REVIEW ARTICLE

Scavenger functions of the liver endothelial cell

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INTRODUCTION

The liver of adult mammals regulates homeostasis by adding and extracting substances to or from the blood. To this end the liver supplies the blood with certain substances, such as the bulk of the plasma proteins, but sequesters other components, such as various waste products, to maintain a normal physiological equilibrium. This dual function is divided between the different populations of hepatic cells. Thus, the hepatocytes, which constitute 92.5% and 65% of the total hepatocellular volume and number, respectively (Blouin *et al.*, 1977; Seglen, 1976) are the main site of protein synthesis. The sinusoidal cells distribute numberwise as follows: liver endothelial cells, 70%; Kupffer cells, 20%; stellate cells, 10% (Knook & Sleyster, 1976; Knook *et al.*, 1977), and pit cells, < 1% (Wisse *et al.*, 1976). The populations of liver endothelial cells and Kupffer cells make up the hepatic reticuloendothelial system, which clear the blood from various particulate and soluble matter. The importance of the hepatocytes in the intermediary metabolism and synthesis of macromolecules has been appreciated for a long time. Likewise, the function of the Kupffer cells, the greatest reservoir of macrophages in the body, in the clearance of various circulating particles has been well established. Recently, however, increased attention has been paid to the liver endothelial cells as the site of uptake of various soluble macromolecules and nano-particles (Brouwer *et al.*, 1988; Kirn *et al.*, 1986; Knook & Wisse, 1982; Wisse *et al.*, 1989).

It is the purpose of this review to show that the liver endothelial cells make up an important part of the hepatic reticuloendothelial system in that they are specialized in receptor-mediated endocytosis of a number of macromolecules which are taken up only to a minor extent, or not at all, by other types of liver cells.

MORPHOLOGY AND CHARACTERIZATION

Prior to Aterman's and Wisse's important electron microscopic studies (Aterman, 1963; Wisse, 1970, 1972), very little was known about the endothelial cells of the hepatic sinusoid. In fact, the mere existence of a sinusoidal cell distinct from the Kupffer cell was for long a subject of controversy, and several studies suggested that since most hepatic sinusoidal cells are able to endocytose, the cells must be functional variants of the same cell type (Bankston & Pino, 1980; Benacerraf, 1964; Nicolescu & Rouiller, 1967). From his thorough studies on rat liver endothelial cells, Wisse concluded: (1) that the cells

compose the sinusoidal lining; (2) they contain sieve plates with fenestrations (Figs. 1 and 2; $\sim 0.1 \mu\text{m}$ openings through the cytoplasm, connecting the sinusoidal lumen with the space of Disse); (3) their cytoplasm contains bristle-coated micropinocytic vesicles ($0.18 \mu\text{m}$ in diameter) and smooth macropinocytic vesicles ($0.7 \mu\text{m}$ in diameter), both of which are part of the endocytic apparatus; (4) the cells do not contain: worm-like structures (which are frequently seen in Kupffer cells), a marginal fold (present in ordinary vascular endothelial cells), a basement membrane, fenestrations closed by diaphragms, smooth micropinocytic vesicles, or rod-shaped tubulated bodies (Weibel–Pallade bodies) present in extrahepatic endothelial cells; (5) no transitional stages of mixed endothelial and Kupffer cell feature are seen. Widmann *et al.* (1972) and Wisse (1974) stained sections of glutaraldehyde-fixed liver for endogenous peroxidase, and found that this was a method to distinguish Kupffer cells, which were positive, from the liver endothelial cells, which gave a negative reaction.

Although most of what we know about liver endothelial cells stems from experiments with rats, an array of other species, including man, other rodents, ruminants, dogs, amphibians, fishes and lamprey, have been observed to contain a fenestrated endothelium in their hepatic sinusoids.

ISOLATION AND CULTIVATION

Preparation of a single-cell suspension is a prerequisite to isolate the various liver cell populations. To this end, a minced preparation of the organ may be stirred with collagenase, and then with Pronase (Seljelid & Smedsrød, 1981). Albeit simple, this method gives a relatively low yield due to the mechanical damage of mincing, and both parenchymal and endothelial cells are destroyed during the process. Dispersion by perfusion of the liver with enzyme(s) is more frequently used. The enzymes used may be collagenase (Seglen, 1976), Pronase (Knook & Sleyster, 1976), or a combination of the two enzymes (Berg & Boman, 1973; Munthe-Kaas *et al.*, 1975). The liver endothelial cells contained in the resulting cell suspension may be isolated by centrifugation techniques, and in some instances followed by cultivation.

Knook & Sleyster (1976) introduced centrifugal elutriation of Pronase-treated cells to produce highly enriched liver endothelial cells in suspension, and the cells so prepared could be cultivated on a substratum of collagen (de Leeuw *et al.*, 1982). However, since Pronase

Abbreviations used: PIHNP, N-terminal propeptide of procollagen type III; VLDL, LDL, HDL, very low, low and high density lipoprotein; apo, apolipoprotein.

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Fig. 1. Scanning electron micrographs of rat liver

Following perfusion fixation with glutaraldehyde through the portal vein, small pieces of liver tissue were critical point dried. Surfaces resulting from fracturing such critical point dried specimens were coated with a thin layer of gold and examined in the scanning electron microscope. The low power micrograph (a) shows plates of parenchymal cells (P) with sinusoids (S) between them. Note that some red blood cells (thick arrows) are present in the sinusoids. The high power magnification (b) shows endothelial surface (E) as viewed from the inside of a sinusoid. Note the fenestrae (thin arrows) in the endothelium, and the adjacent parenchymal microvilli (*) in the space of Disse. Bar is 50 μm in (a) and 5 μm in (b).

was used, surface receptors for endocytosis were recovered only after 24 h in the presence of serum. Although Praaning-van Dalen & Knook (1982) reported that Pronase, when used at 10 °C, rendered endocytic activity intact, Eskild *et al.* (1989) noted a 40 % reduction of receptor-mediated endocytosis. Therefore, Pronase should be avoided when preparing liver cells for function studies *in vitro*.

Preparation of functionally intact liver endothelial cells without the use of Pronase was reported by Steffan *et al.* (1981) who employed collagenase perfusion, density centrifugation in Metrizamide, and centrifugal elutriation. The cells could be established in monolayer cultures on a growth substrate of collagen. Smedsrød *et al.* (1982) and Smedsrød & Pertoft (1985) developed a method to obtain monolayer cultures of highly enriched liver endothelial cells by collagenase perfusion, low-speed centrifugation to remove most of the parenchymal cells, density separation in Percoll of the cells in the resulting supernatant to generate a band of purified sinusoidal cells, and finally selective adherence and serum-free cultivation of Kupffer and endothelial cells on growth substrates of tissue culture plastics and fibronectin, respectively. Liver endothelial cells so prepared retain their *in vivo* functions (Pertoft & Smedsrød, 1987). Finally, Blomhoff *et al.* (1984c) used collagenase perfusion, treatment with enterotoxin from *Clostridium perfringens* (which is specifically toxic to the hepatocytes), density centrifugation, and centrifugal elutriation to prepare highly purified and functionally intact liver endothelial cells, which could be established in serum-free monolayer culture on a growth substrate of fibronectin.

