inclusions.
    - 3.  **$\alpha$ -1-antitrypsin deficiency:** Enlarged K containing phagolysosomes-Perisinusoidal fibrosis.<sup>22</sup>
    - 4. **Hemochromatosis:** Hypertrophic K with a) heterogeneous lysosomes containing dense granules of hemosiderin, packed with small electron-dense ferritin particles b) free ferritin particles in the cytoplasm, sometimes arranged as paracrystalline accumulation; intracytoplasmic hemosiderin and ferritin membrane-bound granules in E; perisinusoidal fibrosis.<sup>19</sup>
    - 5. **Amyloidosis:** Deposition of amyloid fibrils in a widened DS (linear deposit in general, rarely globular). Amyloid deposit consists in randomly oriented, rigid nonbranching (5–15  $\mu$ m diameter) fibrils and some "doughnut" rod component; amyloid fibrils in K or not; depression of sinusoidal membrane of H and atrophy of Hmv; persistence of some Co fibers in DS.<sup>20</sup>

(continued)

**TABLE 1.** Human liver sinusoids: Major ultrastructural abnormalities (continued)

**6. Hepatic nonamyloid light chain deposition:** Finely granular and electron-dense material in DS. Sometimes fibrillar and parallel arrangement; peliosis and perisinusoidal fibrosis (rare).<sup>21</sup>

<sup>1</sup>Only disorders in which electron microscopic findings are known are included. BIm, basement membrane-like material; Co, collagen; E, endothelial cell; F, fibroblast-like cell; H, hepatocyte; Hmv, hepatocyte microvilli; K, Kupffer cell; L, lymphocyte; M, macrophage; MF, myofibroblast-like cell; P, pit cell; PS, perisinusoidal cell (fat-storing cell, Ito cell); RER, rough endoplasmic reticulum; S, sinusoid.

<sup>2</sup>Phillips, 1987b.

<sup>3</sup>Bioulac-Sage, 1988b.

<sup>4</sup>Panner and Hanss, 1968.

<sup>5</sup>Lapis and Schaff, 1979; Bardadin and Scheuer, 1984; Bardadin and Desmet, 1985; Phillips et al., 1987b.

<sup>6</sup>Vos (de) and Desmet, 1979; Jezequel et al., 1983; Raymond et al., 1987; Phillips et al., 1987b; Dubuisson, 1987.

<sup>7</sup>Sztark et al., 1986b.

<sup>8</sup>Horn et al., 1987.

<sup>9</sup>Takahashi et al., 1987; Bioulac-Sage, 1988d.

<sup>10</sup>Schaffner and Popper, 1963; Minato et al., 1983; Lamouliatte et al., 1985; Bioulac-Sage, 1986a; Phillips et al., 1987b.

<sup>11</sup>Bioulac-Sage, 1986b; Bioulac-Sage, 1988b.

<sup>12</sup>Callea et al., 1982.

<sup>13</sup>Hruban, 1979; Tobe et al., 1985; Tabarin et al., 1986; Tabarin et al., 1987.

<sup>14</sup>Bioulac-Sage, 1986d; Zafrani et al., 1987; Bioulac-Sage, 1988d; Zafrani and Feldmann, 1988.

<sup>15</sup>Fennel and Vierling, 1985; Phillips, 1987b; Bioulac-Sage, 1988d.

<sup>16</sup>Phillips et al., 1987b; Dubuisson et al., 1988.

<sup>17</sup>Bioulac-Sage, 1988d.

<sup>18</sup>Elleder, 1984.

<sup>19</sup>Hausmann et al., 1976; Iancu et al., 1978.

<sup>20</sup>Skinner et al., 1966; Pfeifer and Alterman, 1979; Johannessen, 1979; Pfeifer et al., 1983.

<sup>21</sup>Droz et al., 1984; Phillips et al., 1987b.

