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Removal of cellular debris formed in the Disse space in patients with cholestasis *

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Summary. Using electron microscopy, we investigated how cellular debris, formed in the Disse space during cholestasis, was cleared. Ten patients with cholestasis of varied origin and severity were studied and compared with 10 controls without liver disease. In cholestatic patients, sinusoidal cells contained variable amounts of amylase PAS-positive material. In clean perfusion-fixed sinusoids the endothelial cells often appeared swollen and active, with few fenestrations. Hepatocyte blebs and cellular debris were sometimes seen in the Disse space. Two mechanisms were apparently involved in the clearing process: phagocytosis by macrophages either infiltrated into the Disse space, or forming the barrier; and the passage of debris from the Disse space into the sinusoidal lumen through the endothelial wall. Debris was either forced through enlarged pores or through the wall, with a progressive invagination followed by an outpouching in the lumen. The force, possibly provided by endothelial massage, may not be sufficient to push out cellular debris from the Disse space; morphological data seemed to indicate that endothelial damage may be a necessary factor. Debris present in the lumen was phagocytized by numerous active macrophages. Cellular debris was not observed in the Disse space of control patients.

Key words: Cellular debris – Kupffer – Endothelial cells

Introduction

In some liver diseases, for example in pure cholestasis, hepatocellular damage seems to be minimal, as shown by the normal or subnormal level of aminotransferases and the absence of necrosis under light microscopy. However the presence of abundant amylase (diastase) PAS-positive (DPAS) material in sinusoidal cells indicates phagocytosis of hepatocellular remnants by Kupffer cells or macrophages (Desmet 1972).

Kupffer cells anchored to the endothelial wall are largely exposed to the blood stream. Occasionally they form part of the sinusoidal barrier (Gendrault et al. 1982). Phagocytosis of necrotic material implies either the infiltration of macrophages into the Disse space or the passage of cellular debris through the endothelial wall.

The object of this electron microscopic study was to describe the process involved in the clearing of cellular debris formed in the Disse space during human cholestasis and trapped behind a non-ruptured endothelial wall.

Material and methods

Ten patients with cholestasis of varied origin and severity were studied. Relevant clinical and biochemical data are presented in Table 1. Aminotransferases were below X 2N and X 4N in 4 and 6 patients respectively. In 3 patients levels were above X 10N.

Four patients had a percutaneous biopsy for diagnostic purposes; 6 had a wedge liver biopsy performed during abdominal surgery for the relief of mechanical cholestasis. Ten patients without clinical symptoms of liver disease and with normal liver function tests were used as controls. None were alcoholic; all had a wedge liver biopsy taken during abdominal surgery performed for gallbladder lithiasis (4 cases), carcinoma of the colon or stomach (5 cases) and peptic ulcer (1 case).

Part of the biopsy was fixed and processed for routine histology. Paraffin sections were stained with haematoxylin-eosin and PAS after amylase (diastase) digestion (DPAS).

Part of the biopsy was perfusion-fixed with 1.5% glutaraldehyde (Sztark et al. 1986a, b). On 1 μm sections, mid zonal areas with well-preserved hepatocyte plates and clear sinusoid lining architecture (with no evidence of necrosis) were selected for ultra-thin sections. Grids were double-contrasted with ura-

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Table 1. Clinical and biochemical data

Cases	Age	Sex	Diagnosis	Bilirubin ($N<17$ μmol/L)	ALAT ($N<40$ UI/L)	ASAT	Alkaline phosphatase ($N<80$ UI/L)
1	72	F	Periampullary neoplasm	28	27	37	48
2	82	M	Choledocholithiasis	68	54	46	271
3	41	F	Benign recurrent cholestasis	120	52	45	190
4	22	M	Hepatitis (unknown origin)	343	54	86	118
5	60	M	Chronic pancreatitis	184	96	127	125
6	45	M	Periampullary neoplasm	184	96	195	576
7	53	M	Cancer (head) of pancreas	200	128	270	986
8	25	F	Drug hepatitis	451	685	580	89
9	35	M	Chronic pancreatitis*	82	590	1195	725
10	72	F	Chronic active hepatitis (non A, non B)	173	1525	925	270