Most of our present knowledge about the scavenger function of liver endothelial cells has been obtained from experiments with surface cultures or suspensions of these cells prepared according to the principles outlined above. The approximate yields and purities of liver endothelial cells obtained in these preparation procedures are compared in Table 1.

CHARACTERISTICS WHICH INDICATE THAT LIVER ENDOTHELIAL CELLS ARE GEARED TO A SCAVENGER FUNCTION

Wisse (1972) observed that intravenously injected Thorotrast was very rapidly internalized into small coated and larger uncoated vesicles of liver endothelial cells, and suggested that "the cells participate in the continuous uptake and digestion of plasma proteins and may thus fulfill an important function in the metabolic activity of the liver". In much of the following it will become clear that Wisse was correct in his speculations. There are several reasons to believe that the liver endothelial cells are well furnished to endocytose and metabolize biological material.

Firstly, their strategic location in the wall of the hepatic sinusoid, as well as their large luminal surface, endow the liver endothelial cells with a unique possibility to take up circulating macromolecules brought to the liver through the portal vein.

Secondly, the presence of numerous coated pits and vesicles points towards an active endocytosis. The coated pits expose cationic residues (Ghitescu & Fixman, 1984) which may facilitate the specific endocytosis of various negatively charged macromolecules, including ligands for the hyaluronan/chondroitin sulphate and scavenger receptors (see outline below).

Finally, the specific activities of several lysosomal enzymes is higher in liver endothelial cells than in other liver cells (Knook & Sleyster, 1980; Rodén *et al.*, 1989). For instance, the specific activities of acid phosphatase, acid DNAase, β -N-acetylglucosaminidase, β -glucuronidase and arylsulphatase A and B have been reported to be higher in liver endothelial cells than in Kupffer cells, and much higher than in parenchymal cells. In addition, the activity of acid cholesteryl esterase is high in liver endothelial cells (van Berkel *et al.*, 1980; Nagelkerke *et al.*, 1985). Although part of the lysosomal enzymes may derive from clearance of circulating enzymes (see outline below), the high content of such enzymes reflect the

Table 1. Yield and purity of liver endothelial cells in different preparative procedures

The cells were grown in monolayer culture for 20 h, identified, and counted.

Method	$10^{-6} \times$ Yield (liver endothelial cells/ g of rat liver)	Purity (%)	Reference
Collagenase/enterotoxin, density gradient, elutriation	6.2	n.d.*	Blomhoff <i>et al.</i> (1984c)†
Collagenase/Pronase, density gradient, elutriation	7.0	85–95‡	van Bossuyt <i>et al.</i> (1988)
Collagenase, density gradient, selective adherence	15–20	90–95§	Pertoft & Smedsrød (1987), Gustafson <i>et al.</i> (1989)

* Not determined.

† See calculations in Pertoft & Smedsrød (1987).

‡ Purity determined by transmission electron microscopy; positive reaction of endogenous peroxidase used to distinguish Kupffer cells.

§ Purity determined by light microscopy: liver endothelial cells distinguished by their specific accumulation of fluorescently labelled ovalbumin; Kupffer cells distinguished by their positive peroxidase reaction; fat-storing cells distinguished by their content of autofluorescent vitamin A.

fact that the cells actively degrade endocytosed material.

METABOLISM OF CONNECTIVE TISSUE COMPONENTS

Even if a large amount of information has accumulated regarding a major role of liver endothelial cells in the *catabolism* of connective tissue components (Table 2), it should be noted that some reports also have dealt with the *production* of matrix macromolecules, and we have therefore included a discussion of this subject. There is also a growing literature on the *pathological metabolism* of connective tissue components in the liver sinusoids.

Production of connective tissue components by liver endothelial cells

Irving *et al.* (1984) reported that monolayer cultures of purified liver endothelial cells displayed type IV collagen phenotype, as determined by immunofluorescence, and Clement *et al.* (1985, 1986) found low amounts of types I, III, and IV collagen, and fibronectin, in the Golgi/endoplasmic reticulum areas of liver endothelial cells *in vivo*, indicating synthesis. By immunogold/electron microscopy Geerts *et al.* (1986) observed procollagen III peptides in vesicles of liver endothelial cells *in situ*. Since the nature of these vesicles could not be identified, no conclusion could be drawn as to whether the procollagen peptides were secreted or taken up. Recently, cultured guinea pig liver endothelial cells were found to synthesize and secrete cellular fibronectin (Rieder *et al.*, 1987). Incorporation of [³⁵S]methionine and immunoprecipitation showed that fibronectin was indeed produced by synthesis *de novo*, and the authors suggested that turning on the fibronectin production by liver endothelial cells could be an important event in the development of liver fibrosis. Another factor which may control capillarization of liver sinusoids seen in fibrotic livers is synthesis of type IV collagenase in both Kupffer and endothelial cells (Kashiwazaki *et al.*, 1987).

Apart from the studies which clearly showed synthesis *de novo* of fibronectin, none of the other studies prove that the connective tissue components identified by immunohistochemistry are really synthesized by liver endothelial cells. In fact, since the cells are so actively engaged in receptor-mediated endocytosis of various connective tissue macromolecules (see outline below), it may well be that antigens of such substances observed intracellularly by immunohistochemical techniques merely reflect internalized material.

Endocytosis of connective tissue macromolecules

Hyaluronan and chondroitin sulphate. Intravenously injected hyaluronan, a major connective tissue polysaccharide consisting of repeating units of glucuronic acid and *N*-acetylglucosamine (for a review see Laurent *et al.*, 1986a), was shown by Fraser *et al.* (1981) to be rapidly taken up ($t_{1/2} = 2.5$ min), mainly by the non-parenchymal liver cells. Blocking of the uptake by an excess of unlabelled hyaluronan suggested that the uptake was receptor-mediated. Subsequent studies using monolayer cultures of purified cells showed that liver endothelial cells, but not Kupffer or parenchymal cells, avidly endocytose the polysaccharide (Eriksson *et al.*, 1983; Smedsrød *et al.*, 1982, 1984). Intravenous administration of [¹⁴C]- or [³H]hyaluronan, followed by cell

separation or electron microscopic autoradiography, respectively, confirmed that the liver endothelial cells are the site of clearance of this substance from the circulation (Fraser *et al.*, 1985; Smedsrød *et al.*, 1986). Recently, Dahl *et al.* (1988), using hyaluronan coupled to the adduct ¹²⁵I-labelled tyramine cellobiose, which accumulates intralysosomally after endocytosis and degradation of the carrier-polysaccharide, also found the major part of the label in the liver endothelial cells. These results thus show that these cells constitute the most important scavenger system for circulating hyaluronan.