**TABLE 2.** Mechanisms and consequences of sinusoidal lesions (hypothesis)<sup>1</sup>

### Mechanisms

#### Endothelial cells

- Detergent action of bile salts (cholestasis)
  - Immune toxicity
  - . Increased expression of class I/II antigens (CMH) (alcohol, liver transplantation, viral hepatitis)
  - . Antigenicity of Mallory bodies (alcoholic hepatitis): recruitment of neutrophils leading to local cytotoxic damage
  - Hypoxemia (zone 3) (alcohol, shock)
  - Increased portal blood pressure (postsinusoidal hypertension)
  - Direct toxicity of drugs/toxic/secretion products (i.e., malignant cells)
  - Secondary to massive hepatocyte damage
- Kupffer cells (see above)

### Consequences

#### Endothelial cells

- Disruption of the wall: predisposes to hepatocellular injury
- Hyperactivity: involvement in liver fibrosis

#### Kupffer cells

- Depletion or inhibition: predispose to hepatocellular injury
- Depletion: predisposes to hepatocellular carcinoma
- Hyperactivity: may promote hepatic fibrosis; release of material toxic for hepatocytes

#### Perisinusoidal cells

- Hyperactivity: involvement in liver fibrosis

#### Pit cells

- Depletion or decreased activity: loss of tumor surveillance
- Capillarization of sinusoids: Hepatocyte death (extension of the extracellular matrix) or transformation into biliaryhepatocytes and biliary cells

#### Infiltration of the space of Disse (red blood cells, amyloidosis):

Hepatocyte atrophy

Extensive perisinusoidal fibrosis: Impairment of exchanges between blood and hepatocytes

<sup>1</sup>Popper and Acs, 1985; Lough et al., 1987; Brouwer et al., 1988; Jones and Summerfield, 1988.

**TABLE 3.** Lipid overload in perisinusoidal cells related to an impaired release of vitamin A

### Observations

- Lipid overload in perisinusoidal cells (PS) is observed (1- $\mu$ m-thick section, TEM) in many circumstances apparently not linked to hypervitaminosis A<sup>1</sup>. In our experience, it includes: diabetes type I and II<sup>2</sup> (Bernaua et al., 1982; Balazs, 1985; Latry, 1987), malignant liver diseases<sup>3</sup> (in nontumoral tissue during hepatocellular carcinoma and liver metastases) (Bioulac-Sage et al., 1988b), non-Hodgkin's malignant lymphoma (Bioulac-Sage et al., 1988; Zafrani, 1988), miscellaneous<sup>4</sup> (Bioulac-Sage et al., 1988) (intestinal and biliary fistulae, alcohol, hepatocellular damage etc.).

- In primary biliary cirrhosis, decreased serum retinol binding protein (s-RBP) and serum vitamin A levels with normal or elevated liver vitamin A level are associated with normal s-RBP and cellular retinol binding protein (CRBP) immunostaining of hepatocytes and increased CRBP of PS whose relative number is increased (Nyberg et al., 1988).

### Hypothesis<sup>5</sup>

- Decreased capacity of the liver to synthesize or secrete s-RBP (decreased number of hepatocytes, i.e., in hepatocellular damage and cell death or functional block) leads to accumulation of vitamin A in PS (related to an apparent increased amount of CRBP in PS?).
- Defective retransport of vitamin A from PS to hepatocytes.

### Consequence

Is the excessive storage of vitamin A inside PS responsible for perisinusoidal fibrosis, as it is for hypervitaminosis A (whatever its mechanisms)?

<sup>1</sup>Overload is often irregular from one area to another; the relative number of PS is increased. The composition of the lipid has not been investigated.

<sup>2</sup>Overload is frequently observed.

<sup>3</sup>Overload is seen in about 20% of cases.

<sup>4</sup>The number of cases examined is too small to draw any firm conclusion; however, hepatocellular damage, whatever its causes, seems to be frequently associated with lipid overload.

<sup>5</sup>In hypervitaminosis A, liver disease might affect the capacity of this organ to export vitamin A, thereby enhancing its local toxicity (Leo, 1988).

Sinusoids and sinusoidal cell ultrastructural abnormalities are frequently observed in liver diseases and in extrahepatic diseases. Unfortunately, they are seldom specific or useful for diagnostic purposes. From this perspective, the present results were quite disappointing. On the other hand, this extensive morphological study has been helpful in some circumstances: 1) in determining the nature of lesions (Table 1): Among the newest findings were a) the identification of endothelialitis lesions after orthotopic liver transplantation, b) the massive damage of endothelial cells occurring in fulminant hepatitis, and c) the increase in the number of pit cells in various conditions, but more particularly in patients with metastasis (Kaneda et al., 1984; Bioulac-Sage et al., 1988a; Bioulac-Sage et al., 1988d); 2) in describing new morphological entities, such as perisinusoidal cell lipid overload associated with capillarization of sinusoids in liver diseases rather than hypervitaminosis A (Table 3) or identifying new etiologies (i.e., thrombocytopenic purpura and cancer) for perisinusoidal fibrosis in patients with normal liver histology (Table 4); 3) for understanding certain pathophysiological mechanisms, including a) maintenance of gross sinusoidal architecture despite destruction of the endothelial cells and infiltration of the space of Disse by red blood cells and macrophages in fulminant hepatitis (Fig. 5); b) the role of angiogenic processes in sinusoidal dilatation during postsinusoidal hypertension; and c) the potential relationship between sinusoidal dilata-

