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Liver cell shedding and phagocytic reaction in alcoholic liver disease

An ultrastructural study

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Summary. Sinusoidal macrophages were studied by light and electron microscopy in 49 liver biopsies from alcohol-abusers with a variety of alcohol-related liver lesions or with near-normal livers. Changes were related to those in nearby hepatocytes. A reduction in the number of macrophages was noted in the more severely damaged livers. Hepatocytes formed blebs at their sinusoidal poles, and these protruded into the space of Disse and into the sinusoidal lumen. It is postulated that reduced phagocytic activity in the livers of patients with severe alcohol-related liver disease leads to increased shedding of hepatocellular material into the circulation. This may promote the development of autoimmune reactions directed against hepatocytes.

Key words: Alcoholic liver disease – Ultrastructure – Phagocytosis – Cell shedding

Introduction

The principal components of alcoholic liver disease are fatty liver, alcoholic hepatitis characterised by hepatocellular ballooning, Mallory body formation, infiltration by neutrophil leucocytes and fibrosis, and cirrhosis; the latter usually develops as a result of alcoholic hepatitis (International Group 1981; Hall 1987). The biochemical consequences of alcohol abuse are also well-documented (Lieber 1986). However, the exact mechanisms by which hepatocytes degenerate and collagen accumulates remain unclear, and the sequence of events in alcoholic hepatitis is uncertain; these doubts reflect the lack of a satisfactory experimental model. Because light microscopic observation of alcoholic hepatitis reveals changes at the sinusoidal aspects of the hepatocytes, and because an apparent reduction in the number of active sinusoidal macrophages has been demonstrated both by scintiscanning (Lahnborg et al. 1981) and immunocytochemically (Mills and Scheuer 1985), the present study focused on events at the sinusoidal aspect of the hepatocytes as seen by electron microscopy; particular attention was paid to the relationship between sinusoidal macrophages and hepatocellular alterations.

Material and methods

Fortynine liver biopsies were selected from filed material (1978–1986) on the basis that the patients were alcohol abusers, that biopsy had shown a range of abnormalities associated with alcohol abuse or alternatively near-normal liver, that material was available for both light and electron microscopy, and that the latter material was technically satisfactory. Five patients showed little or no histological abnormality, 14 had fatty livers, 14 had features of alcoholic hepatitis without obvious cirrhosis, and the remaining 16 had cirrhosis with or without alcoholic hepatitis. All specimens had been fixed in formol saline or buffered formalin and embedded in paraffin for light microscopic examination using standard methods (haematoxylin and eosin, periodic acid-Schiff method after diastase digestion (diastase-PAS), chromotrope aniline blue, Perls' stain for iron and Gordon and Sweets' method for reticulin fibres). All specimens were stained immunocytochemically for muramidase (lysozyme) by a standard peroxidase-antiperoxidase method with pre-digestion of sections with 0.1 percent trypsin for 15 min. Immunocytochemical reagents were obtained from Dakopatts.

For electron microscopy, small portions of each biopsy specimen (2–5 blocks) were fixed in cacodylate-buffered 3 percent glutaraldehyde, and embedded in Lemix resin. Ultrathin sections were examined in a Philips 201 transmission electron microscope. In each case serial sections (3–8 grids per block) were studied, care being taken to include all zones of the hepatic acinus.

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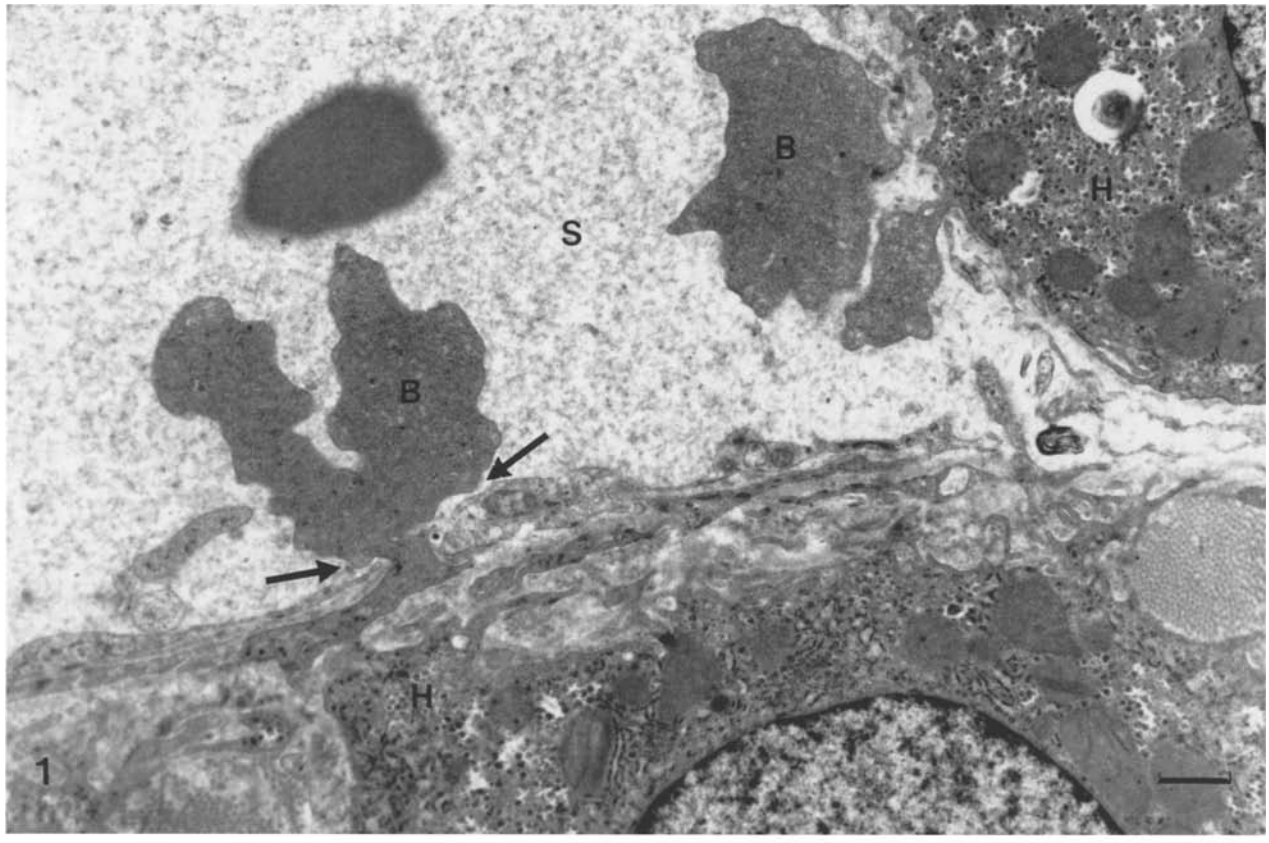