The specificity and binding affinity of the hyaluronan receptor has been studied on cultured liver endothelial cells (Smedsrød *et al.*, 1984; Laurent *et al.*, 1986b; Raja *et al.*, 1988). The binding affinity increases with increasing chain length of hyaluronan, as shown by the finding that the K_d values for an oligosaccharide consisting of 35 monosaccharides and a molecule of M_r 6.4×10^6 are 0.2 μ M and 9 pM, respectively (Laurent *et al.*, 1986b; Table 2). The same study ruled out any major contribution of co-operative multiple-site attachment of high- M_r ligands to the cell surface. The finding that each cell could bind about 10^5 oligosaccharides, but only about 10^3 molecules with M_r 6.4×10^6 , was explained by mutual exclusion of large molecules from the cell surface.

Chondroitin sulphate, and to a lesser extent dermatan sulphate, inhibit binding and uptake of hyaluronan, suggesting that the receptor recognizes a common determinant in these three closely related polysaccharides (Smedsrød *et al.*, 1984). Chondroitin sulphate was found to bind with a 3-fold higher affinity than does hyaluronan of the same degree of polymerization (Laurent *et al.*, 1986b). The finding that neither heparan sulphate nor heparin were inhibitory supports the conclusion that the recognized determinant is not only a high net negative charge (Smedsrød *et al.*, 1984). Chondroitin sulphate, both as free chains and as proteoglycan, is endocytosed via the same receptor which recognizes hyaluronan (Smedsrød *et al.*, 1985b).

Based on the results obtained *in vitro*, it was calculated that the efficiency of endocytosis in liver endothelial cells is more than sufficient to clear the blood from the hyaluronan which normally enters the circulation from the tissues (Smedsrød *et al.*, 1984). This has recently been confirmed by experiments *in vivo* on sheep and humans (Lebel, 1989; Lebel *et al.*, 1989). In both species the clearance of hyaluronan from the blood stream follows Michaelis-Menten kinetics. In 12 humans the mean serum concentration of hyaluronan was 0.031 μ g/ml, while K_m was 10-fold higher or 0.34 μ g/ml. The mean endogenous input into the circulation was 34 mg/day while the maximal metabolic rate (V_{max}) was 384 mg/day. The liver endothelial cells therefore have a great reserve capacity for uptake of hyaluronan.

It is interesting to note that, since binding affinity increases with increasing chain length of hyaluronan, the population of circulating polysaccharide will be relatively enriched in low- M_r species. This principle was directly shown by Fraser & Laurent (1989) and Dahl *et al.* (1988). The first authors showed in rabbit that hyaluronans with M_r values of 100 000 and 5×10^6 exhibited half-lives in plasma of 7.0 and 1.8 min, respectively, and the latter authors found that hyaluronan of M_r 10 000 had a significantly longer serum half-life in the rat than hyaluronan of M_r 250 000 (2.7 min versus 0.9 min, respectively).

Table 2. Parameters of binding and uptake in liver endothelial cells of various connective tissue macromolecules

Ligand	Receptor type	Dissociation constant (K_d) (μM)	$10^{-3} \times$ Molecules bound/cell	Endocytic efficiency (pl of medium/min per cell)
Hyaluronan (~ 35 monosaccharides per chain)†	HA receptor	0.2	120	n.d.*
Hyaluronan (M_r 4×10^5)‡	HA receptor	6×10^{-5}	8.1	2
Hyaluronan (M_r 6.4×10^6)†	HA receptor	9×10^{-6}	0.9	n.d.
Chondroitin sulphate (M_r 2×10^4)‡	HA receptor	2.5×10^{-3}	63	1.2
Chondroitin sulphate proteoglycan (M_r 2×10^6)§	HA receptor	$(1.1-29) \times 10^{-5}$	1.8-4.2	5.8
Collagen type I§	Collagen receptor	$(1.3-7.1) \times 10^{-3}$	81-300	3.7
Collagen type II	Collagen receptor	n.d.	n.d.	n.d.
Collagen type III	Collagen receptor	n.d.	n.d.	n.d.
Collagen type IV	Collagen receptor	n.d.	n.d.	n.d.
Collagen type V	Collagen receptor	n.d.	n.d.	n.d.
Collagen type XI	Collagen receptor	n.d.	n.d.	n.d.
Bovine PIIINP¶	PIIINP receptor	1×10^{-3}	420	n.d.
Rat PIIINP¶	PIIINP receptor	n.d.	160	4

* Not determined.
† Laurent *et al.* (1986b).
‡ Smedsrød *et al.* (1984).
§ Smedsrød *et al.* (1986).
|| Smedsrød (1989).
¶ Smedsrød (1988).

In the early autoradiographic studies by Fraser *et al.* (1985) it could be visualized that hyaluronan was endocytosed into vesicles in the liver endothelial cells. To document this process, chondroitin sulphate proteoglycan was coupled to colloidal gold and incubated with liver endothelial cells, and the marker was chased in the electron microscope for ultrastructural characterization of binding and endocytosis (Smedsrød *et al.*, 1988b). With this electron-dense probe, binding to the cell surface was observed only in coated regions, and after rapid internalization (less than 1 min) the ligand was transported via small coated vesicles to larger uncoated macropinocytic vesicles. Only after 40–60 min was the marker transferred to acid phosphatase-containing vesicles, indicating that some modification of the ligand had to take place prior to fusion of lysosomes with the macropinocytic vesicles (Smedsrød *et al.*, 1988b). McGary *et al.* (1989) have shown that the receptors responsible for the uptake apparently are recycled back to the cell surface during the continuous endocytosis of the polysaccharide. This indicates that the uptake of hyaluronan and chondroitin sulphate by liver endothelial cells is mediated by an ordinary endocytic receptor pathway.

Hyaluronan biosynthetically labelled in the acetyl groups with ^3H and in the sugar rings with ^{14}C is rapidly degraded by liver endothelial cells to its constituent monosaccharides, glucuronic acid and *N*-acetylglucosamine, with no intermediate residues (J. R. E. Fraser, personal communication). Gulonic acid was identified as a metabolite of glucuronic acid. The deacetylation of *N*-acetylglucosamine can only occur after phosphorylation to *N*-acetylglucosamine 6-phosphate, and phosphorylated intermediates of the sugar have been found in foetal liver after intravenous injection of labelled

hyaluronan (Fraser *et al.*, 1989). It was recently shown by Rodén *et al.* (1989) that the key enzyme, *N*-acetylglucosamine 6-phosphate deacetylase, is present in large amounts in the liver endothelial cells as well as in Kupffer cells, but not in hepatocytes.