**TABLE 4.** Perisinusoidal fibrosis in patients with normal liver histology<sup>1</sup>**Disease associated with perisinusoidal fibrosis<sup>2</sup>**

- Diabetes of either type I or II (Bernauau, 1982; Balazs and Halmos, 1985; Latry et al., 1987), thrombocytopenic purpura (idiopathic or not) (Degott et al., 1985; Lafon et al., 1987), hypervitaminosis A (Hruban, 1974; Bioulac-Sage, 1988b), drugs (Zafrani, 1983), alcohol (Lieber and Leo, 1986), miscellaneous<sup>3</sup> (Bioulac-Sage, 1988b): gallbladder lithiasis, in the non tumoral (and noncirrhotic) tissue of patients with benign liver cell adenoma, focal nodular hyperplasia, hepatocellular carcinoma, metastases, extrahepatic cancer, polycystic liver disease.
- Major ultrastructural abnormalities** (Bioulac-Sage et al., 1988d)
- . Widening of the space of Disse (more collagen bundles, more fibrils and amorphous material)
  - . Transformation of perisinusoidal cells independently of their lipid load
  - Thick, long and sometimes digitated processes (giving the impression of several layers)
  - Processes (and cell body) containing either more RER (fibroblastic aspect) or filaments, occasionally condensed near the plasma membrane (myofibroblastic aspect) or both (fibromyofibroblastic aspect)
  - . Increased deposit of basement membrane-like material: in general in between the endothelial cell and the perisinusoidal cell; occasionally beneath the perisinusoidal cell and, in rare cases, close to the sinusoidal membrane of the hepatocyte.
  - . Hyperactivity of Kupffer cells

<sup>1</sup>By light microscopy (hematoxylin-eosin), liver histology was normal with the exception of eventual mild fibrosis of hepatic vessels, steatosis, and hypertrophy of perisinusoidal cells.

<sup>2</sup>Does not include patients with hematological disorders for whom blood cells are often seen in portal tracts and/or sinusoids.

<sup>3</sup>In these cases the incidence of perisinusoidal fibrosis is unknown.

tion, peliosis hepatitis, perisinusoidal fibrosis or tumors and drugs or toxic compounds (Table 5).

Perhaps the most important point in interpreting ultrastructural lesions of hepatic sinusoids is to consider them a reflection of "the roles of cell-cell interactions and of hormones and the matrix components that are the vehicles of those interactions" (Reid et al., 1988). The liver represents a complex bioecological system that interacts with the other ecosystems within the individual (Rojkind and Greenwel, 1988). Thus, it seems appropriate that sinusoids and sinusoidal cells, which are in contact with the blood, react to any systemic abnormality, however mild. This is further illustrated in hematological disorders that elicit sinusoidal ultrastructural alterations despite normal liver function tests and hepatic histology. Regardless of the specific hemopathy, the same general features are apparent, i.e., the attachment of blood cells to sinusoidal cells, Kupffer cell activation, sinusoidal dilatation, and perisinusoidal fibrosis. Autoimmune hemolysis represents another example wherein Kupffer cells exhibit a marked phagocytosis of red blood cells. Nevertheless, there remains a plethora of diseases in which the sinusoids have not been subjected to any investigation.