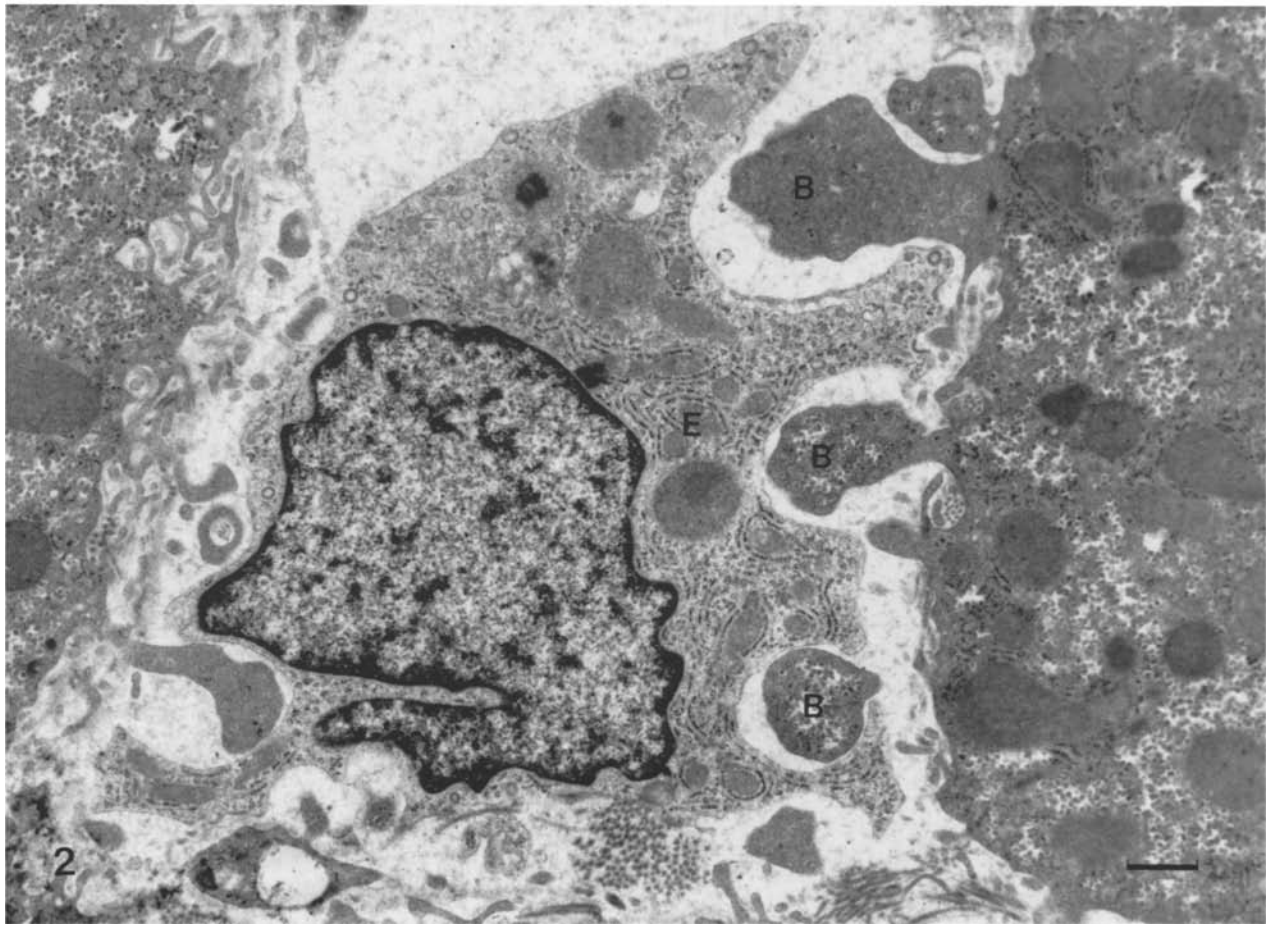
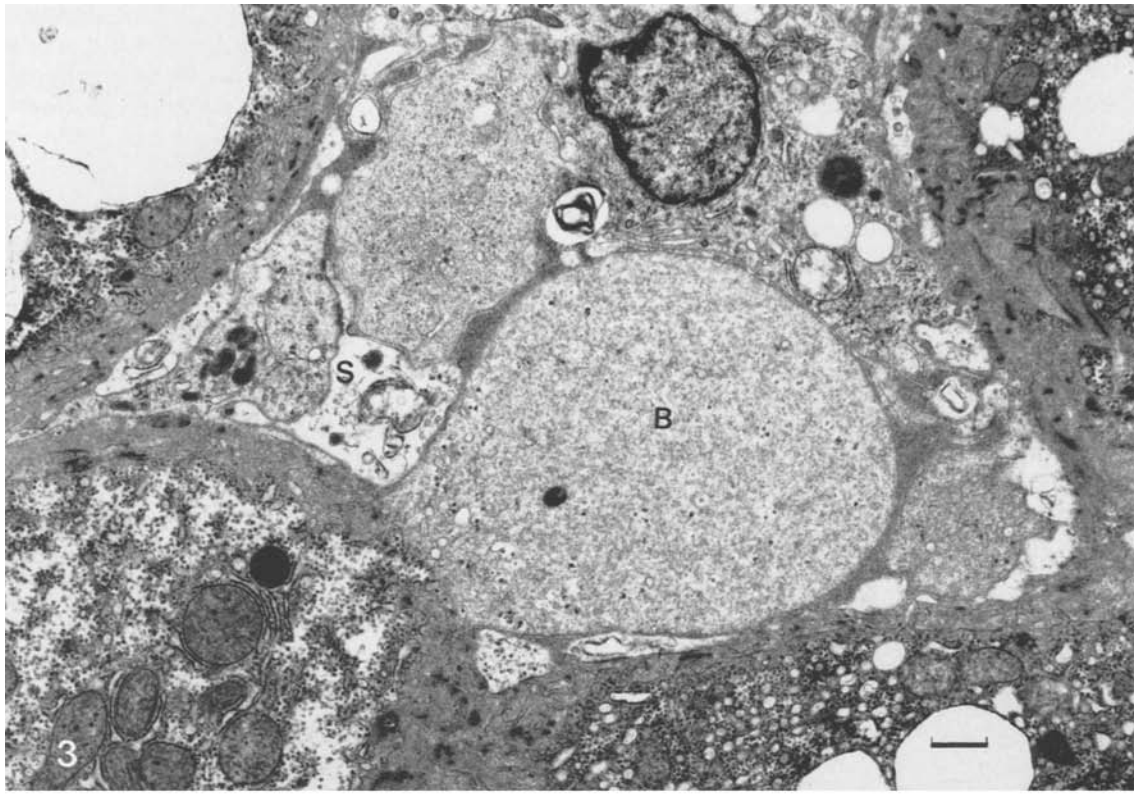