When hyaluronan labelled with ^3H in acetyl groups or ^{14}C in the sugar moieties was given to liver endothelial cells in culture, the label was recovered quantitatively as [^3H]acetate or [^{14}C]lactate, respectively (Smedsrød *et al.*, 1984). It was therefore concluded that the cells operate on a mainly anaerobic metabolism. Co-operation between liver endothelial cells and parenchymal cells has been suggested based on the finding that ^3H from intravenously injected [^3H]hyaluronan rapidly reappears in the blood as $^3\text{H}_2\text{O}$. It is thought that [^3H]acetate is transferred to the parenchymal cells and oxidized immediately after degradation of the polysaccharide in the liver endothelial cells.

In a drug delivery model study, intravenously administered microparticles of chondroitin sulphate were shown to be taken up exclusively in Kupffer cells (Laakso & Smedsrød, 1987). Studies *in vitro* with cultured cells showed ingestion of these particles in Kupffer cells, whereas only binding was observed to liver endothelial cells. These findings reflect a general rule: liver endothelial cells take up soluble matter or very small particles, whereas Kupffer cells take up larger particles, even if the particles expose mainly determinants which are recognized by the former cell type.

In summary, numerous studies have shown that liver endothelial cells carry common receptors for circulating hyaluronan and chondroitin sulphate. Due to high receptor number, rapid receptor recycling, and high receptor–ligand affinity, hyaluronan is cleared extremely efficiently from the blood.

Fig. 2. Scanning electron micrographs of cultured rat liver endothelial cells

Cells, suspended in serum-free medium, were seeded in fibronectin-coated dishes, washed after 2 h of incubation, maintained for 24 h in serum-free medium, and prepared for scanning electron microscopy. The low power micrograph (a) shows a well spread liver endothelial cell with a nuclear area (N) surrounded by an extremely thin peripheral cytoplasm. Close inspection of (a) reveals that fenestrae are scattered all over the surface. Close up (b) of outlined area in (a) shows fenestration consisting of similarly sized holes (arrows). Bar is 5 μm in (a) and 1 μm in (b).

Collagen. Intravenously injected type I collagen α -chains were cleared by the sinusoidal cells of rat liver with a serum half life of 2–3 min (Smedsrød *et al.*, 1985a). Low- M_r degradation products appeared in the circulation after about 10 min. Studies with cultured liver cells showed that the uptake was 10 times higher per liver endothelial cell than per Kupffer cell, and virtually nothing was taken up by the parenchymal cells. While Kupffer cells *in vitro* secrete enzymes which effectively degrade α 1 chains, liver endothelial cells take up the ligand via receptor-mediated endocytosis, and degrade it intralysosomally. The receptor is specific for α 1-chains or gelatin, but does not recognize native collagen. Since the large split products of collagen released from the connective tissue by mammalian collagenase under normal physiological conditions are readily gelatinized at body temperature, it was suggested that this uptake system

may be an important clearance mechanism for fragments of collagen released during the normal turnover of connective tissue (Smedsrød *et al.*, 1985a). Recently, Smedsrød (1989) reported that the receptor which recognizes collagen type I mediates endocytosis and intralysosomal degradation of types II, III, IV, V, XI, and perhaps IX, collagen as well. Experiments with cultured liver endothelial cells showed that ligands for the hyaluronan/chondroitin sulphate receptor or for the mannose/*N*-acetylglucosamine receptor could not compete with the binding and endocytosis of gelatin. Moreover, since all experiments were carried out in the complete absence of serum, no serum factors were required for binding and uptake. Fibronectin was not involved, as revealed by findings that neither fibronectin nor anti-fibronectin antibody interfered with the binding. Binding experiments indicated that gelatinized collagen binds to liver endothelial cells via two different receptor populations, one with K_d 7.1 nM (8.1×10^4 molecules bound per cell), and the other with K_d 1.3 nM (3×10^5 molecules bound per cell). Uptake studies showed that the endocytic efficiency corresponded to 3.7 pl of medium being cleared/min per cell at a ligand concentration of 0.25 $\mu\text{g/ml}$ (Smedsrød *et al.*, 1986).

The finding that gelatin is cleared directly and specifically from the circulation via receptors on liver endothelial cells contrasts with the idea that plasma fibronectin acts as an opsonin for uptake of gelatin colloids in the hepatic Kupffer cells (Yamada, 1983). Much of the data on which this hypothesis is based have been obtained by incubating liver slices with gelatinized material coated or not with fibronectin (Martinez-Hernandez, 1984). It has been customary to interpret binding of, for instance, fibronectin-coated gelatin-latex to such slices as uptake in Kupffer cells. However, a recent report revealed that the particles were bound, but not internalized, and since not only Kupffer, but also endothelial and parenchymal, cells express receptors for binding to fibronectin fixed to a surface, it may well be that the liver slice assay reflects binding mainly to the two latter cell types. Moreover, the high background binding to liver slices of control particles coated only with gelatin (Martinez-Hernandez, 1984) probably reflects the fact that both endothelial and parenchymal cells bind to gelatin surfaces (Rubin *et al.*, 1981).

In summary, endocytic receptors on liver endothelial cells mediate the very efficient clearance of denatured collagen from the blood. The receptor recognizes denatured but not native collagen of types I, II, III, IV, V, and XI. The uptake results from a direct ligand–receptor interaction, in which fibronectin plays no role.

Procollagen peptide. Collagen is synthesized as a procollagen molecule which is processed extracellularly by specific proteases to yield one *N*-terminal and one *C*-terminal peptide, and one central collagen molecule. The two terminal peptides so produced have to be disposed of, whereas the central collagen molecule is incorporated into the connective tissue matrix. Purified preparations of the *N*-terminal propeptide of collagen type III (PIIINP) were recently found to be effectively endocytosed and degraded almost exclusively by liver endothelial cells (Smedsrød, 1988). The receptor on rat cells does not distinguish between rat or bovine PIIINP. Neither gelatinized type III collagen, hyaluronan, chondroitin sulphate, formaldehyde-treated serum albumin, nor

mannose were inhibitory, showing that the receptor has a unique specificity for PIINP. The serum half-life of intravenously injected propeptide is about 2 min. Each liver endothelial cell can bind $(2-4) \times 10^5$ molecules of PIINP with a dissociation constant of about 1 nM (Table 2). The efficiency of endocytosis, expressed as volume of medium cleared/min per cell, was calculated to be 4 pl, which is in the same order of magnitude as endocytosis of gelatinized collagen, hyaluronan, and chondroitin sulphate proteoglycan (Smedsrød *et al.*, 1986). This clearance capacity is well in excess of the need to take up procollagen peptides released to the blood under physiological conditions.

Fibronectin and laminin. Several laboratories have reported that freshly isolated liver endothelial cells prepared by means of collagenase perfusion attach and spread on tissue culture plastics, without the need for a coat of adhesive protein on the dishes (de Leeuw *et al.*, 1982; Steffan *et al.*, 1981). However, since the cells in these studies were seeded in medium supplied with 10–20 % serum, it cannot be excluded that a serum component(s) such as fibronectin would mediate the adherence of the cells. In fact, when isolation, seeding and cultivation are carried out in the complete absence of serum, such as with the method described by Smedsrød & Pertoft (1985), a coat of fibronectin, fragments of fibronectin, or laminin on the growth substrate is a requirement for efficient attachment and spreading. Therefore, one would expect that the cells carry receptors for these glycoproteins. Although soluble fibronectin is not taken up by these cells (Smedsrød *et al.*, 1985a), Johansson *et al.* (1987) recently found evidence of a fibronectin receptor which mediates cell attachment, but not endocytosis.