F = female; M = Male
* Acute episode of obstructive Jaundice

Table 2. Light microscopy results

Cases	Cholestasis	Sinusoidal phagocytosis (DPAS staining)	Necrosis/inflammation
1	0	0	0/++
2	+(CL)	+(CL)	0
3	++(C and ML)	+++(C and ML)	0
4	+++(at random)	+++(at random)	+/+
5	+++(C and ML)	+++(C and ML)	0
6	+++(C and ML)	+++(C and ML)	0
7	++ (CL)	++ (CL)	0
8	+ (CL)	+ (CL)	++/+
9	++ (CL)	++ (CL)	0/+
10	0/+	0/+	+/+++

Results were graded according to severity from 0 to 3+++
CL = (centrolobular); ML = (mediolobular)

nyl acetate and lead citrate and observed under a Philips EM 301 (Centre de Microscopie Electronique, Université de Bordeaux II, France). Only results concerning the removal of hepatocyte blebs and cellular debris in the Disse space will be presented here.

Results

On light microscopy, cholestasis was of varying severity and in most cases was present in zone 3 of the acinus. It was grossly correlated to the accumulation of DPAS positive material in the sinusoids (Table 2, Fig. 1). Focal areas of damaged or necrotic cells were seen in 3 patients. In controls, there was no sign of cholestasis and DPAS staining was barely visible.

On electron-microscopy the sinusoidal barrier in controls was formed by the fenestrated endothelial wall reinforced by processes of perisinusoidal cells, or in a few cases by Kupffer cells (Fig. 2a). Neither hepatocyte blebs nor cellular debris or macrophages were seen in the Disse space.

In cholestatic patients, the sinusoidal wall contained few fenestrae; exchanges therefore appeared more difficult. Endothelial cell bodies were frequently swollen, distorted and bulged into the lumen. They contained numerous round or oval electron dense bodies of irregular shape and size (which can fuse into compound bodies), a prominent RER and well defined Golgi profiles; coated or uncoated vesicles were seen in endothelial cell bodies and their processes. Numerous hepatocyte blebs and cellular debris were observed in the Disse space but their size and number were highly variable from one sinusoid to another and from one case to another. Numerous macrophages (or Kupffer cells) obstructed the sinusoidal lumen and occasionally formed part of the barrier.

With the electron microscope it was possible to understand where phagocytosis of debris occurred (Fig. 2b):

1) phagocytosis in the Disse space (Figs. 3–4)

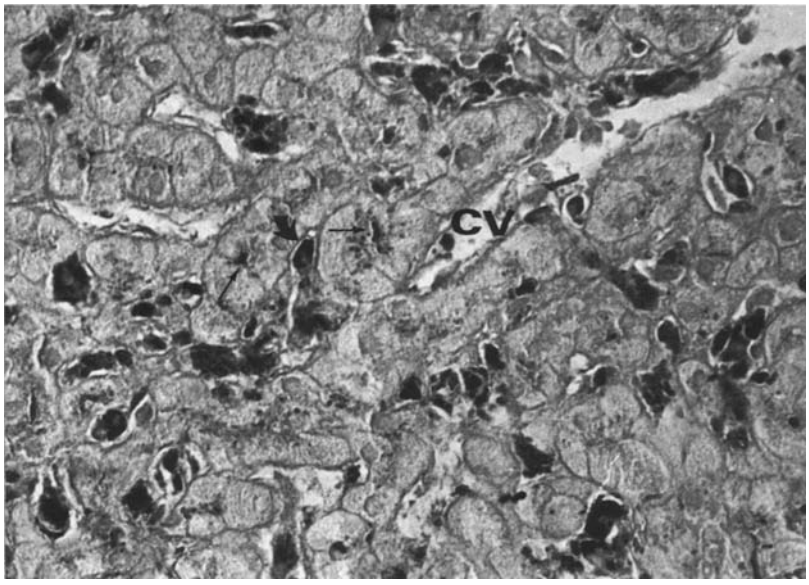

Fig. 1. Light microscopy. DPAS stain. Accumulation of DPAS positive material (*large arrow*) in sinusoids around the central vein (CV). Bile plugs (*small arrows*) are visible in dilated bile canaliculi. ×475