Fig. 1. Cytoplasmic blebs (*B*) protruding through endothelial fenestrae (*arrows*) into sinusoidal lumen (*S*). The larger blebs are seen to be connected with the bodies of two hepatocytes (*H*). ×9750; *bar* = 1 μm

Fig. 2. Several cytoplasmic blebs (*B*) invaginate the cytoplasm of an endothelial cell (E). ×9450; *bar* = 1 μm



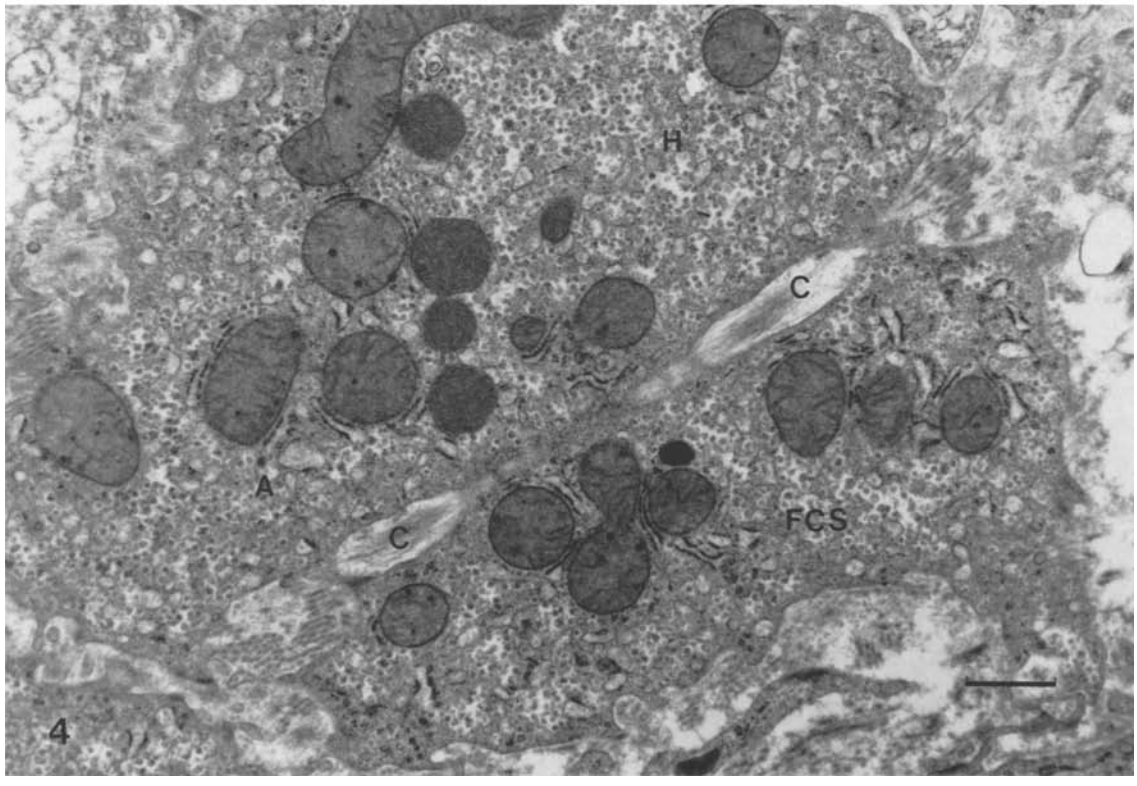


Fig. 3. A large bleb (*B*), of decreased electron density compared with the remaining cytoplasm of a hepatocyte, protrudes into a sinusoid (*S*). ×8500; *bar* = 1 μm

Fig. 4. Focal cytoplasmic shedding (*FCS*). The fragment is separated from the main body of the hepatocyte (*H*) by collagen fibres (*C*). ×13850; *bar* = 1 μm

Results

Light microscopy

Previously made diagnoses of the various forms of alcoholic liver disease were confirmed using standard criteria. Sinusoidal cells were most easily identified in diastase-PAS-stained sections. Phagocytic cells sometimes occupied a central position straddling the sinusoidal lumen. Some contained lipid droplets. Endothelial cells formed a flat sinusoid-lining layer. Their cell bodies often contained refractile iron-positive or negative granules.

The number of sinusoidal phagocytes appeared to be reduced in nearly all biopsies. Four biopsies with histological cholestasis were an exception; in these biopsies, pigment-laden phagocytes were prominent whereas in a further 7 cholestatic biopsies they were inconspicuous. In livers with fatty change but no alcoholic hepatitis, the reduced number of phagocytes, some of them containing vacuoles as well as diastase-PAS-positive material, was distributed throughout the acini. A greater reduction in phagocyte numbers was observed in alcoholic hepatitis, especially when fibrosis was severe. Remaining phagocytes were mostly seen in acinar zone 3 and in fibrous scars. Some had formed fat granulomas around lipid droplets within scars. The number of phagocytes was also reduced in most livers with cirrhosis, with or without features of alcoholic hepatitis. Two cirrhotic livers with canalicular cholestasis were an exception. Diastase-PAS-positive cells in the nodules of cirrhotic livers were usually smaller than the phagocytes seen in normal or fatty livers, resembling circulating monocytes in shape.