Because liver function results from a complex number of interactions between nonparenchymal cells, hepatocytes, and the extracellular matrix they secrete, it should be impossible to detect hepatocyte damage without at least a minimal sinusoidal lesion (Rojkind and Greenwel, 1988). In our experience, this hypothesis has been confirmed. For example, in cholestasis

**TABLE 5.** Primary lesions of hepatic sinusoids (Zafrani, 1988)**Main entities with etiologic factors****Sinusoidal dilatation**

- Perivenular: congestive heart failure; obstruction of large and/or small hepatic veins (i.e., Budd-Chiari syndrome, venoocclusive disease).
- Periportal, mediolobular: oestropregestative oral contraception; pregnancy
- Randomly: neoplastic and granulomatous diseases

**Peliosis hepatitis**

- Chronic wasting diseases: tuberculosis; cancer
- Drugs and toxic: androgenic steroids; medroxyprogesterone acetate; tamoxifen; vinyl chloride; arsenic; thorium dioxide; corticosteroids; vitamin A; azathioprine

**Perisinusoidal fibrosis**

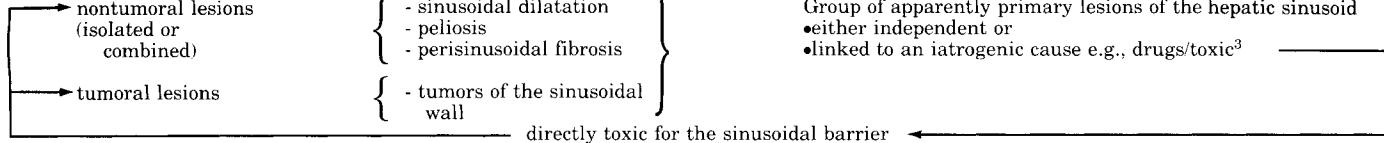
- Drugs and toxic: arsenic; vinyl chloride; cupric sulfate; thorium dioxide; antimetabolites (methotrexate, mercaptopurine); immunosuppressive agents (azathioprine)

**Tumors of the sinusoidal wall**

- Vascular tumors of probable endothelial cell origin: hemangioma - infantile hemangioendothelioma - angiosarcoma<sup>1</sup>; epithelioid hemangioendothelioma

**Sinusoidal lesions in hematologic disease**

- Dilatation and cystic peliotic cavities in massive involvement of the liver by hematologic malignancies with fast cellular growth and in extensive metastatic diseases
- Sinusoidal dilatation and perisinusoidal fibrosis in peripheral T-cell lymphoma, in chronic lymphocytic leukemia of T-cell origin and in hairy cell leukemia (associated with angiomatic lesions)<sup>2</sup>

**Hypothesis**

<sup>1</sup>Drugs implicated in the production of sinusoidal dilatation, peliosis, and perisinusoidal fibrosis are also considered to be responsible for angiosarcoma.

<sup>2</sup>In acquired immunodeficiency syndrome, a condition in which T-lymphocytes may infiltrate the hepatic sinusoids, sinusoidal dilatation, and peliosis are observed.

<sup>3</sup>Including, in hematologic disorder, a toxic effect either of intrasinusoidal abnormal cells or some of their secretion products.

even a mild hepatocellular alteration, e.g., dilatation of bile canaliculi, is associated with endothelial and Kupffer cell changes. Although hepatocytes appear somewhat abnormal in liver cell adenoma and in well-differentiated hepatocellular carcinoma, there are striking abnormalities of endothelial cells and the extracellular matrix. It is likely that the next step in liver pathology will be a better understanding of the complex cell-cell and cell-matrix interactions in order to unravel the mechanisms responsible for diseases (such as perisinusoidal fibrosis, metastatic infiltration) and to find the therapeutic means to block such abnormal processes.