Recent work by Smedsrød *et al.* (1989) showed that native complexes of laminin and nidogen, as well as the separated proteins, are rapidly eliminated from the blood mainly by the liver endothelial cells. The large fragments of laminin carrying the cell adhesion activity were cleared much slower from the blood, indicating that the uptake of laminin did not occur via cell adhesion receptors.

These findings show that recognition, binding, and endocytosis of a soluble ligand may not necessarily be mediated by the same receptor which anchors the cells to the same molecule coating the growth substrate.

Liver endothelial cells in pathological conditions

Increased serum levels of connective tissue macromolecules in liver disease. Patients with liver cirrhosis have significantly increased serum levels of hyaluronan (Engström-Laurent *et al.*, 1985; Frébourg *et al.*, 1986) and PIINP (Rohde *et al.*, 1979). It has been generally held that these elevated levels reflect increased hepatic synthesis of connective tissue components. However, based on the knowledge that liver endothelial cells are important scavengers, dysfunction of these cells have recently been implicated as a cause of decreased hepatic clearance and thus abnormally high serum levels of connective tissue molecules. Alcorn & Chojkier (1987) recently commented on the current belief that the serum level of PIINP can be used as a reliable non-invasive measure of hepatic fibrosis. The authors concluded that as long as total metabolism of these peptides is largely unknown, it is incorrect to suggest that hepatic fibrosis *per se* is the direct cause of the increased serum con-

centration of PIINP. Further, it is unlikely that production of hyaluronan in the fibrotic liver could be the cause of the increased serum level, in view of the already large production of hyaluronan elsewhere in the body. However, increased lymph flow from the splanchnic area in liver cirrhosis could possibly carry more hyaluronan to the circulation (Engström-Laurent & Laurent, 1989). The relative contributions of increased supply and decreased uptake, respectively, to the increased concentration of these substances in blood is at present unknown.

Extrahepatic conditions giving increased serum levels of connective tissue components. There are several conditions with increased extrahepatic production of hyaluronan which lead to increased serum levels of this polysaccharide, including various inflammatory diseases, e.g. rheumatoid arthritis (Engström-Laurent & Hällgren, 1985), certain tumours such as mesothelioma (Dahl & Laurent, 1988), and Werner's syndrome (Laurent *et al.*, 1987). Lebel (1989) and Lebel *et al.* (1989) showed that the hyaluronan uptake from plasma obeys Michaelis-Menten kinetics, and it can be deduced that there will be an increased steady state level of hyaluronan in blood with increased input into the circulation.

METABOLISM OF LIPOPROTEINS

Serum lipoprotein levels are closely linked to the development of atherosclerosis. The lipoprotein levels are not only a function of the rate of synthesis, but to a great extent dependent on the rate of removal of lipoproteins from the circulation (Havel, 1985). The liver is the only organ that can metabolize and excrete cholesterol, and it also plays a dominant role in the removal of cholesterol-containing lipoproteins from serum (Cooper, 1985). There is now growing evidence that liver endothelial cells are very efficient in the binding and uptake of lipoproteins *in vivo*.

Naturally occurring lipoproteins

In the rat, non-parenchymal liver cells have been shown to take up intravenously injected lipoproteins, labelled biosynthetically in the cholesterol (ester) moiety (van Berkel & van Tol, 1979). Very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) were all taken up by the non-parenchymal cells, and this uptake was 3–4 times higher per mg of cell protein as compared to parenchymal cells. However, in the total uptake by the liver the parenchymal cells dominated as they greatly outnumber the non-parenchymal cells.

The experiments by van Berkel & van Tol (1979), which showed the main uptake of VLDL in the parenchymal cells, were made after a 30 min circulation period when most of the injected radioactivity had been cleared from the plasma. However, in similar experiments performed with shorter circulation times, Gustafson *et al.* (1989) found that the liver endothelial cells were responsible for the initial uptake of ^{125}I -tyramine-cellobiose-labelled VLDL in the rat. In this study the cells seemed to be very active in binding VLDL but less active in the degradation, as indicated by the large proportion of radioactivity associated with liver endothelial cells at 5 min after injection and the relatively small increase in total accumulated radioactivity from 10

to 30 min after injection. It was concluded that the relatively low initial binding and uptake in parenchymal cells is probably due to the fact that VLDL must interact with non-parenchymal cells before it can be taken up by the hepatocytes. Autoradiographic studies support the hypothesis of sinusoidal cell involvement in the early events of VLDL uptake by the liver (Stein *et al.*, 1974). It has been proposed that VLDL binds to hepatic lipase, an enzyme known to be exclusively located on the liver endothelial cell surface (Kuusi *et al.*, 1979) and reported to be active in the HDL, VLDL and chylomicron-remnant metabolism (Borensztajn & Kotlar, 1981).

Uptake *in vitro* of VLDL in liver endothelial cells can be inhibited by VLDL, chylomicrons, and HDL containing apolipoprotein (apo) E (Gustafson *et al.*, 1985, 1986). VLDL and HDL from patients with defective apo E and LDL, that only contain minute amounts of apo E, are poor competitors. VLDL preincubated with apo E shows increased uptake by the cells. These results suggest that the uptake of lipoproteins by liver endothelial cells is strongly dependent on functionally active apo E. Apo C III, present in triacylglycerol-rich lipoproteins and HDL, is, in experiments with purified lipoproteins, also able to interact with the apo E binding site but does not seem to mediate endocytosis of lipoprotein particles (Gustafson *et al.*, 1985, 1986). Rat liver endothelial cells have approximately 1×10^6 apo E/CIII binding sites per cell with a dissociation constant of 400–500 nM.

Gustafson *et al.* (1988) have characterized the apo E binding proteins of liver endothelial cells by immunoprecipitation of cell-surface labelled proteins in intact cells in culture. Three proteins with molecular masses of 60, 110 and 170 kDa were specifically precipitated by apo E and monospecific polyclonal antibodies towards apo E and only in the presence of divalent cations. The 110 and 170 kDa proteins have the same molecular masses as the LDL (B/E) receptor with and without its carbohydrate unit, and the 60 kDa protein might be the so-called apo E receptor, also known as the chylomicron-remnant receptor (Shelburne *et al.*, 1980).