The number of muramidase-positive cells approximately reflected the numbers of phagocytes as assessed in diastase-PAS-stained sections; less muramidase-positive cells were seen in the more severely damaged livers. In the near-normal and mildly fatty livers, muramidase-positive cells were regularly distributed throughout the acini. In more severely fatty livers, the remaining muramidase-positive cells were mainly seen where fatty change was less severe, for example in acinar zones 2. A few formed small intra-acinar aggregates. In contrast to fatty livers, in which portal muramidase-positive cells were absent or few, portal tracts and scars in livers with alcoholic hepatitis contained more of these cells. Two different patterns were seen in cirrhotic livers; in inactive cirrhosis a few muramidase-positive cells were scattered throughout the nodules and septa, while in the presence of alcoholic hepatitis there were more positive cells within fibrous septa but few in the parenchyma. Somewhat more muramidase-positive cells were seen in the presence of cholestasis, but never as many as in the near-normal livers.

Electron microscopy

The space of Disse and sinusoids

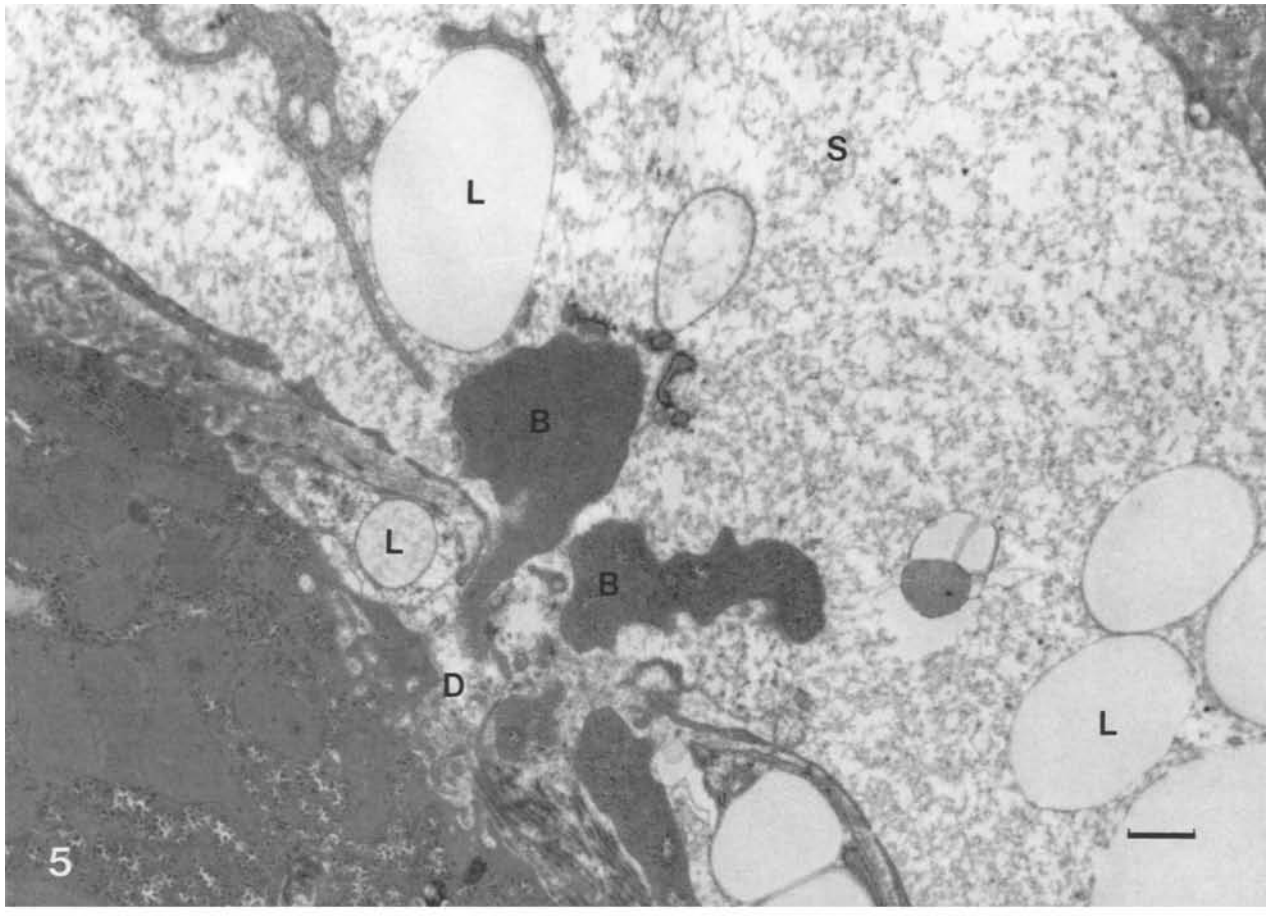
While all features of the hepatic parenchyma were surveyed, particular attention was paid to the sinusoids and sinusoidal aspects of the hepatocytes. The most striking feature seen in this location was the presence of hepatocellular debris, organelles and portions of intact hepatocytes, lying within the space of Disse and in the sinusoidal lumen. This was seen in all the disease groups as well as in the near-normal livers, and took the following forms:

(1) Blebs and fragments of amorphous membrane-limited liver-cell cytoplasm (Fig. 1). These fragments, seen in the space of Disse or sinusoid, contained few or no organelles. Some appeared to be lying free while others were in continuity with underlying hepatocytes, having apparently formed by broadening and elongation of their sinusoidal microvilli. Blebs composed of swollen hepatocyte cytoplasm penetrated the endothelial fenestrae or invaginated the endothelial cell bodies (Fig. 2), and some appeared to lie within them. Hepatocytes involved in this process showed no other degenerative features, and were not rich in lipid droplets; small blebs were found in all forms of alcoholic liver disease, but were most prominent where hepatocellular damage was least pronounced. All acinar zones were involved.

Larger blebs, also continuous with underlying hepatocytes, often showed an abrupt reduction in electron density compared with the rest of the cytoplasm (Fig. 3). Some contained scanty mitochondria or other organelles. Such blebs were often seen in severely damaged livers, in areas of alcoholic hepatitis or scarring. However, ballooned hepatocytes in alcoholic liver disease had smooth outlines and were devoid of blebs. Bleb formation was especially severe in cholestatic livers, in which the process could also be seen at the canalicular poles or lateral borders of hepatocytes, with associated loss of junctional complexes and dilatation of the perisinusoidal space.

