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Structure and Function of Sinusoidal Lining Cells in the Liver*

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ABSTRACT

The hepatic sinusoid harbors 4 different cells: endothelial cells (100, 101), Kupffer cells (96, 102, 103), fat-storing cells (34, 51, 93), and pit cells (14, 107, 108). Each cell type has its own specific morphology and functions, and no transitional stages exist between the cells. These cells have the potential to proliferate locally, either in normal or in special conditions, that is, experiments or disease. Sinusoidal cells form a functional unit together with the parenchymal cells. Isolation protocols exist for all sinusoidal cells.

Endothelial cells filter the fluids, exchanged between the sinusoid and the space of Disse through fenestrae (100), which measure 175 nm in diameter and are grouped in sieve plates. Fenestrae occupy 6–8% of the surface (106). No intact basal lamina is present under these cells (100). Various factors change the number and diameter of fenestrae [pressure, alcohol, serotonin, and nicotin; for a review, see Fraser et al (32)]. These changes mainly affect the passage of lipoproteins, which contain cholesterol and vitamin A among other components.

Fat-storing cells are pericytes, located in the space of Disse, with long, contractile processes, which probably influence liver (sinusoidal) blood flow. Fat-storing cells possess characteristic fat droplets, which contain a large part of the body's depot of vitamin A (91, 93). These cells play a major role in the synthesis of extracellular matrix (ECM) (34, 39–41). Strongly reduced levels of vitamin A occur in alcoholic livers developing fibrosis (56). Vitamin A deficiency transforms fat-storing cells into myofibroblast-like cells with enhanced ECM production (38).

Kupffer cells accumulate in periportal areas. They specifically endocytose endotoxin (70), which activates these macrophages. Lipopolysaccharide, together with interferon γ, belongs to the most potent activators of Kupffer cells (28). As a result of activation, these cells secrete oxygen radicals, tumor necrosis factor, interleukin 1, interleukin 6, and a series of eicosanoids (28) and become cytotoxic against tumor cells [e.g., colon carcinoma cells (19, 22, 48)]. Toxic secretory products can cause necrosis of the liver parenchyma, which constitutes a crucial factor in liver transplantation (55).

Pit cells possess characteristic azurophylic granules and display a high level of spontaneous cytolytic activity against various tumor cells, identifying themselves as natural killer cells (10). The number and cytotoxicity of pit cells can be considerably enhanced with biological response modifiers, such as Zymosan or interleukin 2 (8). Pit cell proliferation occurs within the liver, but recent evidence indicates that blood large granular lymphocytes develop into pit cells in 2 steps involving high- and low-density pit cells (88). Kupffer cells control the motility, adherence, viability, and cytotoxicity of pit cells (89), whereas cytotoxicity against tumor cells is synergistically enhanced (80, 81).

Keywords. Kupffer cell; endothelial cell; fat-storing cell; pit cell; microscopy; ultrastructure; review; cell biology

INTRODUCTION

In and around the hepatic sinusoids, we encounter 5 cell types: Kupffer cells, endothelial cells, fat-storing cells, pit cells, and parenchymal cell (Fig. 1). Each of these cells shows a characteristic fine structure, which is well preserved on the condition that the tissue is fixed by perfusion, preferably with a low concentration of buffered and isotonic glutaraldehyde solution (100, 105). Relative volumes were measured by morphometric methods in rat liver (2) and illustrate the relative importance of the following components in the tissue: the sinusoidal lumen (10.6%), the space of Disse (4.9%), the volumes of parenchymal cells (78%), endothelial cells (2.8%), Kupffer cells (2.1%), and fat-storing cells (1.4%). These data refer to volumes, not numbers of cells. Sinusoidal cells contribute 26% to the total cell membrane surface (mainly endothelial cells), 58% to the volume of pinocytotic vesicles (mainly endothelial cells), 43% to the total lysosomal volume (mainly Kupffer cells + endothelial cells), and 55% to the total fat droplet volume (mainly fat-storing cells) (2). These data show that sinusoidal cells, occupying only 6.3% of the tissue volume, might play an important role in pinocytosis, digestion of endocytosed material, and storage of vitamin A in fat droplets of fat-storing cells.

Endothelial cells, fat-storing cells, and parenchymal cells are sessile cells. In contrast, Kupffer cells and pit cells seem to be mobile cells, adhering to the endothelial lining. Each cell type seems to be autoproliferative, but proliferation can be considerably increased by biological response modifiers (4, 5), interleukins (8), or partial hepatectomy (5). Recruitment of Kupffer cells and pit cells from extrahepatic sources, like the bone marrow, seems to occur (7, 8). The origin and differentiation of endothelial cells and fat-storing cells are much less debated, although considerable attention has been paid to the distribution, proliferation, and changes of fat-storing cells during liver damage, disease, or fibrosis (34, 38, 47, 59, 65, 76, 91, 93, 98, 109). Each of the sinusoidal cells has different functions. They also take different positions but