LDL that carry a single protein molecule, apo B, are mainly taken up via B/E-receptors of the liver. Nenseter *et al.* (1988) found both B/E-receptor dependent and independent uptake of 125 I-tyramine-cellobiose-labelled LDL in rabbit liver endothelial cells after 24 h of circulation *in vivo*. The liver endothelial cell uptake was relatively small compared to the uptake in parenchymal cells, but was increased to the same level as the uptake in parenchymal cells after cholesterol feeding. Receptor-mediated uptake of LDL occurs also in rat liver endothelial cells, e.g. Handley *et al.* (1981) observed binding and internalization of LDL conjugated to colloidal gold, and S. Gustafson (unpublished work) noted uptake of 125 I-tyramine-cellobiose-labelled LDL *in vivo* by rat liver sinusoidal endothelial cells.

To summarize, liver endothelial cells seem to have proteins on their surface that are able to bind chylomicrons, VLDL, LDL, and HDL containing apo E. The cells also carry hepatic lipase, show efficient endocytosis of the lipoprotein particles, and have a high acid cholesteryl esterase activity.

Modified lipoproteins

The term 'scavenger receptor' was coined to denote the receptor in macrophages responsible for endocytosis of a number of macromolecules having a high net negative

charge as a common feature, for instance acetylated LDL (Goldstein *et al.*, 1979; Brown *et al.*, 1980). Administration of acetylated LDL to macrophages in culture can transform the cells into cholesterol-filled foam cells (Brown & Goldstein, 1983) resembling the foam cells, of macrophage origin, present in early atherosclerotic lesions (Schaffner *et al.*, 1980). Acetylated LDL, administered intravenously or to cell cultures, are taken up almost exclusively by the liver endothelial cells (Nagelkerke *et al.*, 1983; Blomhoff *et al.*, 1984a). Pitas *et al.* (1985) reported that intravenously injected acetoacetylated LDL bound almost exclusively to coated regions of liver endothelial cells, suggesting that the receptor-ligand complex is endocytosed via coated pits, similar to the uptake of LDL via LDL receptors. The cholesterol esters are rapidly hydrolysed in the liver endothelial cells while re-esterification is very limited (Nagelkerke *et al.*, 1985). The free cholesterol might be directly transported to the parenchymal cells for conversion to bile acids.

The removal of modified lipoproteins by liver endothelial cells is important in order to reduce the uptake of these atherogenic lipoproteins by peripheral macrophages.

The liver sieve

It has been suggested that the open fenestrae of the liver endothelial cells serve to exchange soluble and particulate material between the blood and the space of Disse (Wisse *et al.*, 1985). Therefore, since the diameter of the holes in rat cells vary from about 100 to 200 nm (Wisse *et al.*, 1985), a filtration effect is thought to exist, with particles above this size (i.e. large chylomicrons) being unable to pass across the liver endothelium. Evidence for this idea has been presented by Naito & Wisse (1978) and de Zanger & Wisse (1982), who found that chylomicrons larger than fenestrae remained in the circulation. However, other findings by Wisse & McCuskey (1986) indicate that simple sieving is not sufficient to regulate the uptake of chylomicrons. The authors proposed the hypothesis that forced sieving and 'endothelial massage' performed by blood cells travelling through the sinusoids allow even large chylomicrons to enter the space of Disse. It has also been clearly shown that the removal of chylomicrons from the circulation is due to uptake via specific receptors recognizing apo E (see the section on metabolism of naturally occurring lipoproteins above). Individuals with defect apo E accumulate chylomicron remnants in plasma (Cooper, 1985), remnants that easily penetrate the endothelium.

UPTAKE OF DENATURED PROTEINS

In addition to the receptor for acetylated LDL, liver endothelial cells carry calcium-independent scavenger receptors for endocytosis of denatured proteins such as formaldehyde-treated albumin (Blomhoff *et al.*, 1984b,c; Eskild & Berg, 1984; Eskild *et al.*, 1986; Smedsrød *et al.*, 1985c). A protein of apparent M_r 125000 which binds this ligand in a saturable manner has been purified from octylglucoside extracts of rat liver sinusoidal cells (Horiuchi *et al.*, 1985b,c). The binding molecule consists of two non-covalently linked glycoprotein subunits with M_r 53000 and 30000. Monospecific antibodies against the binding protein effectively blocked binding of radio-

labelled formaldehyde-treated albumin to sinusoidal cell membranes, indicating that the binding protein was identical to the scavenger receptor for denatured proteins on intact liver endothelial cells.

Although similar in some respects, the receptors for acetylated LDL and formaldehyde-treated albumin seem to differ in other respects. Thus, acetyl-LDL inhibits binding of ^{125}I -labelled formaldehyde-treated albumin to rat sinusoidal cells, whereas binding of ^{125}I -labelled acetyl-LDL is not inhibited by formaldehyde-treated albumin or antibodies raised against the receptor for formaldehyde-treated albumin (Horiuchi *et al.*, 1985a). However, both receptors are similarly sensitive to polyanionic compounds such as dextran sulphate and poly(L-glutamic acid). In contrast with these results is the finding by Eskild *et al.* (1986) that endocytosis of the polyanion chondroitin sulphate in cultured liver endothelial cells can be completely blocked by the presence of formaldehyde-treated albumin, while the polysaccharide does not inhibit uptake of formaldehyde-treated albumin. These puzzling results could possibly be explained by binding of ligands to other structures than endocytic receptors. The results could, however, also be explained by the presence of several receptors and crossreactivity of the ligands. Clearly, more studies are needed to elucidate the relationship between the mechanism of uptake of acetylated LDL, formaldehyde-treated albumin, and connective tissue polysaccharides.

Neither acetylated LDL, formaldehyde-treated albumin nor any of the other modified macromolecules mentioned above are normal constituents of the body. Therefore, the question as to the physiological significance of the receptor(s) has yet to be resolved. It has been shown that cultured extrahepatic endothelial cells (Henriksen *et al.*, 1981, 1987) and aggregating platelets (Fogelman *et al.*, 1980) may convert LDL into a ligand for the macrophage scavenger receptor. However, the physiological relevance of these phenomena remains to be shown.

ENDOCYTOSIS VIA THE MANNOSE/ N-ACETYLGLUCOSAMINE RECEPTOR

The discovery of hepatic lectins, or carbohydrate-specific receptors, was made by Ashwell & Morell (1974) who found that the mammalian liver recognizes and endocytoses circulating galactose/*N*-acetylgalactosamine-terminated glycoproteins. The specific uptake mechanism is mediated by the parenchymal cells which carry on their surface galactose/*N*-acetylgalactosamine-specific receptors (Kawasaki & Ashwell, 1976). This idea of a specific recognition of carbohydrates by liver was extended when Stockert *et al.* (1976) reported on a hepatic uptake system specific for *N*-acetylglucosamine-terminated glycoproteins. Yet another hepatic lectin-like activity was reported by Brown *et al.* (1978) who showed that mannose-terminated glycoproteins are taken up by a specific mechanism in rat liver. Isolation of liver cells showed that the mannose-terminated yeast invertase was taken up via a mannose-specific mechanism mainly by non-parenchymal liver cells (Madnick *et al.*, 1978). Steer & Clarenburg (1979) then made the remarkable observation that hepatic sinusoidal cells, but not parenchymal cells, recognize and endocytose both mannose- and *N*-acetylglucosamine-terminated glycoproteins, apparently via the same bind-