Fig. 5. A sinusoid (*S*) contains blebs (*B*) and isolated lipid droplets (*L*). Lipid is also seen in the space of Disse (*D*) and is thought to have entered the sinusoid via endothelial pores. ×9000; *bar* = 1 μm

Fig. 6. A Kupffer cell (*K*) is characterized by marked irregularity of cell and nuclear shape, and a content of primary and secondary lysosomes. It occupies a central position in the sinusoidal lumen. ×8500; *bar* = 1 μm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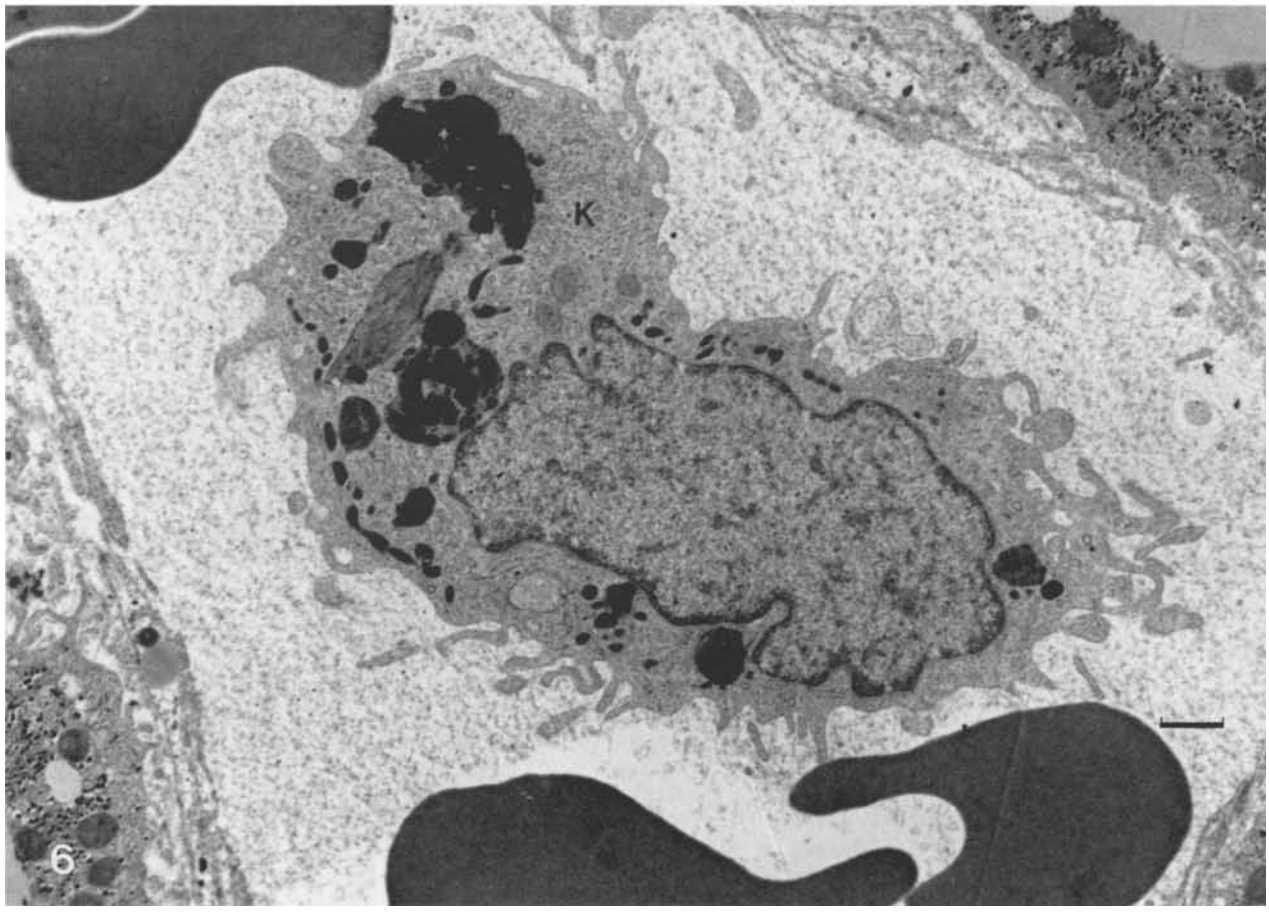
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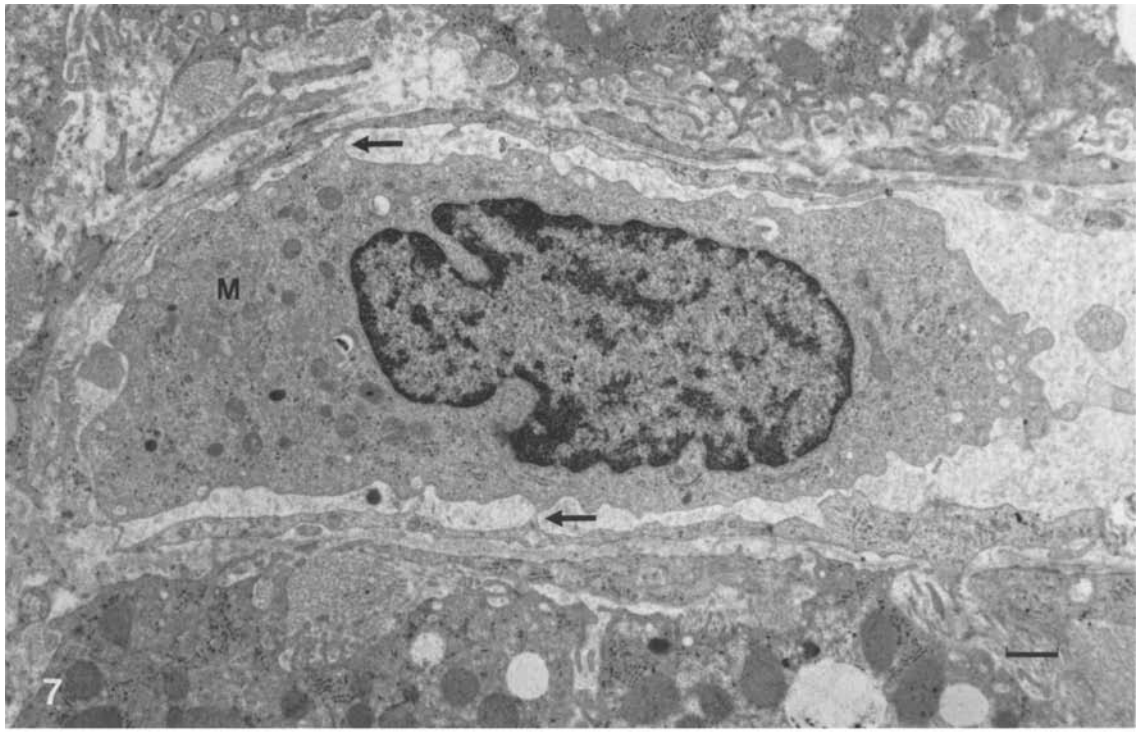