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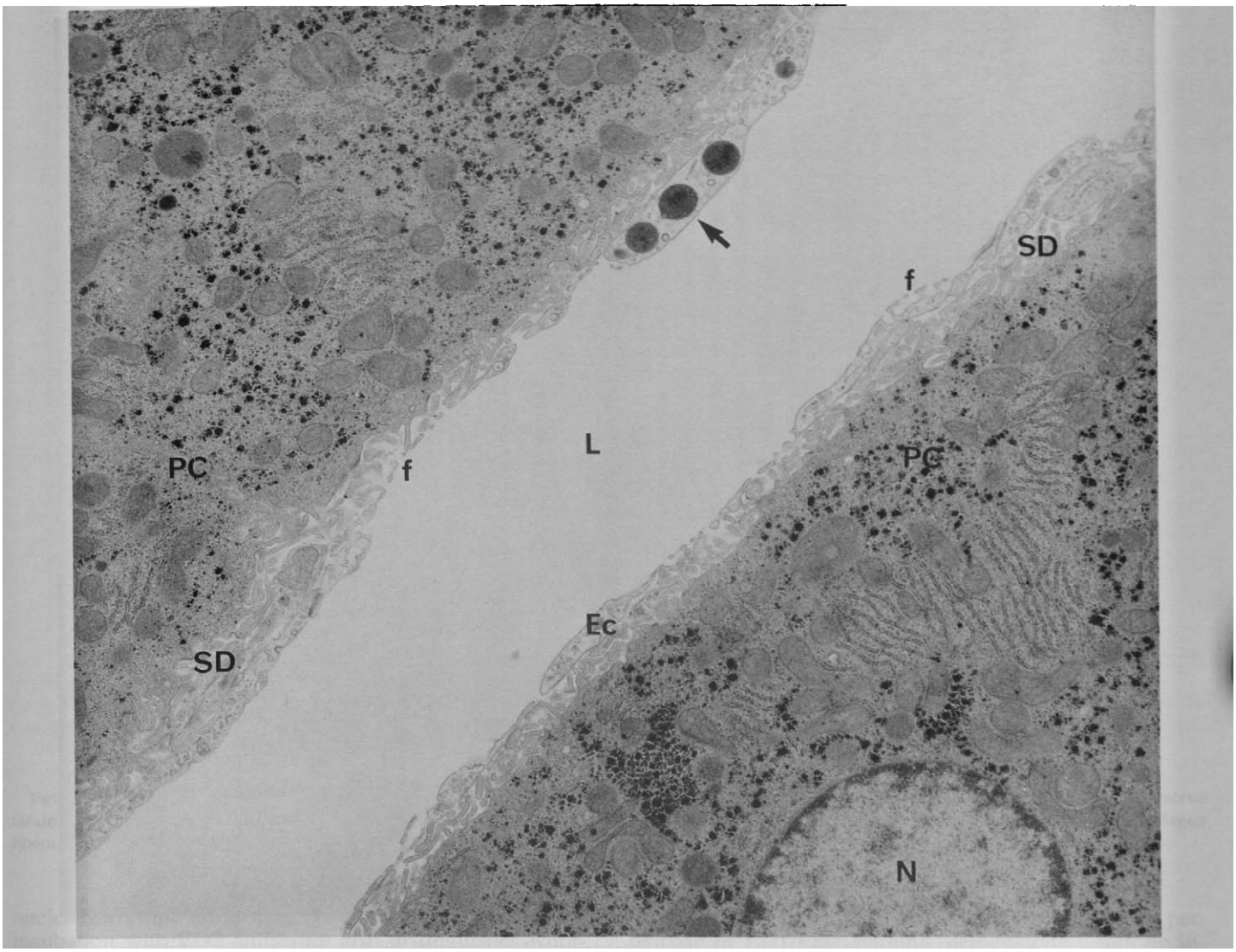

FIG. 1.—A transmission electron micrograph of a longitudinal section through a rat liver sinusoid. The wall of the sinusoid is composed of a thin layer of fenestrated endothelium, which is covering the space of Disse, filled by microvilli extending from the parenchymal cell surface. The endothelial lining shows the presence of dense bodies or lysosomes (arrow). The parenchymal cells contain glycogen, a nucleus, endoplasmic reticulum, and numerous organelles. ×9,200. Abbreviations used in Figs. 1–5: Ec = endothelial cell; f = fenestrae; L = sinusoidal lumen; N = nucleus; PC = parenchymal cell; SD = space of Disse.

form a functional unit together with parenchymal cells. In experiments and disease, these cells display different and cell-specific reactions, as an expression of their individual contributions to liver function. Comparable, but not equal, cells are found in other tissues. Research has been stimulated by new techniques for the isolation, purification, and culture of all 4 sinusoidal cells (1, 10, 16, 26, 54, 83). Present research is focusing on the network of cytokines that is supported by these cells and that they use to communicate in normal, experimental, and disease conditions (28, 29).

CELLULAR STRUCTURE

Endothelial cells are sessile cells (see Fig. 2) constituting the sinusoidal wall, the endothelium, or endothelial lining (Fig. 1). Endothelial cells form a fenestrated lining containing open (undiaphragmed) fenestrations of 0.17 μm in diameter (100). Fenestrae are grouped, and form sieve plates, separated by cytoplasmic arms. A lobular gradient of decreasing fenestrae diameter is compensated by an inverse gradient of fenestrae number (106). The porosity of the endothelial lining approaches about 10%, meaning that only a limited surface of the lining is available for free exchange. Free passage of fluid, solutes, and small particles is secured by the absence of a basement membrane underlying the endothelial lining. Both fenestrae and sieve plates are delineated by a cytoskeleton ring, composed of a hitherto unknown cytoskeletal protein (15, 17, 86, 87). At the cell membrane and in the adjacent, peripheral cytoplasm, a multitude of coated pits