ing sites. Because of the then recent finding that alveolar macrophages carry receptors for mannose-terminated glycoproteins (Stahl *et al.*, 1978), most authors at that time thought that the hepatic mannose-binding site was expressed only on the Kupffer cells (Schlesinger *et al.*, 1978). The first indication that the liver endothelial cells were involved in this activity was reported by Hubbard *et al.* (1979), who presented a detailed electron microscopic autoradiographic study on the cellular site of uptake of glycoproteins with different terminal carbohydrate residues. Results from this work showed that the liver endothelial cells, on a cellular basis, are 2–6 times more active than the Kupffer cells in the uptake of mannose-terminated glycoproteins. Since then, several studies *in vitro* and *in vivo* have unequivocally shown that the liver endothelial cells are the main site of uptake of mannose- and *N*-acetylglucosamine-terminated glycoconjugates (Eskild *et al.*, 1986; Niesen *et al.*, 1984; Isaksson *et al.*, 1983; Praaning-van Dalen *et al.*, 1987; Smedsrød *et al.*, 1985c, 1988a; Summerfield *et al.*, 1982).

Although an intracellular mannose-binding protein has been isolated from the liver of rabbit (Kawasaki *et al.*, 1978), rat (Mizuno *et al.*, 1981; Townsend & Stahl, 1981; Wild *et al.*, 1983), and man (Wild *et al.*, 1983), there is no report of the isolation of the cell surface receptor for mannose from liver endothelial cells. Recently, the mannose receptor was isolated from rat (Haltiwanger & Hill, 1986a) and rabbit (Wileman *et al.*, 1986; Lennartz *et al.*, 1987b) alveolar macrophages as well as human placenta (Lennartz *et al.*, 1987a). It is a 175–180 kDa membrane glycoprotein, and contains both *O*-linked and complex *N*-linked oligosaccharides. The isolated binding protein shows similar binding characteristics to the receptor on intact cells. The binding is strongly Ca^{2+} -dependent, with half-maximal binding at 0.72 mM-Ca^{2+} , which is well within physiological concentrations. The binding is maximal at pH 7.0 and absent below pH 5.0–5.5.

Polyclonal antibodies raised against this pulmonary macrophage mannose receptor also bind to Kupffer and liver endothelial cells (Haltiwanger & Hill, 1986a,b). To date, this is the only evidence published that the mannose receptor of alveolar macrophages and liver endothelial cells are the same proteins. These immunohistochemical observations differ from several recent uptake studies showing that ligands for the liver mannose receptor are taken up mainly or almost exclusively by the liver endothelial cells (see Table 3). More studies are needed to explain why the mannose receptor is functionally present mainly on the liver endothelial cells, whereas a great proportion of the receptor antigen can be demonstrated on the Kupffer cells as well.

Magnusson & Berg (1989) found that the mannose receptor, which is apparently present in 20000–25000 copies on the surface of liver endothelial cells, bind ovalbumin with a dissociation constant of $6 \times 10^{-8} \text{ M}$. The authors further found that the mannose receptor cycles between the cell surface and an intracellular compartment at a remarkably high speed: the half-life of the receptors located on the surface is about 10 s. This explains why ligands for this receptor are so efficiently cleared from the circulation.

Most studies on the hepatic mannose receptor have been carried out with unphysiological ligands, such as modified glycoproteins, neoglycoproteins, ovalbumin (see Fig. 3), mannan, invertase, or horseradish per-

Table 3. Ligands known to be taken up by the hepatic mannose receptor

Abbreviations: NPC, non-parenchymal liver cells (mixture consisting mainly of Kupffer and liver endothelial cells); LEC, liver endothelial cells. This list covers most of the studies in which the site of uptake was specified to cell type (NPC or LEC).

Ligand	Uptake mainly in	Reference
Agalacto-orosomucoid	NPC	Steer & Clarenburg (1979)
	LEC	Hubbard <i>et al.</i> (1979)
	NPC	Schlesinger <i>et al.</i> (1978)
	LEC	Praaning-van Dalen <i>et al.</i> (1987)
	LEC	Summerfield <i>et al.</i> (1982)
Ahexasamino-orosomucoid	NPC	Steer & Clarenburg (1979)
	LEC	Hubbard <i>et al.</i> (1979)
	LEC	Praaning-van Dalen <i>et al.</i> (1987)
α -Mannosidase	NPC	Ullrich <i>et al.</i> (1979)
β -Glucuronidase	LEC	Hubbard <i>et al.</i> (1979)
	NPC	Achord <i>et al.</i> (1978)
	NPC	Schlesinger <i>et al.</i> (1978)
β -Hexosaminidase	NPC	Steer <i>et al.</i> (1979)
	LEC	Isaksson <i>et al.</i> (1983)
β -N-Acetylglucosaminidase	NPC	Ullrich <i>et al.</i> (1979)
	NPC	Schlesinger <i>et al.</i> (1978)
Glycosylated salivary amylase	LEC	Niesen <i>et al.</i> (1984)
Horseradish peroxidase	LEC	Praaning-van Dalen <i>et al.</i> (1987)
Invertase	NPC	Madnick <i>et al.</i> (1978)
Lactoperoxidase	NPC	Hildenbrandt & Aronson (1985)
Mannan	NPC	Steer & Clarenburg (1979)
Mannobioseaminated ribonuclease A dimer	NPC	Steer & Clarenburg (1979)
Ovalbumin	LEC	Smedsrød <i>et al.</i> (1985c)
	LEC	Magnusson & Berg (1989)
Ribonuclease B	NPC	Brown <i>et al.</i> (1978)
	NPC	Schlesinger <i>et al.</i> (1978)
Tissue plasminogen activator	LEC	Smedsrød <i>et al.</i> (1988a)
	LEC	Kuiper <i>et al.</i> (1988)
	NPC	Saunders <i>et al.</i> (1985)
Uteroferrin	NPC	Saunders <i>et al.</i> (1985)
<i>Candida albicans</i>	LEC	Schwocho & Moon (1981)

oxidase, whereas relatively few such studies have dealt with ligands which may enter the portal vein under physiological conditions, such as lysosomal enzymes, glycosylated salivary amylase, uteroferrin, tissue plasminogen activator, *Candida albicans* (Table 3). Clearly, the mannose receptor functions as a scavenger receptor, removing enzymes which would otherwise accumulate in blood and be harmful to the organism. In addition, the liver endothelial cells may arrest circulating cells of the yeast *Candida albicans* by a mannose-specific binding mechanism (Schwocho & Moon, 1981). A direct morphological support of this concept was presented by Sawyer *et al.* (1976) who used scanning electron microscopy to show that the yeast cells adhered to liver endothelial cells, but not to Kupffer cells. A similar mannose-specific mechanism has recently been suggested for the attachment of the protozoan parasite *Leishmania donovani* (Chakraborty & Das, 1988). Thus, the mannose receptor of liver endothelial cells appears to promote adherence of pathogens.