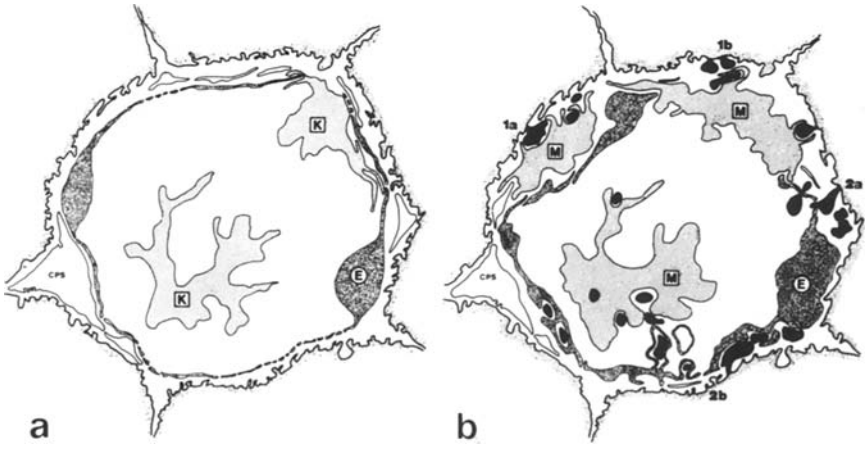

Fig. 2. Schematic presentation of sinusoidal cells in control (**a**) and cholestatic (**b**) patients. In **a** the endothelial wall is formed by fenestrated endothelial cells (*E*) and occasionally by Kupffer cells (*K*). Some Kupffer cells are also present in the lumen. No blebs or cellular debris are seen in the Disse space or in the sinusoidal lumen. In **b**, hepatocyte blebs or cellular debris (*in black*) are phagocytized by macrophages (*M*) infiltrated in the Disse space (*1a*) or by macrophages (or Kupffer cells) forming part of the sinusoidal barrier (*1b*). Blebs or debris pass from the Disse space into the lumen through the endothelial wall (*E*) either through enlarged pores (*2a*) or through the wall with a progressive invagination followed by an outpouching in the lumen (*2b*)

– macrophages infiltrated in the Disse space beneath the endothelial barrier had direct access to debris (Fig. 3),
– macrophages forming the sinusoidal barrier, that is to say in direct contact with the Disse space, also had direct access (Fig. 4).

2) Phagocytosis in the sinusoidal lumen (Figs. 5–6).

Phagocytosis by the numerous macrophages (or Kupffer cells) in the lumen occurred only if debris had crossed the barrier. There was indeed morphological evidence of the passage of debris from the Disse space into the lumen through the endothelial wall: passage either through enlarged fenestrae (Fig. 5), or by progressive invagination of debris in the wall, followed by outpouching in the sinusoidal lumen (Fig. 6). According to the plane of sectioning, debris appeared to be either imprisoned in the wall or surrounded by the endothelial membrane.

By electron microscopy it was difficult to measure exactly the importance of the clearing process because it varied from one sinusoid to another and – a fortiori – to evaluate the preponderance of one mechanism over another. It appeared however that the clearing process was correlated to the quantity of DPAS positive material present in the