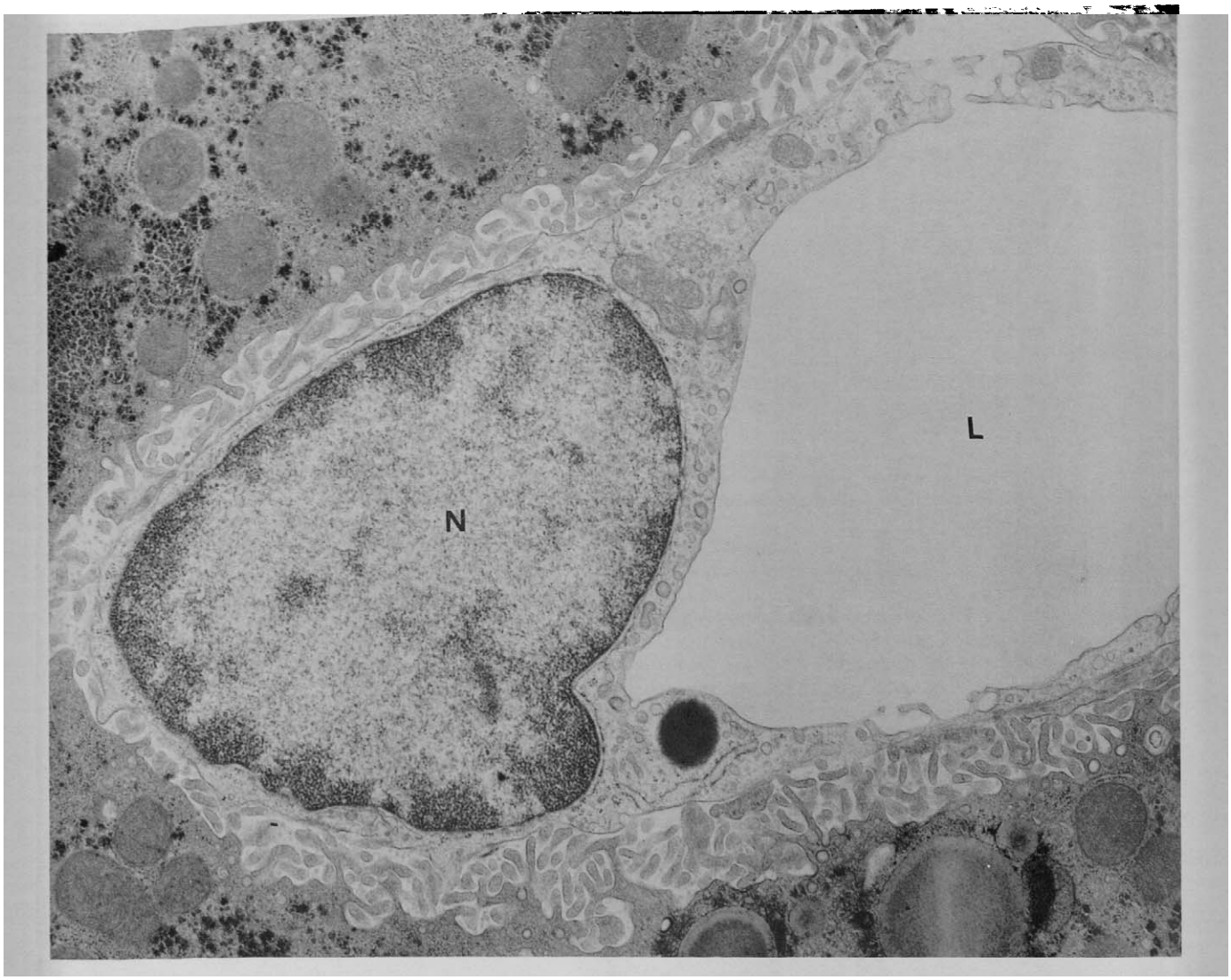

FIG. 2.—An endothelial cell with limited perinuclear cytoplasm, containing a few organelles, such as mitochondria, a lysosome, and a few cisternae of endoplasmic reticulum. The endothelial cell rests on the microvilli filling the space of Disse. ×16,000.

in different stages of pinching off can be observed (Fig. 2). A peculiar vacuole, connected to a bending of the cellular membrane, can sometimes be observed. This structure is probably a macropinocytotic vesicle, thought to contribute to the process of endocytosis, but was hitherto described by only 1 author (101). In the perikaryon, we recognize numerous lysosomes of almost equal diameter but varying densities (101). The nucleus of endothelial cells sometimes contains a sphaeridium (a peculiar body in the nucleus), whereas in the cytoplasm a special smooth endoplasmic reticulum can be observed, connected to the rough endoplasmic reticulum (RER) (101). This RER sometimes closely approaches the cell membrane, showing a lack of ribosomes at the face paralleling the cellular membrane (101). Incidental fragments of basal lamina-like material can be observed (100, 101) in-between endothelial cells and fat-storing cells. These fragments are probably explaining the incidental positive immunostaining of basal lamina components such as laminin (35, 36, 58).

Kupffer cells are resident macrophages, adhering to the endothelial lining and giving the impression that they have variable shapes, probably meaning that they are motile and are capable of migrating (102, 103) (Fig. 4). Kupffer cells are stellate cells (Sternzellen), which are preferentially located in the periportal sinusoids, as is clear from their lobular distribution, that is, 43, 28, and 29% for portal, midzonal and central areas, respectively. Kupffer cells mostly show an abundant cytoplasm, containing lysosomes or dense bodies with varying diameters and densities. Peculiar structural details of Kupffer cells are a fuzzy cell coat, wormlike structures, annulate lamellae, and fuzzy-coated vacuoles, which can be seen after special preparatory conditions (96, 101, 103). After incubation with buffered diaminobenzidin and hydrogen peroxide, these cells show a peroxidase-positive RER and