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### REFERENCES

- Balabaud, C., Boulard, A., Quinton, A., Saric, J., Bedin, C., Boussarie, L., and Bioulac-Sage, P. (1988) Light and transmission electron microscopy of sinusoids in human liver. In: *Sinusoids in Human Liver: Health and Disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 87-110.
- Balazs, M., and Halmos, T. (1985) Electron microscopic study of liver fibrosis associated with diabetes mellitus. *Exp. Pathol.*, 27:153-162.
- Bardadin, K.A., and Scheuer, P.J. (1984) Endothelial cell changes in acute hepatitis. A light and electron microscopic study. *J. Pathol.*, 144:213-220.
- Bardadin, K.A., and Desmet, V.J. (1985) Ultrastructural observations on sinusoidal endothelial cells in chronic active hepatitis. *Histopathology*, 9:171-181.
- Bernau, D., Guillot, R., Durand, A.M., Raoux, N., Gabreau, T., Passa, P., and Feldmann, G. (1982) Ultrastructural aspects of the liver perisinusoidal space in diabetic patients with and without microangiopathy. *Diabetes*, 31:1061-1067.
- Bioulac-Sage, P., Balabaud, C., Dubroca, J., Boussarie, L., Grimaud, J.A., Latry, P., Lamouliatte, H., and Quinton, A. (1986a) Sinusoids and the disse space in patients with liver diseases. In: *Marker Proteins in Inflammation*. Vol. 3, J. Bienvenu, J.A. Grimaud, P. Laurent, eds. Walter de Gruyter & Co., Berlin, pp. 417-431.
- Bioulac-Sage, P., Lamouliatte, H., Saric, J., Merlio, J.P., and Balabaud, C. (1986b) Ultrastructure of sinusoidal cells in a benign liver cell adenoma. *Ultrastruct. Pathol.*, 10:49-54.
- Bioulac-Sage, P., Latry, P., Dubroca, J., Quinton, A., and Balabaud, C. (1986c) Pit cell in human liver. In: *Cells of the Hepatic Sinusoid I*. A. Kirn, D.L. Knook and E. Wisse, eds. Kupffer Cell Foundation, Rijswijk, pp. 415-420.
- Bioulac-Sage, P., Roux, D., Quinton, A., Lamouliatte, H., and Balabaud, C. (1986d) Ultrastructure of sinusoids in patient with agenetic myeloid metaplasia. *J. Submicrosc. Cytol.*, 18:815-821.
- Bioulac-Sage, P., Boulard, A., Rossignol, D., Bernard, P., Le Bail, B., Quinton, A., and Balabaud, C. (1988a) The increase in the number of liver sinusoidal pit cells in four patients with primary or metastatic cancer of the liver. *J. Submicrosc. Cytol.*, 20:335-340.
- Bioulac-Sage, P., Quinton, A., Saric, J., Grimaud, J.A., Mourey, M.S., and Balabaud, C. (1988b) Chance discovery of hepatic fibrosis in a patient with asymptomatic hypervitaminosis A. *Arch. Pathol. Lab. Med.*, 112:505-509.
- Bioulac-Sage, P., Lafon, M.E., Le Bail, B., and Balabaud, C. (1988c) Perisinusoidal and pit cells in liver sinusoids. In: *Sinusoids in human liver: Health and disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 39-62.
- Bioulac-Sage, P., Lafon, M.E., Le Bail, B., Boulard, A., Dubuisson, L., Quinton, A., Lamouliatte, H., Saric, J., and Balabaud, C. (1988d) Ultrastructure of sinusoids in liver disease. In: *Sinusoids in Human Liver: Health and Disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 223-278.
- Bouwens, L., Geerts, A., Van Bossuyt, H., and Wisse, E. (1987) Recent insights into the function of hepatic sinusoidal cells. *Neth. J. Med.*, 31:129-148.
- Brouwer, A., Wisse, E., and Knook, D.L. (1988) Sinusoidal endothelial cells and perisinusoidal fat-storing cells. In: *The liver: Biology and Pathobiology*. I.M. Arias, H. Popper, W.B. Jakoby, D. Schachter, and D.A. Shafritz, eds. Second Edition, Raven Press Ltd, New York, pp. 665-682.
- Calleja, F., Mebis, J., and Desmet, V.J. (1982) Myofibroblasts in focal nodular hyperplasia of the liver. *Virchows Arch.*, 396:155-166.