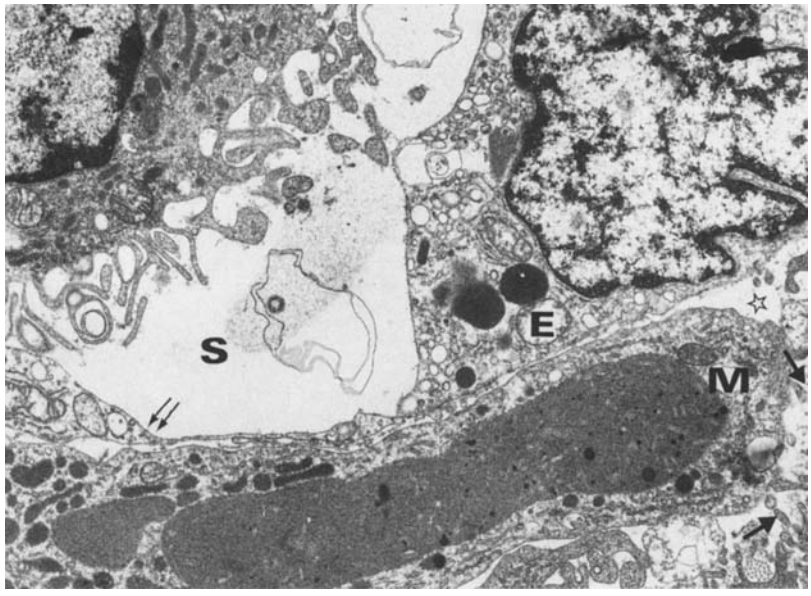

Fig. 3. Electron microscopy. Macrophages infiltrated in the Disse space (corresponding to Fig. 2b, *1a*). S = sinusoidal lumen – E = endothelial cell – *Double arrow* = endothelium lining – M = macrophages – *Arrow* = hepatocyte microvilli – White star = Disse space. × 8900

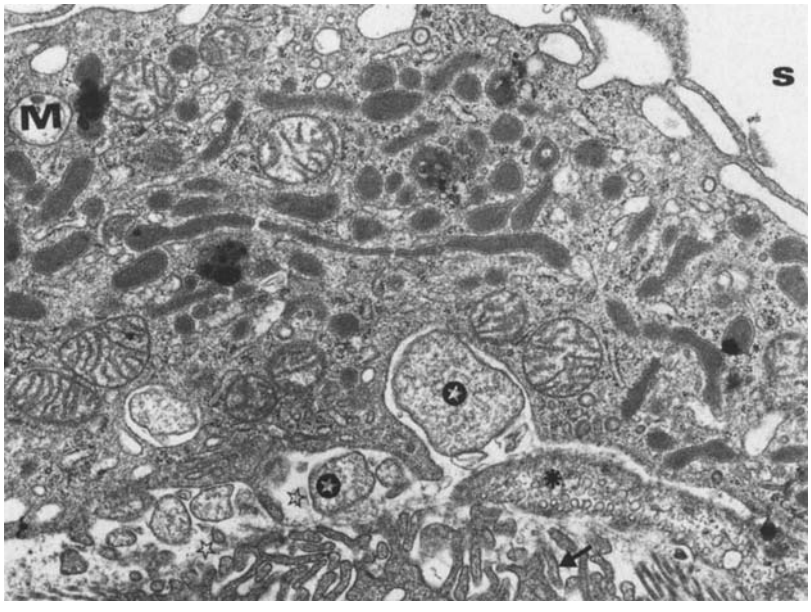

Fig. 4. Electron microscopy. Macrophages forming the sinusoidal barrier (corresponding to Fig. 2b, *1b*). Same legend as above – *White star in black circle* = cellular debris. × 17200

sinusoidal cells. The invagination process was a constant observation although all the stages were not always observed.

In addition, it must be stressed that macrophages were only rarely seen in the Disse space, that numerous sinusoids did not contain cellular debris or contained cellular debris in the Disse space without visible evidence of the clearing mechanism involved.

Occasionally the endothelial wall was ruptured, especially in patients with very high levels of bilirubin and aminotransferases, letting through large quantities of cellular debris. Even in these cases, the invagination and outpouching process was visible close to the rupture.

Discussion

Observation of the ultrastructure of the liver in cholestasis has been focused on canaliculi and hepatocyte organelles with little attention paid to the sinusoidal membranes of the hepatocytes, the Disse space and the sinusoidal cells (Phillips et al. 1979; Steiner et al. 1962).