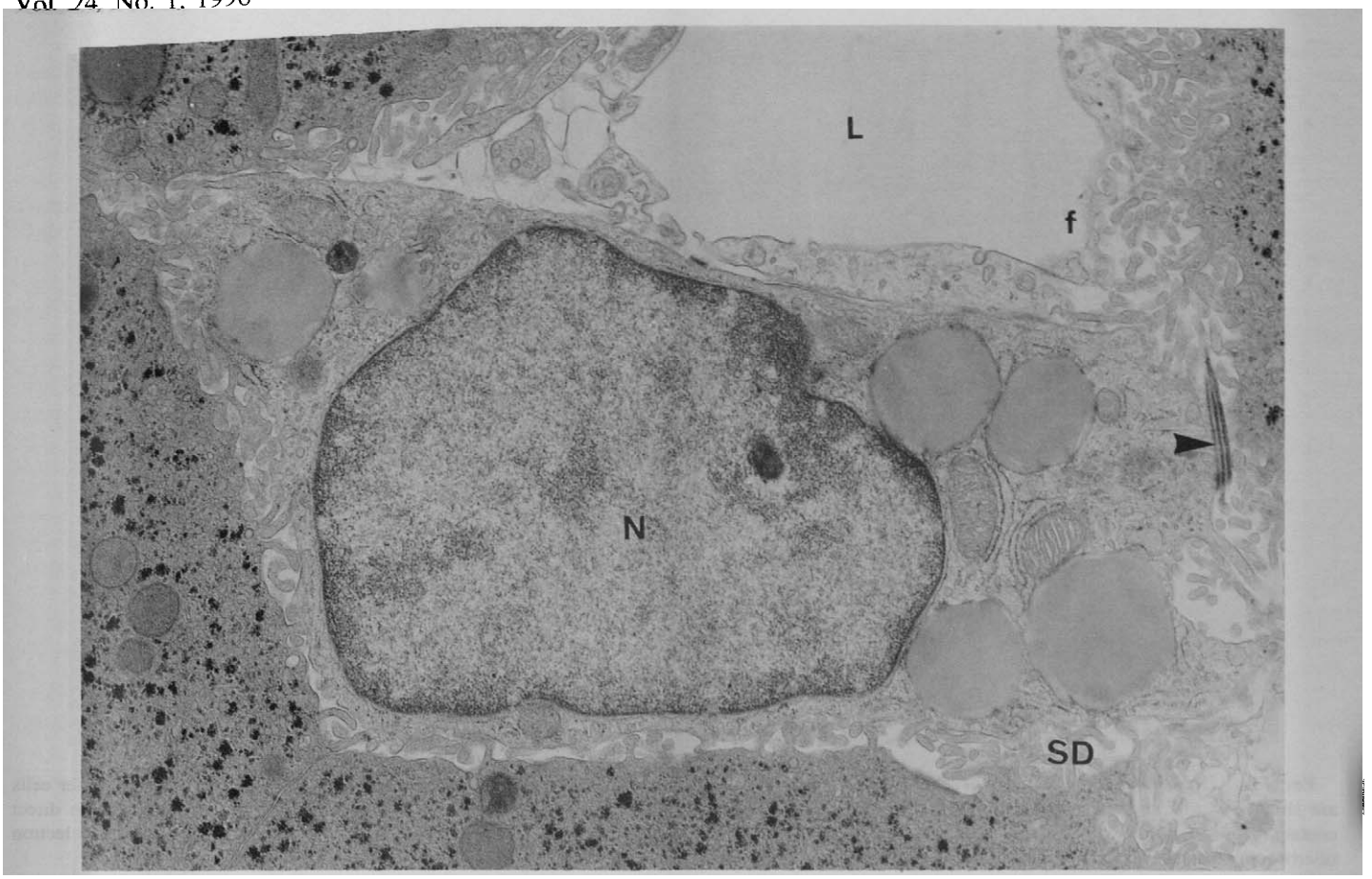

FIG. 3.—A fat-storing cell, as usual situated within the space of Disse, that is, covered by the endothelial lining. In the cytoplasm we observe fat droplets and cisternae of the endoplasmic reticulum, besides the nucleus. Usually, fat-storing cells are neighbored by several bundles of collagen fibers, of which only a small representation is shown here (arrowhead). ×13,200.

nuclear envelope in rat liver (101). Immunocytochemical incubation with ED2 antiserum also distinguishes Kupffer cells from monocytes and other cells within the liver tissue (21). Kupffer cells occur in numbers such as 1.4–2 × 10^7 cells/g liver (3). In the intact, normal liver, Kupffer cells exclusively phagocytose large particles *in vivo* (e.g., 0.8 μm latex) and the endocytotic mechanisms involve the fuzzy cell coat, pseudopods, wormlike structures, and vacuoles, together with many lysosomes (104). After destruction of Kupffer cells, or in single-cell cultures, other liver cells might also show the capacity to endocytose large particles (23). Kupffer cells are long-living cells, as demonstrated by experiments using vinblastin and ^{3}H-thymidine injections, which show a low mitotic index of 0.06% in the normal rat liver (3). After partial hepatectomy, zymosan, or interleukin 2 (IL-2) the mitotic index of Kupffer cells is enormously enhanced, resulting in a strong growth of the population (4, 5, 8). In a number of these experiments, the mitotic potential of Kupffer cells could explain the growth of the populations. However, in most cases a fraction of the resident macrophages (25% after zymosan) was recruited from extrahepatic sources, probably the bone marrow (4). Also, monocytes and monocyte-derived macrophages, which can be distinguished from Kupffer cells by peroxidase or ED1/ED2 staining, demonstrate a mitotic index within the liver of about 2.5% at 2–5 days after zymosan (11). Monocytes can invade the liver tissue but never reach a level exceeding 30% of the total number of macrophages (11). Transitional stages between monocytes and Kupffer cells are never seen (11, 102, 103). Further experiments indicated that the liver-resident macrophages or Kupffer cells stop proliferating when the liver itself is irradiated. Shielding the liver, and irradiation of the bone marrow together with splenectomy, do not inhibit the growth of the Kupffer cell population, as does liver irradiation (7). This means that in most cases Kupffer cell proliferation represents the predominant source for the growth of the population.

Fat-storing cells have been given many names: Ito cells, perisinusoidal cells, parasinusoidal cells, stellate cells, lipocytes, sinusoidal pericytes, and so on. Fat-storing cells are also sessile cells, containing characteristic fat droplets, which are clearly visible at low magnification (51) (Fig. 3). These fat droplets contain vitamin A, which is demonstrated by autofluorescence (91, 93). The number and volume of these fat droplets seem to depend on the physiological status of the cells (91). Pathological