- Degott, C., Capron, J.P., Bettan, L., Molas, G., Bernau, D., Potet, F., Feldmann, G., and Benhamou, J.P. (1985) Myeloid metaplasia, perisinusoidal fibrosis, and nodular regenerative hyperplasia of the liver. *Liver*, 5:276-281.
- Droz, D., Noel, L.H., Carnot, F., Degos, F., Ganeval, D., and Grunfeld, J.P. (1984) Liver involvement in nonamyloid light chain deposits disease. *Lab. Invest.*, 50:683-689.
- Dubuisson, L., Bioulac-Sage, P., Boussarie, L., Quinton, A., Saric, J., de Mascarel, A., and Balabaud, C. (1987) Removal of cellular debris formed in the Disse space in patients with cholestasis. *Virchows Arch. A*, 410:501-507.
- Dubuisson, L., Bedin, C., Boussarie, L., Balabaud, C., and Bioulac-Sage, P. (1988) Liver sinusoids and hemodynamic disturbance. In: *Sinusoids in human liver: health and disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 279-298.
- Elleder, M. (1984) Ito cells in lysosomal storage disorders. An ultrastructural study. *Virchows Arch.*, 46:13-19.
- Fennell, R.H., and Vierling, J.M. (1985) Electron microscopy of re-jected human liver allografts. *Hepatology*, 5:1083-1087.
- Gendrault, J.L., Montecino-Rodriguez, F., and Cinquabre, J. (1982) Structure of the normal human liver sinusoid after perfusion fixation. In: *Sinusoidal Liver Cells*. D.L. Knook and E. Wisse, eds. Elsevier Biomedical Press, Amsterdam, pp. 93-100.
- Gendrault, J.L., Steffan, A.M., Bingen, A., and Kirn, A. (1988) Kupffer and endothelial cells. In: *Sinusoids in human liver: health and disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 17-38.
- Hausmann, K., Wulffkel, U., Dullmann, J., and Kuse, R. (1976) Iron storage in macrophages and endothelial cells. *Histochemistry, ultrastructure, and clinical significance*. Blut, 32:289-295.
- Horn, T., Christoffersen, P., and Henriksen, J.H. (1987) Alcoholic liver injury defenestration in non cirrhotic livers. A scanning electron microscopic study. *Hepatology*, 7:77-82.
- Hruban, Z., Russell, R.M., Boyer, J.L., Glagov, S., and Bagheri, S.A. (1974) Ultrastructural changes in livers of two patients with hypervitaminosis A. *Am. J. Pathol.*, 76:451-468.
- Hruban, Z. (1979) Ultrastructure of hepatocellular tumors. *J. Toxicol. Environ. Health*, 5:403-431.
- Iancu, T.C., Lichterman, L., and Neustein, H.B. (1978) Hepatic sinusoidal cells in iron overload. Ultrastructural observations. *Isr. J. Med. Sci.*, 14:1191-1201.
- Jezequel, A.M., Librari, M.L., Mosca, P.G., and Novelli, G. (1983) The human liver in extrahepatic cholestasis: ultrastructural morphometric data. *Liver*, 3:303-314.
- Jezequel, A.M. (1985) Some aspects of ultrastructural pathology. In: *Liver and Biliary Disease*. R. Wright, G.H. Millward-Sadler, K.G.M.M. Alberti and S. Karan, eds. Baillière Tindall, London, pp. 517-536.
- Johannessen, J.V. (1979) The liver, the gallbladder and biliary ducts. In: *Electron Microscopy in Human Medicine*. Vol. 8, J.V. Johannessen, ed. McGraw-Hill International Book Company, New York.
- Jones, E.A., and Summerfield, J.A. (1985) Functional aspects of hepatic sinusoidal cells. *Semin. Liver Dis.*, 5:157-174.
- Jones, E.A., and Summerfield, J.A. (1988) Kupffer cells. In: *The Liver: Biology and Pathobiology*. Second Edition. I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz, eds. Raven Press Ltd, New York, pp. 683-704.
- Kaneda, K., Kurioka, N., Seki, S., Wake, K., and Yamamoto, S. (1984) Pit cell-hepatocyte contact in autoimmune hepatitis. *Hepatology*, 4:955-958.
- Lafon, M.E., Bioulac-Sage, P., Grimaud, J.A., Boussarie, L., Merlio,