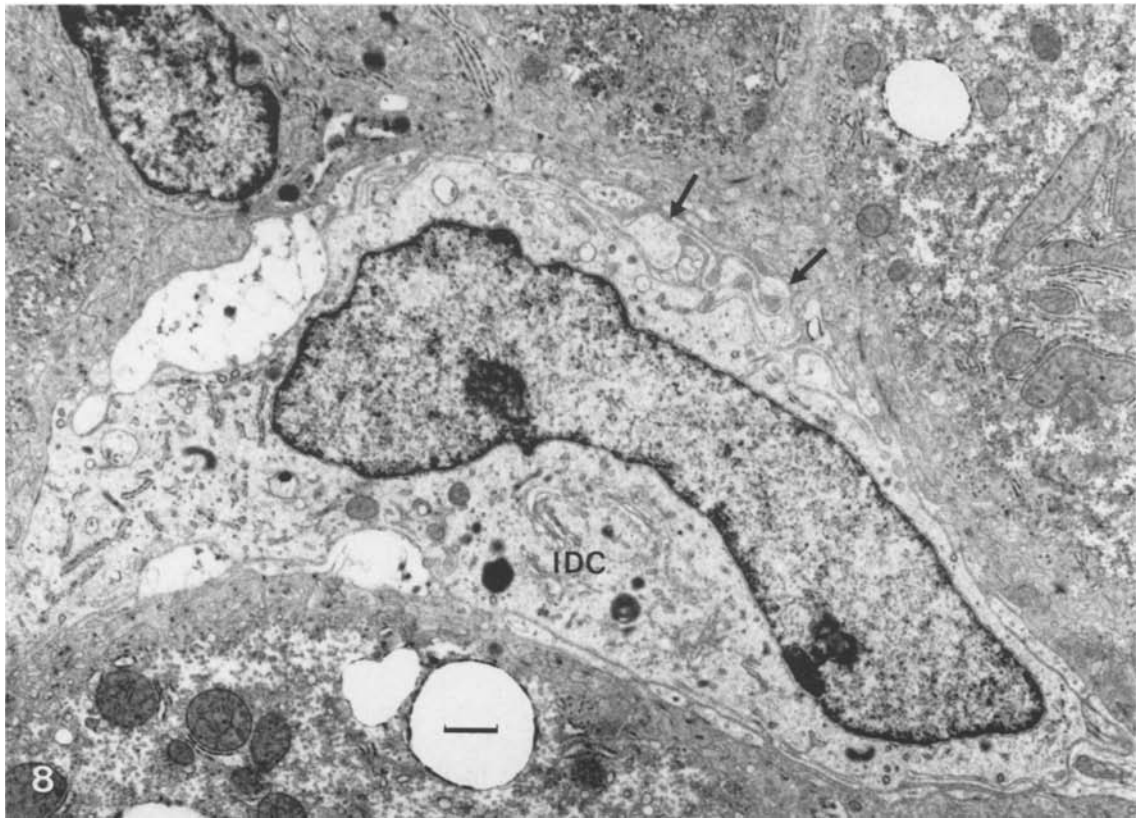

Fig. 7. A monocytoid cell (*M*) within a sinusoid. Although the cell resembles a circulating monocyte, irregularity of the cell borders in relation to adjacent cells suggests anchoring within liver tissue (*arrows*). There is little evidence of phagocytic activity. $\times 7750$; *bar* $= 1$ μm

Fig. 8. An interdigitating cell (*IDC*) within a sinusoid. Many processes extend from the cell membrane (*arrows*). Lysosomes are scanty. $\times 8000$; *bar* $= 1$ μm