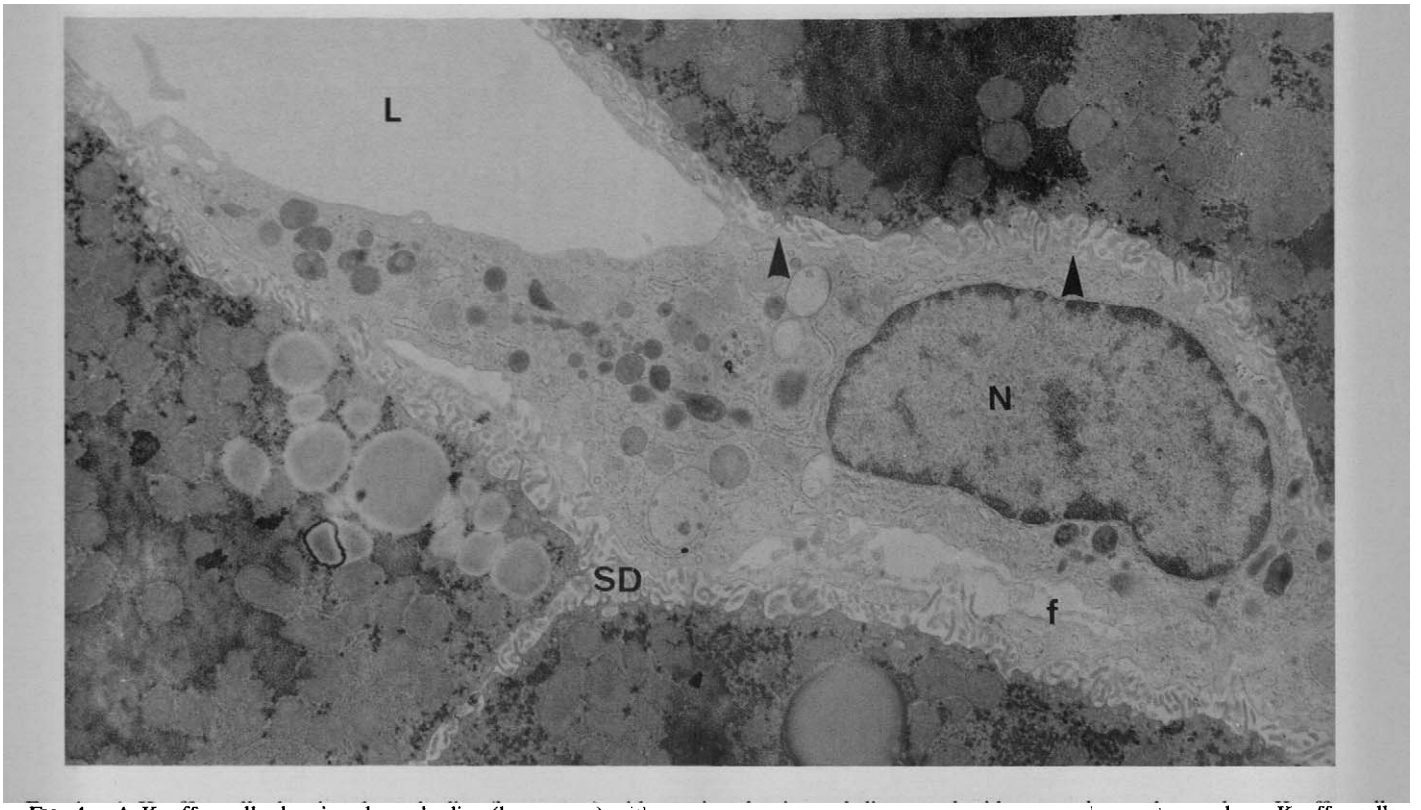

FIG. 4.—A Kupffer cell, showing dense bodies (lysosomes) with varying density and diameter, besides vacuoles, and a nucleus. Kupffer cells are situated within the sinusoid and are adhering to and sometimes replacing the endothelial lining. Kupffer cells are sometimes seen in direct contact with the microvilli of the parenchymal cells (arrowhead). Their stellate shape is mostly not well demonstrated in transmission electron microscopy, because of the 2-dimensional limitations of the ultrathin section. ×6,800.

conditions result in either an abundance or absence of these droplets. There are 2 types of fat droplets, one with a limiting membrane and one without (92). Fat-storing cells take position in the space of Disse, which means that they are always separated from the sinusoidal lumen by a thin layer of the endothelium (77, 100). Fat-storing cells are preferentially located in the periportal area (93). Fat-storing cells sometimes bear a cilium, but it is not clear whether all cells have one, or how this cilium is positioned, and what purpose it serves. Fat-storing cells have strongly developed intermediate filaments, such as desmin, which can be used for specific immunostaining of these cells in rat liver (82, 109). Unfortunately, the desmin staining cannot be applied on human liver. In normal and pathological human liver, fat-storing cells can be immunostained for α-smooth muscle actin (31), which stains only activated cells. Fat-storing cells have branching cytoplasmic processes, together forming a kind of cylindrical basket surrounding the sinusoid, as was beautifully shown by the Golgi impregnation studies of Wake et al (94, 95, 97, 98). During cell culture, and in experimental and pathological conditions, fat-storing cells can develop into myofibroblast-like cells, showing enhanced extracellular matrix (ECM) production and decreased number and volume of fat droplets (99).

Pit cells (108) show the morphology of large granular lymphocytes (52). These cells contain azurophylic granules, a characteristic that can be used to distinguish these lymphoid cells from other (agranular) lymphocytes in smears, cytospins, or other light microscopical preparations (Fig. 5). Granules in human and mouse pit cells are smaller and lower in number, which hampers the study of these cells in these species. Besides the granules, pit cells contain a Golgi apparatus, multivesicular bodies (108), and characteristic rod-cored vesicles (52). Pit cells show cytoplasmic polarity, meaning that they have their organelles situated at one side of the nucleus, the other side showing hyaloplasm, which is filled by a fine fibrillar actin meshwork not containing any organelles. Pit cells have a variable form and are apparently motile cells, like Kupffer cells. Pit cells adhere to the endothelial lining and are sometimes seen in contact with Kupffer cells. Pit cells occur in a frequency of 13.7/mm^2 frozen section of normal liver (57). The lobular distribution of pit cells is unequal: the periportal region contains 17.1 pit cells/mm^2, whereas the centrolobular region contains 10.8 pit cells/mm^2 (57). Pit cell numbers relate to Kupffer cell numbers as 1–10 (10). Pit cells have been immunophenotyped as OX-8$^+$, OX-19$^-$, asialo-GM1$^\pm$, and 3.2.3$^+$ (12, 57). With this 3.2.3 monoclonal antibody, the number of pit cells in the liver of a 270-g rat was estimated to be 34.10^6 cells (57). The specific granules of pit cells are also found in pit cells of the blood, which contain fewer but larger granules. Pit cells do not show pinocytosis or phagocytotic activities. The granules are comparable to the granules of endocrine cells and are supposed to contain per-