The well-developed lysosomal apparatus of liver endothelial cells may partly be the result of the supply of lysosomal enzymes (which often retain their enzymic activity for days after they have been endocytosed) from the circulation, as suggested by Schlesinger *et al.* (1978). Moreover, the uptake of maternal uteroferrin by the fetal liver has been suggested to supply the fetal liver with iron (Saunders *et al.*, 1985). Conversely, the uptake of tissue plasminogen activator is an important mechanism to

remove this substance from the circulation, thus protecting the organism against bleeding complications.

UPTAKE OF IMMUNE COMPLEXES

It has been clearly documented that liver endothelial cells *in vitro* bind IgG-coated erythrocytes (Pulford & Souhami, 1981; Steffan & Kirn, 1982), and there is good evidence that the binding is specifically mediated by an IgG-Fc receptor (Shaw *et al.*, 1984; Smedsrød *et al.*, 1982). Skogh *et al.* (1985) found *in vivo* that immune complexes formed between IgG and highly dinitrophenylated albumin were taken up preferentially by Kupffer cells, whereas a more-light dinitrophenylation directed the complexes to liver endothelial cells. Although the study showed that the cells may be important in the uptake of IgG immune complexes, binding to a true Fc receptor or a non-specific hydrophobic interaction between the Fc region and the cell surface were left as open possibilities. van der Laan-Klamer *et al.* (1985) injected immune complexes composed of bovine serum albumin and IgG, and observed uptake in both Kupffer and endothelial cells. Insoluble complexes were taken up largely by Kupffer cells, whereas soluble complexes were located preferentially in liver endothelial cells. Ultrastructural evidence suggested that both cell types degraded endocytosed immune complexes. Based on the fact that (1) immune complexes taken up by liver endothelial cells did not fix complement, and (2) aggregated

Fig. 3. Light-microscopic micrographs of cultured rat liver endothelial cells

Freshly established cultures of liver endothelial cells were incubated for 24 h in serum-free medium containing ovalbumin (0.1 $\mu\text{g}/\text{ml}$) labelled with fluorescein isothiocyanate, washed, and prepared for light microscopy by glutaraldehyde fixation. The labelled ligand, which carries terminal mannose residues, is endocytosed by mannose-specific receptors present on the liver endothelial cells, but not on other liver cells (Smedsrød & Pertoft, 1985; Smedsrød *et al.*, 1985c). Under these conditions, liver endothelial cells only accumulate the fluorescent dye in their endocytic and lysosomal vesicles [appearing as phase-dense black perinuclear vesicles in the phase contrast microscope (a), and as white vesicles in the fluorescence microscope (b)]. Note the non-endothelial cell visible only in the phase contrast microscope (arrow). Bar is 50 μm .

IgG could inhibit the uptake, it was concluded that uptake was mediated by Fc receptors in both cell types (van der Laan-Klamer *et al.*, 1985, 1986a-c). Muro *et al.* (1987) arrived at the same conclusion by applying peroxidase-antiperoxidase IgG complexes to frozen sections of normal liver of humans and rats. The sinusoidal lining was continuously stained, and by using cell markers it was found that both Kupffer and endothelial cells bound immune complexes.

All studies reported on the hepatic distribution of the receptor for complement component C3b have demonstrated that the Kupffer cells are the only cells in liver with such a receptor *in vitro* (Pulford & Souhami, 1981; Smedsrød *et al.*, 1985c) as well as *in vivo* (Pertoft & Smedsrød, 1987). Therefore, the Fc receptor is probably the only immunological endocytosis receptor present on liver endothelial cells.

TRANSPORT OF PROTEINS THROUGH THE LIVER ENDOTHELIUM

Extrahepatic endothelium has been reported to transport substances across the vascular wall by a process termed transcytosis. Morphologically, this process is characterized by invagination and formation of an endocytic vesicle from the luminal plasma membrane. The vesicle then travels across the cell and empties by exocytosis on the abluminal side. Few reports exist describing transcytosis in liver endothelial cells. One laboratory has reported that transferrin (Soda & Tavassoli, 1984), caeruloplasmin (Kataoka & Tavassoli, 1985), transcobalamin (Soda *et al.*, 1985), and insulin (Soda & Tavassoli, 1983) are endocytosed by specific receptors in liver endothelial cells, and then transferred to the parenchymal cells. The results further suggested that the liver endothelial cells are the main site of uptake in liver of these molecules. Based on this the authors suggested that the liver endothelium serves the function of a tissue-blood barrier to these plasma proteins. Moreover, the remarkable observation was made that transferrin and caeruloplasmin were desialylated inside the cells prior to release (Irie *et al.*, 1988; Tavassoli, 1985). The molecules could then be endocytosed by the galactose receptor in parenchymal cells.

In contrast to these observations is the finding by van Berkel *et al.* (1987) that the uptake of transferrin from the rat circulation *in vivo* is mediated quantitatively by parenchymal, Kupffer and liver endothelial cells. The authors also noted that when transferrin was desialylated prior to administration, the uptake was increased in all three cell types. Clearly, the mechanism of hepatic uptake of transferrin is still a matter of controversy.

CONCLUSIONS

The liver endothelial cell has traditionally been regarded solely as a structural element of the wall of the liver sinusoids. However, research during the last decade has revealed that these cells are actively engaged in blood clearance of an array of macromolecules and small particles. They also take part in the regulation of the serum lipoprotein levels. The fact that the major components of connective tissue (hyaluronan, chondroitin sulphate, and several species of collagen) as well as other waste products (i.e. lysosomal enzymes) are eliminated rapidly and almost exclusively from the blood by

receptor-mediated endocytosis in the liver endothelial cells puts forward a novel concept of how the body disposes of soluble waste products which enter the general circulation. Clearly, the term 'hepatic reticuloendothelial system', which has traditionally been used as a synonym for the population of Kupffer cells, must be extended to embody also the population of liver endothelial cells. One may envisage a share of work between the two cell types: Kupffer cells sequester particulate matter, while liver endothelial cells take up material of smaller dimensions. Aschoff (1924), who introduced the term reticuloendothelial system, included in this group of cells the total sinusoidal endothelium.

The concept of 'scavenger receptor' must be changed to include more than the receptor which recognizes acetylated LDL (Brown & Goldstein, 1983) and formaldehyde-treated albumin (Blomhoff *et al.*, 1984b). The present review describes at least three additional receptors which fit into the definition of a scavenger receptor. These receptors recognize gelatinized collagen, PIINP, and hyaluronan and chondroitin sulphate, of which at least the two collagen-peptide receptors are clearly different from the classical scavenger receptor. Future research will no doubt extend the list of physiological ligands which are endocytosed by liver endothelial cells.

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