- J.P., Reiffers, J., and Balabaud, C. (1987) Perisinusoidal fibrosis of the liver in patients with thrombocytopenic purpura. *Virchows Arch. A*, 411:553–559.
- Lafon, M.E., Bioulac-Sage, P., Le Bail, B., Quinton, A., Saric, J., and Balabaud, C. (1988) Nerves and perisinusoidal cells in human liver. In: *Cells of the hepatic sinusoid*. Vol. 2, E. Wisse, D.L. Knook and K. Decker, eds. Kupffer Cell Foundation, Rijswijk.
- Lamouliatte, H., Dubroca, J., Quinton, A., Balabaud, C., and Bioulac-Sage, P. (1985) Sinusoids in human cirrhotic nodules: better identification with the perfusion fixation technique. *J. Submicrosc. Cytol.*, 17:279–286.
- Lapis, K., and Schaff, Z. (1979) Acute viral hepatitis. In: *Electron Microscopy in Human Medicine: The Liver*. Vol. 8, J.V. Johannessen, ed. McGraw-Hill International Book Company, New York, pp. 124–136.
- Latry, P., Bioulac-Sage, P., Echinard, E., Gin, H., Boussarie, L., Grimaud, J.A., and Balabaud, C. (1987) Perisinusoidal fibrosis and basement membrane-like material in the livers of diabetic patients. *Human Pathol.*, 8:775–780.
- Leo, M.A., and Lieber, C.S. (1988) Hypervitaminosis A: A liver lover's lament. *Hepatology*, 8:412–417.
- Lieber, C.S., and Leo, M.A. (1986) Interaction of alcohol and nutritional factors with hepatic fibrosis. In: *Progress in Liver Diseases*. Vol. 8, H. Popper and F. Schaffner, eds. Grune and Stratton, New York, pp. 253–272.
- Lough, J., Rosenthal, L., Arzoumanian, A., and Goresky, C.A. (1987) Kupffer cell depletion associated with capillarization of liver sinusoids in carbon tetrachloride-induced rat liver cirrhosis. *J. Hepatol.*, 5:190–198.
- McCuskey, R.S. (1988) Hepatic microcirculation. In: *Sinusoids in human liver: Health and disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 151–164.
- Milici, A.J., Peters, K.R., and Palade, G.E. (1986) A new structure in fenestrated endothelia. *Cell Tissue Res.*, 244:493–499.
- Minato, Y., Hasumura, Y., and Takeuchi, J. (1983) The role of fat-storing cells in Disse space fibrogenesis in alcoholic liver disease. *Hepatology*, 3:559–556.
- Nolan, J.P., and Cohen, S.A. (1988) The role of hepatic sinusoidal cells in the antitumor defense of the liver. In: *Sinusoids in human liver: health and disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 341–358.
- Nyberg, A., Berne, B., Nordlinder, H., Busch, C., Eriksson, U., Loof, L., and Vahlquist, A. (1988) Impaired release of vitamin A from liver in primary biliary cirrhosis. *Hepatology*, 8:136–141.
- Panner, B.J., and Hanss, R.J. (1968) Hepatic injury in mushroom poisoning: electron microscopic observations of two neo fatal cases. *Arch. Pathol.*, 87:35–45.
- Pfeifer, U., and Aterman, K. (1979) Shedding of peripheral cytoplasm—a mechanism of liver cell atrophy in human amyloidosis. *Virchows Archiv.*, 29:229–243.
- Pfeifer, U. (1983) Ultrastructural pathology of the human liver. In: *Clinical Hepatology*. G. Csomas and H. Thaler, eds. Springer-Verlag, Berlin, pp. 159–194.
- Phillips, M.J., Latham, P.S., and Poucell, S. (1987a) Electron microscopy of human liver diseases. In: *Diseases of the liver*. L. Schiff and E.R. Schiff, eds. J.B. Lippincott Company, Philadelphia, pp. 47–76.
- Phillips, M.J., Poucell, S., Patterson, J., and Valencia, P. (1987b) *The Liver. An Atlas and Text of Ultrastructural Pathology*. Raven Press, New York.
- Popper, H., and Acs, G. (1985) Regulatory factors in pathologic processes of the liver modulators and interacting metabolic networks. *Semin. Liver Dis.*, 5:191–208.
- Raymond, J.M., Dubuisson, L., Quinton, A., Bioulac-Sage, P., and Balabaud, C. (1987) Benign recurrent cholestasis: a light and electron microscopic study with emphasis on sinusoidal cells. *Ultrastruct. Pathol.*, 11:11–17.
- Reid, L.M., Abreu, S.L., and Montgomery, K. (1988) Extracellular matrix and hormonal regulation of synthesis and abundance of Messenger RNAs in cultured liver cells. In: *The Liver: Biology and Pathobiology*. I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter and D.A. Shafritz, eds. Second Edition. Raven Press Ltd, New York, pp. 717–737.