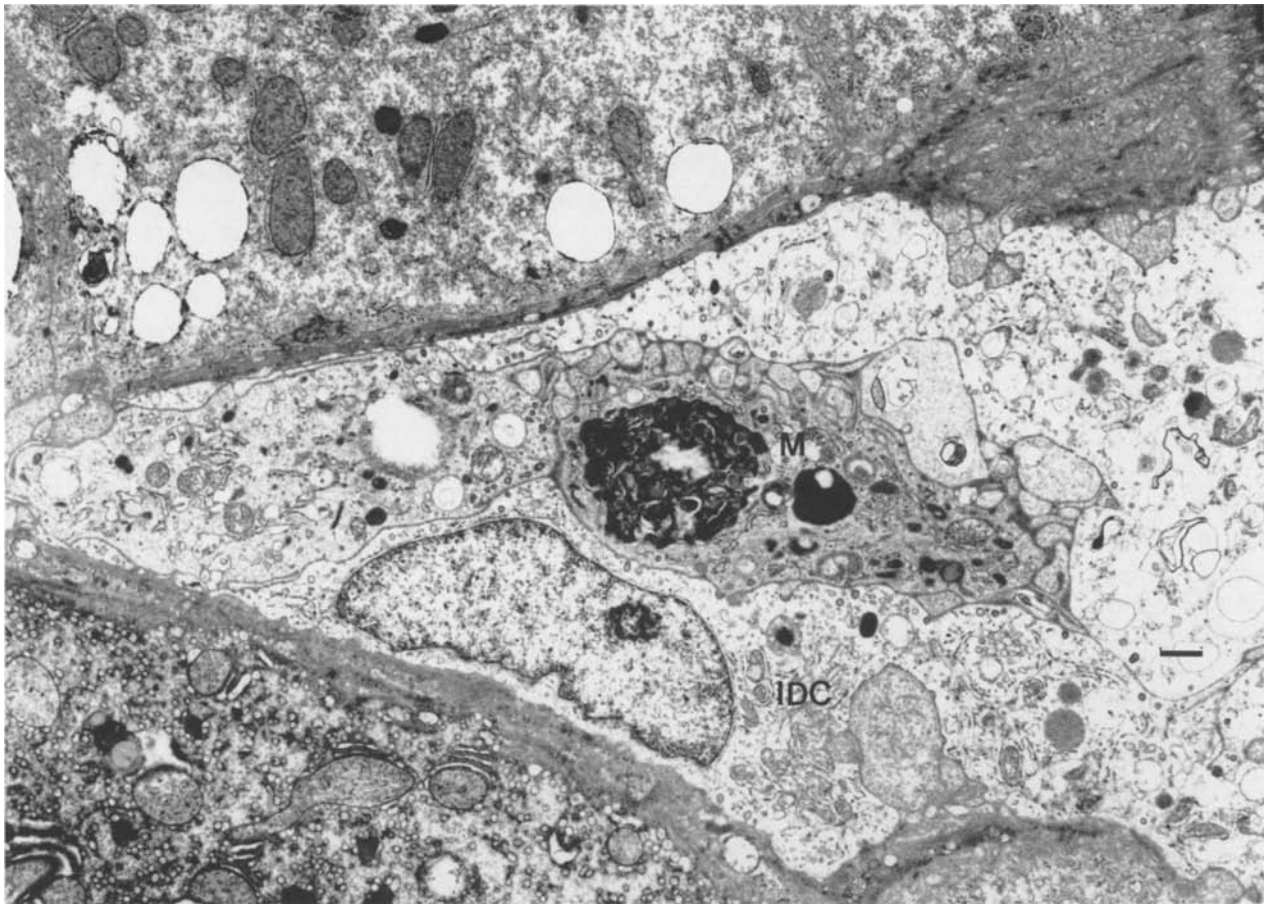

Fig. 9. A granuloma-like aggregate containing a macrophage (*M*) and a cell (*IDC*) with characteristics of an interdigitating cell. ×5600; *bar* = 1 μm

(2) Focal cytoplasmic shedding (Fig. 4). Portions of hepatocyte cytoplasm appeared to have separated from the parent cells, and either lay close to these cells, more or less in their original locations, or protruded from the parent cells in polypoid fashion. The contents of these separated fragments were not noticeably different from those of the rest of the cell, and mitochondria, endoplasmic reticulum, lipid vacuoles and glycogen could be identified. The electron density of the fragments was approximately that of normal liver-cell cytoplasm. The lesion was therefore not considered to represent apoptosis, a process seen infrequently in the material studied. Portions of liver cells resembling these fragments were often seen in the spaces of Disse, sinusoids or terminal hepatic venules, lying among fragmented cell membranes and organelles.

(3) Free-lying lipid vacuoles (Fig. 5). These were found in the space of Disse, sinusoids and terminal hepatic venules.

Macrophages

Two types of cells with ultrastructural characteristics of macrophages were found. *Kupffer cells* had irregular outlines and a ruffled, villous border (Fig. 6). They occupied a central position in the sinusoids, straddling their lumens. Their nuclei were irregular in shape, and their cytoplasm contained variable numbers of primary and secondary lysosomes. *Monocytoid macrophages* (Fig. 7) had more regular outlines and horseshoe-shaped nuclei. They contained variable numbers of lysosomes and a few short strands of rough endoplasmic reticulum. They thus resembled circulating monocytes, differing from them only in their attachment to hepatic cells, their shape and, in some cases, more abundant lysosomes. In addition to these two cell types, there were cells of interdigitating type, with irregular outlines, prominent invaginations of their membranes and little or no evidence of phagocytic activity (Fig. 8); these cells characteristically obliterated sinusoidal lumens.

They formed granuloma-like aggregates together with macrophages and neutrophil leucocytes, especially in livers with alcoholic hepatitis and cirrhosis.

Overall numbers of macrophages varied considerably between the different disease groups. In near-normal livers, Kupffer cells were readily seen in sinusoidal lumens, while circulating monocytes and monocytoid macrophages were sparse. In livers with substantial fatty change monocytoid cells were more abundant. Kupffer cells were present, but in a different location; instead of straddling the lumens of the sinusoids, they were seen within their walls, with their processes or even the whole cell within the space of Disse. Villous projections of Kupffer cell cytoplasm, normally seen on much of the cell circumference, were reduced on the sinusoidal aspect but preserved on cell surfaces facing the space of Disse and hepatocytes. In the most severe examples of fatty change, aggregates of macrophages were present within or outside the sinusoids, sometimes severely reducing or even obliterating their lumens. In some of these aggregates there were macrophage-like cells with scanty lysosomes and prominent interdigitation with neighbouring cells; this suggested a transition from macrophage to interdigitating cell (Fig. 9).

In alcoholic hepatitis macrophages were seen in focal aggregates associated with hepatocellular necrosis, and as part of fat granulomas, but there was an overall decrease in Kupffer cell numbers. Monocytes and monocytoid macrophages, on the other hand, were abundant.

In cirrhotic livers, the macrophage pattern was mixed, with features of both fatty liver and alcoholic hepatitis. In the presence of cholestasis, actively phagocytic macrophages were found, rich in phagosomes and containing biliary material.

Discussion

In this survey of ultrastructural changes in the region of the sinusoidal wall in various forms of alcoholic liver disease, the principal findings were firstly bleb formation and cell shedding at the sinusoidal borders of hepatocytes, with apparent release of cell fragments into the space of Disse, and secondly changes in the numbers, distribution and types of macrophages. Changes in the appearance of endothelial cells and perisinusoidal cells of Ito (lipocytes, fat-storing cells) were also noted but will not be discussed in the present paper.

Cytoplasmic blebs at the sinusoidal poles of hepatocytes and shedding of portions of cytoplasm have been previously described (De Broe et al. 1977; Cossel 1980). In vitro studies have correlated bleb formation with thiol and calcium disturbance and it has been suggested that blebs reflect a change in cytoskeletal structure (Robertson et al. 1978; Jewell et al. 1982). Phillips et al. (1987) documented bleb formation at an ultrastructural level in a variety of liver diseases and considered it to be an indication of hepatocellular degeneration. In patients with cholestasis the cellular debris derived from these extensions of the liver-cell cytoplasm appears to be removed by macrophages (Dubuisson et al. 1987; Raymond et al. 1987). In our own study blebs and fragments were found in both cholestatic and non-cholestatic livers and, with surprising frequency, in near-normal and fatty livers of alcohol abusers as well as in the more severe forms of alcohol-related liver damage. One possible explanation for bleb formation on otherwise normal hepatocytes is that the blebs represent a physiological mechanism whereby the hepatocyte can modify its content of cytoplasm and organelles. The focal cytoplasmic shedding observed in our study may have similar significance. When normal in numbers and activity, macrophages in and near the sinusoidal wall then presumably prevent escape of substantial amounts of hepatocellular cytoplasm into the circulation.