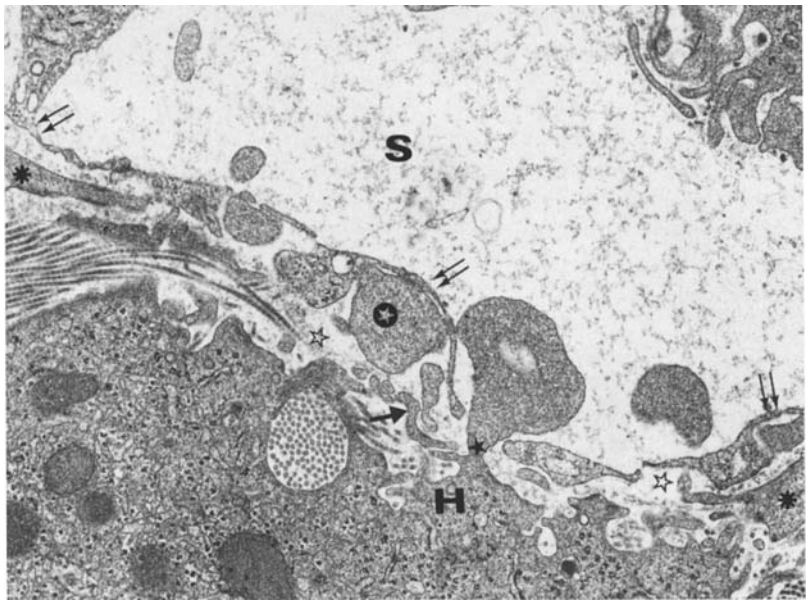

Fig. 5. Electron microscopy. Passage of cellular debris through enlarged pores of the endothelial barrier (corresponding to Fig. 2b, *2a*) Same legend as above – Black star = hepatocyte blebs – H = hepatocyte. × 8900