- Rojkind, M. (1988) Extracellular matrix. In: *The Liver: Biology and Pathobiology*. Second Edition. I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz. eds. Raven Press Ltd, New York, pp. 707–716.
- Rojkind, M., and Greenwel, P. (1988) The liver as a bioecological system. In: *The Liver: Biology and Pathobiology*. Second Edition. I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter and D.A. Shafritz, eds. Raven Press Ltd, New York, pp. 1269–1285.
- Schaffner, F., and Popper, H. (1963) Capillarization of hepatic sinusoids in man. *Gastroenterology*, 44:239–242.
- Sherwood, E.R., and Williams, D.L. (1988) The role of hepatic sinusoidal cells in the antitumor defense of the liver. In: *Sinusoids in Human Liver: Health and Disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 359–368.
- Skinner, M.S., Kattine, A.A., and Spurlock, B.O. (1966) Electron microscopic observations of early amyloidosis in human liver. *Gastroenterology*, 50:243–247.
- Steinhoff, G., Wonigeit, K., and Pichlmayr, R. (1987) Modified donor MHC-expression and replacement of Kupffer cells in human liver grafts. *Hepatology*, 5:S65 (abstract).
- Sztark, F., Bioulac-Sage, P., Latry, P., Quinton, A., Balabaud, C., and Bioulac-Sage, P. (1986a) Perisinusoidal cells in patients with normal liver histology: a morphological study. *J. Hepatol.*, 2:358–369.
- Sztark, F., Latry, P., Quinton, A., Balabaud, C., and Bioulac-Sage, P. (1986b) The sinusoidal barrier in alcoholic patients without liver fibrosis: a morphometric study. *Virchows Arch. A*, 409:385–393.
- Tabarin, A., Bioulac-Sage, P., Merlio, J.P., Lamouliatte, H., Saric, J., and Balabaud, C. (1986) Sinusoids ultrastructure of human hepatocellular carcinoma. *J. Submicrosc. Cytol.*, 18:171–176.
- Tabarin, A., Bioulac-Sage, P., Boussarie, L., Balabaud, C., de Marsacarel, A., and Grimaud, J.A. (1987) Hepatocellular carcinoma developed on noncirrhotic livers. *Arch. Pathol. Lab. Med.*, 111:174–180.
- Takahashi, T., Kamimura, T., and Ichida, F. (1987) Ultrastructural findings on polymorphonuclear leucocyte infiltration and acute hepatocellular damage in alcoholic hepatitis. *Liver*, 7:347–358.
- Tanikawa, K. (1979) Ultrastructural aspects of the liver and its disorders. Igaku-Shoin, Tokyo.
- Tobe, K., Tsuchiya, T., Fujiwara, R., Yamada, G., Nagashima, H., Sasaoka, K., Mimura, H., and Motoi, M. (1985) Kupffer cells in well-differentiated tissue of hepatocellular carcinoma. *Acta Hepatol. Jpn.*, 26:630–637.
- Vos (de), R., and Desmet, V.J. (1979) Ultrastructural aspects of cholestasis. *Acta Gastroenterol. Belg.*, 42:328–333.
- Vuitton, D.A., and Seilles, E. (1988) Immunological aspects of sinusoidal cell functions. In: *Sinusoids in Human Liver: Health and Disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 165–187.
- Wisse, E., Van T Noordende, J.M., Van der Meulen, J., and Daems, W.T. (1976) The pit cell: description of a new type of cell occurring in rat liver sinusoids and peripheral blood. *Cell Tissue Res.*, 173:423–435.
- Wisse, E., and De Leeuw, A.M. (1984) Structural elements determining transport and exchange processes in the liver. In: *Microspheres and Drug Therapy. Pharmaceutical, Immunological and Medical Aspects*. S.S. Davis, L. Illum, J.G. McVie, and E. Tomlinson, eds. Elsevier Science Publishers B.V., Amsterdam, pp. 1–23.
- Wisse, E., de Zanger, R.B., Charels, K., Van Der Smissen, P., and McCuskey, R.S. (1985) The liver sieve: considerations concerning the structure and function of endothelial fenestrae, the sinusoidal wall and the space of Disse. *Hepatology*, 5:683–692.
- Zafrani, E.S., Pinaudeau, Y., and Dhumeaux, D. (1983) Drug-induced vascular lesions of the liver. *Arch. Intern. Med.*, 143:495–502.
- Zafrani, E.S., Degos, F., Guigui, B., Durand-Schneider, A.M., Martin, N., Flandrin, G., Benhamou, J.P., and Feldmann, G. (1987) The hepatic sinusoid in hairy cell leukemia: an ultrastructural study of 12 cases. *Human. Pathol.*, 18:801–807.
- Zafrani, E.S., and Feldmann, G. (1988) Primary lesions of the hepatic sinusoid. In: *Sinusoids in Human Liver: Health and Disease*. P. Bioulac-Sage and C. Balabaud, eds. Kupffer Cell Foundation, Rijswijk, pp. 207–222.