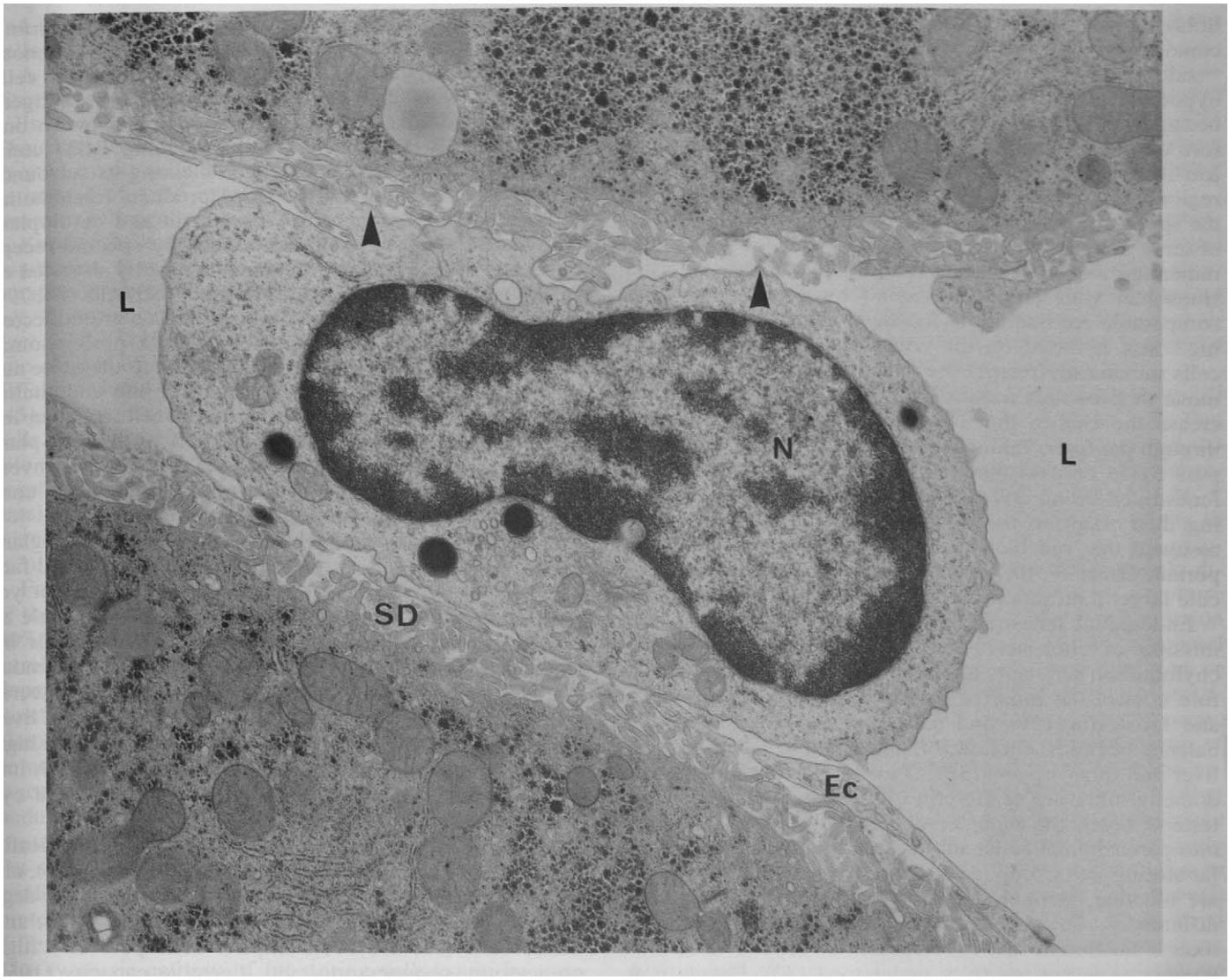

FIG. 5.—A pit cell with its typical dense granules. This pit cell shows close contact to the endothelial lining and is seen to contact microvilli of the parenchymal cells (arrowhead).

forin or substances used during spontaneous tumor cell killing. The granules stain for acid phosphatase, demonstrating that they have a lysosomal nature (10).

CELLULAR FUNCTION

Endothelial cells sieve the fluids, which are exchanged between the sinusoidal lumen and the space of Disse. This sieving probably has a major effect on the liver uptake of particles or rigid droplets, such as lipoproteins, of which chylomicrons and their remnants are involved in the bulk transport of lipids. A realistic filtrating effect could be measured by comparing chylomicrons in the portal blood with those in the space of Disse, demonstrating that the largest particles in the space of Disse were as large as fenestrae (27, 64). The porosity of the lining was found to be in the order of 10%, meaning that 90% of the lining is represented by continuous endothelium (106). Porosity was found to be slightly increased in the centrolobular area, probably compensating for the periportal preference of uptake and transport (106). Endothelial cells are sensitive to alcohol. On a short time basis, alcohol enlarges fenestrae (18, 87). Loss of fenestrae and the formation of a real basal lamina may occur in several diseases, such as alcohol abuse and cirrhosis (32, 47, 50). Endothelial cells in culture respond to the microfilament-blocking agent cytochalasin B by more than doubling the number of their fenestrae (75). Endothelial cells possess a high endocytotic capacity for fluids, molecules (67), and small particles (101, 104).

Liver exchange and transport processes involve the endocytotic capacity of Kupffer cells, endothelial cells, and parenchymal cells, the structural characteristics of the endothelial lining, the space of Disse, and the microvillous surface of the parenchymal cells. The microvilli of the parenchymal cells enlarge the surface by a factor of 5–6 (46), whereas on the other side the endothelial fenestrae limit the free access to the parenchymal cell by a factor of 10 (10%). To overcome the difficulty of bringing fresh

fluids into the space of Disse and to refresh the fluids in contact with the parenchymal cells, the mechanism of "endothelial massage" (106) might be important. In this hypothesis, white blood cells (WBCs) plug the sinusoid because they have an average size of 8.5 μm and therefore do not fit into a sinusoid, which measures from 5.9 μm in the portal region to 7.1 μm in the centrolobular region. As a result, WBCs impress the endothelium *and* the space of Disse. By using *in vivo* microscopy, one can observe interactions between WBCs and the sinusoids, indicating that WBCs plug, deform, and move along the sinusoidal wall (106). A second hypothesis based on comparable considerations has been named "forced sieving" and is based on the consideration that red blood cells unilaterally restrict the space in which lipoproteins move in Brownian motion. Red blood cells therefore increase the chance that lipoprotein droplets will escape through the filter. Taking into account that red blood cells pass by in endless numbers, while gently touching the fenestrated lining and in the meantime constantly adapting their shape to the dimensions of the sinusoid, it is assumed that red blood cells in their turn exert an important effect on the passage of any (aggregated) molecule larger than water through the endothelial filter (106).

Endothelial fenestrae are supposed to control the admission of cholesterol-enriched, vitamin A–containing chylomicron remnants to the space of Disse. They therefore control the input of remnants to parenchymal cells and fat-storing cells and play an important role in the balance of lipids, cholesterol, and vitamin A between the liver and other organs (32). Dynamic changes of the endothelial filter are supposed to influence the plasma cholesterol level, the input of cholesterol and triglycerides into parenchymal cells, and the transport of vitamin A to fat-storing cells. Important factors influencing fenestrae are nicotine, alcohol, serotonin, pantethine, and species differences. To what extent changes in the endothelial filter contribute to the development of fatty liver or atherogenesis is not yet fully understood (32). Recently, it was demonstrated that the transient fatty liver occurring after partial hepatectomy correlated with the enlargement of endothelial porosity and loss of sieve plates (62). Alcoholic defenestration, resulting from chronic alcohol abuse, inhibits retinoid transport to parenchymal and fat-storing cells, which stimulates fat-storing cells to develop into myofibroblasts. The latter cells proliferate and produce more collagen, resulting in perisinusoidal fibrosis. Here again, the role of structural changes in sinusoidal cells versus pathogenic and biochemical changes in parenchymal cells are not fully investigated.

Endothelial cells have a high pinocytotic and a high digestive capacity, as shown by the presence of a multitude of coated pits, vacuoles, and dense bodies. Endothelial cells also possess receptors at their cell membrane, which enable them to clear specific substances from the blood rapidly (74). To this group of substances belong hyaluran and other ECM components, which can be used to specifically label endothelial cells and measure the specific clearance function of liver endothelial cells. Besides this hyaluran receptor, other receptors are described, such as the scavenger [taking up NH_2-terminal propeptide of type I collagen (60) and formaldehyde-treated albumin], collagen α-chain receptor, Fc receptor (85), and mannose receptor (67, 73). The Fc receptor of endothelial cells might be involved in the clearance of soluble antigen/antibody complexes (85). The cooperation between one sinusoidal cell (fat-storing cells) producing ECM components and another sinusoidal cell clearing its surrounding tissue fluid from soluble ECM products (endothelial cells) might be meaningful. Transferrin and ceruloplasmin are taken up by endothelial cells by specific receptors, after which these compounds are partly digested or desialylated and transferred to parenchymal cells (78, 79). After endocytosis by coated pits, materials are collected in fused vacuoles, to be considered as a prelysosomal compartment, before they are transported to the quite numerous lysosomes in the perikaryon of the endothelial cells. Parenchymal cells and endothelial cells together ingest the enormous volume of about 12% of the total plasma volume per day (66). It is not yet discussed or investigated what this means for the turnover of plasma constituents.