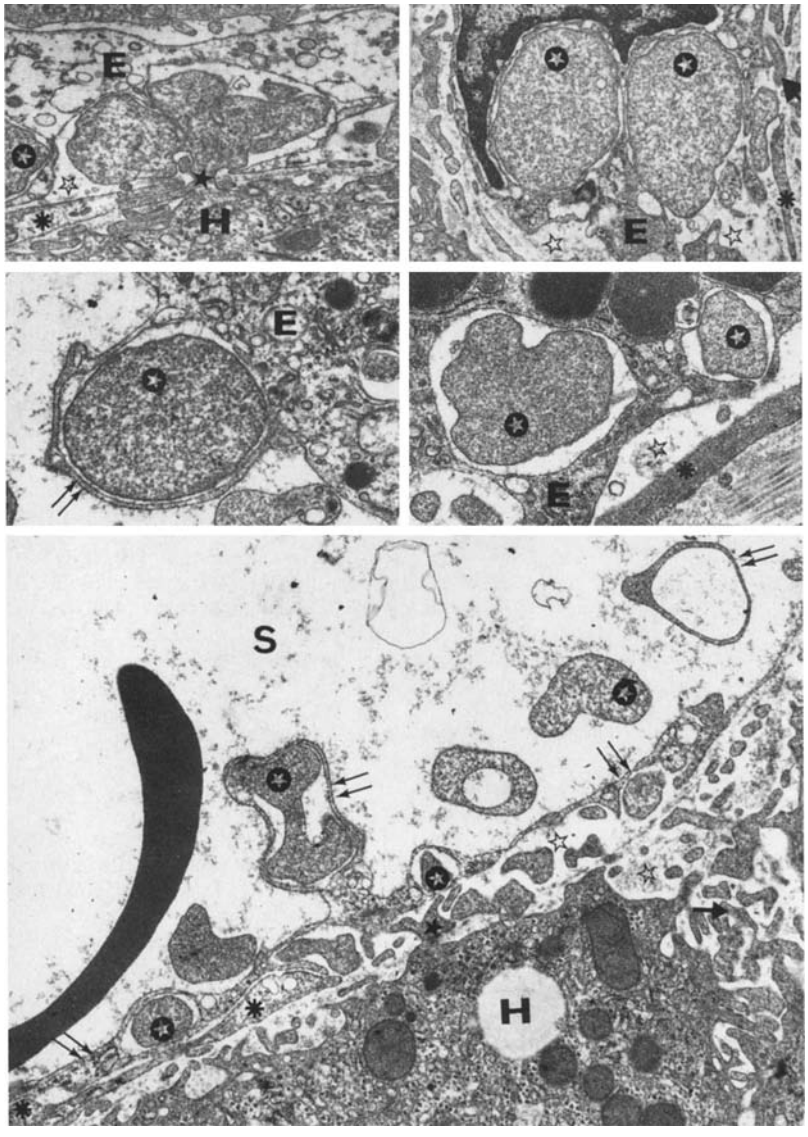

Fig. 6. Electron microscopy. Transmural passage of debris (corresponding to Fig. 2b, *2b*). In the *lower part* are present different steps of the passage of debris through the endothelial wall from bleb formation to invagination and outpouching. × 9300. In the *upper part*: a closer view of the different steps. – Hepatocyte bleb. × 13950 (*top left*) – Debris is pushed against the nucleus of the endothelial wall. × 8100 (*top right*) – Debris is progressively or totally imprisoned in the endothelial wall. × 16350 (*bottom right*) – Debris is surrounded by a thin endothelial rim. × 9700. Same legend as above (*botom left*)

Retention of bile constituents leads to parenchymal damage (Desmet 1972). There is either the formation of blebs, also called hepatocellular clasmatosis (Grimaud and Borojevic 1977), which represents a reversible manifestation of any hepatocellular injury (Desmet and De Vos 1983) or the development of focal necrosis. Both lead to the formation of cellular debris which macrophages are responsible for clearing. The mechanisms involved in this process and, more particularly, how cellular debris gets through a well preserved endothelial wall have not been studied in man in detail, to our knowledge.

Our research has shown that several mechanisms are involved although it is not possible to say which plays the major role or to prove a dynamic process from a series of static, non sequential photographs. If we disregard the presence of macrophages in the Disse space or in the barrier, the question remains how debris, which represents inert material, can reach the lumen. Endothelial cells form the fenestrated sinusoidal wall (Wisse et al. 1985). They are involved in many metabolic functions (for review see Jones and Summerfield 1985) and are capable of endocytosing materials from the blood stream, but this only concerns pinocytosis of very small particles. Kupffer cells are specific agents for the phagocytosis of large particles. The presence of debris inside endothelial cells would therefore more probably indicate transmural passage than phagocytosis.

From our morphological observations, we may speculate that it is the force generated by the endothelial massage (Wisse et al. 1985), which pushes the debris through a fragile endothelial wall that can offer little resistance. The endothelial massage is caused by the passage of white blood cells through the sinusoid. These cells, being larger than the sinusoid, compress the endothelial lining and thus the underlying Disse space as they move along the sinusoid. This constitutes the endothelial "massage". The endothelium, according to its pathological conditions, can offer varying degrees of resistance; from total impermeability to complete rupture. This blind force pushes the debris at random either into the cell body of the endothelial wall, where it will remain or be cleared by the invagination and outpouching mechanism, or into an enlarged pore, or directly into the ruptured wall. The passage of material through the non-ruptured wall could be interpreted as a sign of cellular damage. Under normal conditions, the passage of particles through the endothelial wall occurs only through fenestrae and is limited by the size of these fenestrae (up to 200/μm). Their size in turn is regulated by several factors including blood pressure and hormones (Wisse et al. 1982), and is controlled by microfilaments (Steffan et al. 1986). Although the force is entirely different, the invagination process could perhaps be compared with the migration of haemopoietic cells into the foetal liver through temporary migration pores (Bankston and Pino 1980).

In recent papers on endothelial cells in acute and chronic active hepatitis (Bardadin and Scheuer 1984; Bardadin and Desmet 1985) there has been no mention of the passage of cellular debris from the Disse space into the sinusoidal lumen. From our own experience, transendothelial passage is not specific to cholestasis but is common to all diseases which result in the formation of cellular debris. The shedding of plasma membranes has even been observed in vivo and in vitro in normal patients (De Broe et al. 1977) and in patients with cholelithiasis (Phillips et al. 1983) but it is still not clear how this debris crosses the endothelial wall.

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