In patients with alcohol-related liver disease, however, macrophage activity appears to be reduced (Holdstock et al. 1982). We found decreased numbers of cells with the classical appearances of Kupffer cells, both by light microscopy using diastase-PAS staining and immunocytochemical demonstration of muramidase (lysozyme), and on electron microscopy. Our findings are in keeping with the observation that alcohol abusers with cirrhosis have reduced density of liver scans following injection of radioactive colloids (Lahnborg et al. 1981), and are similar to those in an earlier study of muramidase-positive cells, in which a reduction in such cells was found particularly in alcoholic hepatitis and in cirrhosis (Mills and Scheuer 1985). The effect of a reduction in phagocytic activity in the region of the hepatic sinusoid, in the presence of normal or even perhaps increased cytoplasmic bleb formation and shedding, would probably be to allow increased amounts of hepatocellular material to escape and to reach the general circulation. This may be a factor in promoting the development of autoimmune phenomena in alcoholic liver disease; antibodies to a number of cell components including cytoskeleton have been reported (Kanagasundaram et al. 1977; Zetterman and Sorrell 1981; Kurki et al. 1983; Kurki et al. 1984), and chronic active hepatitis-like lesions have been described in

alcohol abusers (Goldberg et al. 1977; Nei et al. 1983). By contrast, in cholestatic liver diseases, normal or even increased phagocytic activity is presumably adequate to deal with a normal or increased amount of hepatocellular material entering the space of Disse.

Shedding of portions of liver-cell cytoplasm may also affect lipid metabolism, since shed fragments often contained ultrastructurally identifiable lipid. Free lipid droplets were also seen in the space of Disse and vascular lumen. The observed decrease in macrophage numbers would have the effect of allowing more hepatocellular lipid to reach the circulation, thus contributing to hyperlipidaemia.

Muramidase-positive and ultrastructurally typical Kupffer cells are not the only members of the mononuclear phagocyte system to be found in the liver. Contrasting with the reduction in Kupffer cells, there was a concomitant increase in monocytoid cells, whose shape and organelle content suggested only weak phagocytic activity. They probably represent a stage in differentiation towards antigen-presenting interdigitating cells (Bardadin and Desmet 1984). Finally, it is clear that many different factors operate in the various alcohol-related liver diseases, and that many different cell types contribute to their pathogenesis. We have only focused on a small part of this picture.

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References

Bardadin KA, Desmet VJ (1984) Interdigitating and dendritic reticulum cells in chronic active hepatitis. Histopathology 8:657–667

Cossel L (1980) Peripheral cytoplasmic shedding around hepatocytes, apocrine secretions, pathological cell reaction or preparation artefact? Pathol Res Pract 170:298–327

De Broe ME, Wieme RJ, Logghe GN, Roels F (1977) Spontaneous shedding of plasma membrane fragments by human cells in vivo and in vitro. Clin Chim Acta 81:237–245

Dubuisson L, Bioulac-Sage P, Boussarie L, Quinton A, Saric J, de Mascarel A, Balabaud C (1987) Removal of cellular debris formed in the Disse space in patients with cholestasis. Virchows Arch (A) 410:501–507

Goldberg SJ, Mendenhall CL, Connell AM, Chedid A (1977) "Nonalcoholic" chronic hepatitis in the alcoholic. Gastroenterology 72:598–604

Hall P (1987) Alcoholic liver disease. In: MacSween RNM, Anthony PP, Scheuer PJ (eds) Pathology of the liver, 2nd ed., Churchill Livingstone, Edinburgh, pp 281–309

Holdstock G, Leslie B, Hill S, Tanner AR, Wright R (1982) Monocyte function in cirrhosis. J Clin Pathol 35:972–979

International Group (1981) Alcoholic liver disease: morphological manifestations. Lancet i:707–711

Jewell SA, Bellomo G, Thor H, Orrenius S, Smith MT (1982) Bleb formation in hepatocytes during drug metabolism is caused by disturbances in thiol and calcium ion homeostasis. Science 217:1257–1259

Kanagasundaranam N, Kakumu S, Chen T, Leevy CM (1977) Alcoholic hyalin antigen (AHAg) and antibody (AHAb) in alcoholic hepatitis. Gastroenterology 73:1368–1373

Kurki P, Miettinen A, Salaspuro M, Virtanen I, Stenman S (1983) Cytoskeleton antibodies in chronic active hepatitis, primary biliary cirrhosis and alcoholic liver disease. Hepatology 3:297–302

Kurki P, Gripenberg M, Teppo AM, Salaspuro M (1984) Profiles of antinuclear antibodies in chronic active hepatitis, primary biliary cirrhosis and alcoholic liver disease. Liver 4:134–138

Lahnborg G, Friman L, Berghem L (1981) Reticuloendothelial function in patients with alcoholic liver cirrhosis. Scand J Gastroenterol 16:481–489

Lieber CS (1986) Alcohol and the liver: metabolism of ethanol, metabolic effects and pathogenesis of injury. Acta Med Scand (Suppl) 703:11–55

Mills LR, Scheuer PJ (1985) Hepatic sinusoidal macrophages in alcoholic liver disease. J Pathol 147:127–132

Nei J, Masuda Y, Takada A (1983) Chronic hepatitis induced by alcohol. Dig Dis Sci 28:207–215

Phillips MJ, Poucell S, Patterson J, Valencia P (1987) The liver. An atlas and text of ultrastructural pathology. Raven Press, New York

Raymond JM, Dubuisson L, Bioulac-Sage P, Balabaud C (1987) Benign recurrent cholestasis: a light and electron microscopic study with emphasis on sinusoidal cells. Ultrastruct Pathol 11:11–17

Robertson AMG, Bird CC, Waddell AW, Currie AR (1978) Morphological aspects of glucocorticoid-induced cell death in human lymphoblastoid cells. J Pathol 126:181–187

Zetterman RK, Sorrell MF (1981) Immunologic aspects of alcoholic liver disease. Gastroenterology 81:616–624

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