Endothelial cells produce prostaglandin I_2, prostaglandin E_2, thromboxaue A_2, endothelin, Van Willebrand factor, IL-1, IL-6 (68), and interferon (IFN). It is not yet clearly established whether endothelial cells are able to elicit an acute phase reaction in parenchymal cells by their IL-6 production. Also, it is unclear whether endothelial cells can activate Kupffer cells through the secretion of IFN-γ. It is important to know, however, that liver sinusoidal endothelial cells are peculiar cells with a high level of endocytotic and digestive capacity, and with important metabolic products, taking part in the local cytokine cross-talk.

Kupffer cells are resident macrophages and specifically endocytose lipopolysaccharide (LPS; endotoxin) (70), old and foreign cells, parasites, bacteria, viruses, fibrin degradation products (30), particulate substances (103), and tumor cells (33, 69, 108). Kupffer cells possess a high pinocytotic, phagocytotic, and digestive capacity (105). Plasma fibronectin, complement, or immunoglobulins promote the recognition, velocity, and specificity of endocytosis but are not obligatory for Kupffer cell phagocytosis or reticuloendothelial system clearance.

After endotoxin uptake, Kupffer cells become activated and secrete important molecules, such as superoxide, nitric oxide, eicosanoids, platelet activating factor, leukotrienes, ILs, IFN-α/β, and tumor necrosis factor α (TNF-α), some of which are involved in suppressing or accelerating autocrine loops (28). Instead of LPS, Kupffer cells can also become activated by other substances, such as Zymosan, OK 432, bacterie Calmette-Guerin, IFN-γ, colony-stimulating factor, macrophage-activating factor, platelet-activating factor, arachidonic acid, and substances like CCl_4, phorbol myristate acetate, A23187, and acetaminophen. Endotoxin, therefore, seems to be one of the potent activators of Kupffer cells. Although endotoxin is considered to be toxic to cells *in vivo*, it is important to note that endotoxin is not toxic to cells *in vitro*. Endotoxin becomes toxic to cells and tissues because of the products released by Kupffer cells and inflammatory cells. The toxicity of LPS is therefore indirect

and depends on the release of toxic products by Kupffer cells and other cells reaching in a comparable way.

A different form of cytotoxicity of Kupffer cells is directed against tumor cells. It is apparent that Kupffer cells need activation before becoming cytotoxic (20). Their spectrum of cytotoxicity is narrower than the one exerted by pit cells and is operating at a slower pace (48 hr instead of 4–16 hr *in vitro*), as is determined by different experimental setups (6, 19, 22).

Kupffer cells proliferate in a variety of experimental conditions. Kupffer cell mitoses or ^{3}H-thymidine incorporation have been demonstrated after partial hepatectomy, zymosan, OK 432, IL-2, and obstruction of the bile duct (3–5, 7, 102). Kupffer cells can be subfractionated into classes of different diameter, which show functional differences and different locations within the liver lobule (45). Bile acids inhibit the endocytosis of LPS by Kupffer cells, which might result in endotoxemia, which inhibits bile secretion and, in turn, promotes intestinal LPS resorption, creating a vicious cycle (84). In fetal and neonatal liver, Kupffer cells form the center of erythroblastic islands, controlling the development of red blood cells (63).

Fat droplets of rat *fat-storing cells* contain about 75% of the liver content of vitamin A. The liver contains about 90% of the total body vitamin A. The formation, size, number, and vitamin A content of fat droplets seems to depend on physiological circumstances, species, and the input of retinoids (110). After isolation and plating of almost pure populations of fat-storing cells, it could be demonstrated that these cells synthesize and secrete collagen types I and III–VI, fibronectin, laminin, tenascin, undulin, hyaluronic acid, biglycan, decorin, syndecan containing chondroitin, heparan, and dermatan sulfate. Fat-storing cells also synthesize and secrete matrix-degrading metalloproteinases, tissue inhibitor of metalloproteinases, cytokines, and growth factors and express different receptors [for a review, see Geerts et al (34)]. The synthetic activity of fat-storing cells could be measured *in vitro* after culturing the cells at least for a few days. Freshly isolated cells produce protein in quantities that are too small to be measured. Molecular biology methods, such as northern blot, RNAase protection, and polymerase chain reaction are sensitive enough to find messenger RNAs for different ECM components shortly after isolation, enabling the analysis of the ECM products of quiescent fat-storing cells. These methods also allow study of the changes of each individual ECM product in these cells during the development into myofibroblast-like cells (36). As an effect of inflammation, which occurs after parenchymal cell damage in disease or experiments, fat-storing cells can change their position and accumulate in necrotic areas (37). Fat-storing cells change into myofibroblasts, which produce progressively increasing amounts of protein, in particular, collagen types I, III, and IV and fibronectin, laminin, chondroitin, dermatan sulfate, and hyaluronate (38, 43). During this development, fat-storing cells loose their fat droplets and vitamin A, which can be reversed by application of retinoids. Fat-storing cells are most probably activated to develop into myofibroblasts by cytokines and products arising from Kupffer cells (42, 44, 71), inflammatory cells, or acute phase reactants, when liver tissue damage occurs. It was demonstrated that Kupffer cell–conditioned medium contains transforming growth factor β, which is a potent stimulator of collagen synthesis by fat-storing cells (25). It is amazing to conclude that so many and varying pathological events result in the activation of fat-storing cells and development of fibrosis.

As a result of the contractility, which was demonstrated *in vitro* (53) and *in vivo* (111), it might be assumed that fat-storing cells have the capacity to influence sinusoidal blood flow. Another important aspect of fat-storing cell function, which is hitherto not extensively investigated, is the contractility of the fat-storing cells and the possible effect on liver blood flow.

Pit cells have a proven capacity to spontaneously kill a variety of tumor cells in a MHC-unrestricted way, that is, without the involvement of antibodies or without the activation by cytokines or other signals (10). This capacity has been proven for tumor cells including YAC-1 cells, used to test specific natural killer (NK) activity in rat NK cells. Pit cells also possess NC (or natural cytotoxicity) activity, which is a cytolytic activity mainly directed against solid tumor cells, taking longer coculture times to be effective. This probably reflects upregulation of *de novo* synthesis of a cytotoxic factor (6). The level of cytolysis can be enhanced by injecting zymosan or infusing IL-2 (8), IFN-α, or IFN-β. Pit cells also proliferate after these signals and after partial hepatectomy, Zymosan, *P. acnes,* OK 432, and IL-2 (8, 13). Some of these stimulating agents represent particles. These are exclusively taken up by Kupffer cells. This indicates that effects of these particles on pit cells are most probably indirect effects. The stimulating particles most probably activate Kupffer cells, which then release a signal that in its turn activates the pit cells. After IL-2 infusion through the hepatic artery, pit cell counts went up 43 times when compared to normal tissue! In pit cell cultures, the strong locomotory activity, together with chemotactic attraction and a high deformability of pit cells, was observed (9). Pit cell granules probably contain perforin, a pore-forming protein, which kills the tumor cells. Intravenous injections of antibodies against NK cells enhance the metastasis of colon carcinoma cells in the liver (72). Pit cells are indeed able to kill colon carcinoma cells in a syngeneic model consisting of CC531 cells and inbred Wag/Rij rats (61).

Evidence resulting from different experiments indicates that pit cells are derived from blood LGL (88). Pit cells in the liver can be separated into 2 subfractions: low-density (LD) cells and high-density (HD) cells (90). LD cells have more but smaller granules than HD cells. HD cells show a stronger positive reaction for asialo-GM1, whereas the cytotoxicity of LD cells is about twice as strong as HD and 5 times as strong as the one of blood LGL (90). Adoptively transferred fluorescent HD cells could be found back in the liver as apparent LD cells, proving that HD cells develop into LD cells (90). It might therefore be concluded that (a fraction of) blood LGL home to the liver and develop into HD pit cells and later into LD pit cells. Several observations indicate that this

process takes about 2 wk. Recent evidence indicates that pit cells depend on the presence of a healthy population of Kupffer cells (89). In circumstances in which Kupffer cells become activated or increase in number by zymosan and other products, or are removed from the liver through killing by liposomes, the pit cells follow the trend of Kupffer cells with a lag phase of about 1 wk (89). A specific synergism was also found in cytotoxicity experiments, showing that pit cells and Kupffer cells do kill tumor cells better when they act together (80, 81).

Isolation, Purification, and Culture of Sinusoidal Cells

Because of the importance of cell isolation in experiments on the function of sinusoidal cells, we briefly summarize the procedure for obtaining suspensions of relative pure and viable cells (±90%). Many of the specific sinusoidal cell structures, functions, and responses are maintained during isolation and culture, such as the presence of fenestrae, phagocytosis, LPS response, vitamin A fluorescence, and cytotoxicity. The first steps in the isolation procedure are common to endothelial cells, Kupffer cells, and fat-storing cells:

1. isolation of cells starts with a portal liver perfusion with Gey's balanced salt solution (GBSS) without Ca^{++},
2. followed by perfusion with GBSS with Ca^{++} plus collagenase (with or without pronase, depending on the cell type),
3. after which pieces of liver are incubated in the same enzyme solution;
4. the cell suspension is filtered through nylon gauze,
5. and the cells are centrifuged, pelleted, and washed.

Further purification of endothelial cells (16) occurs by isopycnic centrifugation in a 2-step Percoll gradient, followed by selective adhesion, which removes Kupffer cells. Endothelial cells can be plated and cultured on collagen-coated plastic dishes in RPMI medium with the addition of fetal calf serum (FCS) and penicillin/streptomycin. Endothelial cells can be cultured for a few (2) days, after which they start accumulating fluid-filled vacuoles, transforming the cells into foam-cells, which loose viability 1 or 2 days later.

Kupffer cells can be purified with the same procedure as endothelial cells, but elutriation is often used as the final step in purification. Kupffer cells can be cultured in plastic dishes, containing DMEM medium with FCS and penicillin/streptomycin. Kupffer cells can be cultured for 5–10 days, during which they do not change substantially in morphology. Kupffer cells can proliferate in vitro under the influence of CSF-1 (49).

Fat-storing cells are purified by sequential centrifugation in Nycodenz, providing floating fat-storing cells in the top layer. Fat-storing cells can be cultured in plastic dishes with DMEM + FCS + penicillin/streptomycin. Cells can be cultured for many days but transform during culture into myofibroblast-like cells (24, 26), which can be subcultured almost permanently (34).

Pit cells are collected by simple, portal PBS washout with elevated hydrostatic pressure. Cells in the effluent, collected via the cannulated vena cava, are pelleted and layered on Ficoll-Hypaque. After centrifugation, the remaining cells are resuspended and filtered through a nylon wool column to remove adherent cells such as monocytes and adherent lymphocytes. Further purification is performed by gradient centrifugation on Percoll. Cells can be cultured, but after 2 days they die. A great advantage of the isolation method for pit cells is the absence of protein-degrading enzymes. This means that probably no destruction of the cell surface molecules takes place, providing the advantage of studying adhesion molecules, cellular contacts, and cytotoxicity with properly prepared cells (10). A disadvantage is the short lifetime of pit cells. Their viability goes down after 1 day, meaning that cytotoxicity experiments of a duration longer than 16 hr have limited value.

Conclusions: Health Aspects of Sinusoidal Cells

The permeabiblity characteristic of the liver endothelial filter (the liver sieve) is an important factor in the distribution of chylomicron remnants and therefore plays a role in the development of fatty liver, atherogenesis, and vitamin A distribution. The liver filter is probably involved in the pathogenesis of viral infections. Because of the restricting conditions imposed by the liver sieve, endothelial cells also promote the contact between the infecting organism and defensive cells, such as Kupffer cells.

Endotoxemia can develop as a result of reduced Kupffer cell function. On the other hand, patients with generalized infections or trauma have activated Kupffer cells, which secrete factors, causing parenchymal cell necrosis and probably increased discomfort and sickness. Activated Kupffer cells are cytotoxic to foreign cells and certain tumor cells. Kupffer cells are certainly involved in the process of metastasis of intestinal tumors.

Fat-storing cells can transform into myofibroblasts, which proliferate and deposit excessive amounts of collagen and other ECM products; they play a major role in fibrosis and cirrhosis. Fat-storing cells become activated during tissue damage, which activates Kupffer cells, which in their turn further activate fat-storing cells by the release of their products.

Pit cells are a natural defense system of the liver, showing cytotoxic behavior against immigrating tumor cells. It is known that NK cells also play a role in hematopoiesis and in killing virus-infected cells. Evidence for a role of pit cells in viral hepatitis or fetal hematopoiesis is lacking, and these subjects might therefore form "hot" targets for future pit cell research.

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