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Ultrastructural Characteristics of Novel Epithelial Cell Types Identified in Human Pathologic Liver Specimens with Chronic Ductular Reaction

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Previous immunohistochemical studies on human liver biopsies with chronic ductular reaction revealed the presence of "small cells" with bile-duct type cytokeratin profile in the periportal area. This study identified similar cells by electron microscopy. The authors studied 13 human liver specimens with various liver diseases, but all characterized by chronic ductular reaction. In all specimens, variable numbers of "small cells" with common epithelial characteristics were identified in the periportal area. They could be classified into three types. Type I cells showed an oval cell shape and oval nucleus, early or established formation of junctional complexes with adjacent cells, a full assortment of cytoplasmic organelles, and bundles of tonofilaments. Type II cells showed features of bile-duct cell differentiation, including lateral interdigitations, apical microvilli, basal pinocytotic vacuoles, and basement membrane formation. In contrast, type III cells displayed additional features indicating hepatocellular differentiation, such as a more prominent nucleus, formation of a hemicanaliculus, and glycogen rosettes. It is concluded that these small cells of epithelial nature display variable differentiation characteristics of either bile-duct type cells or hepatocytes. These findings support the existence of bipotential progenitor epithelial cells in human liver. They may have implications for liver regeneration and carcinogenesis. (Am J Pathol 1992, 140:1441–1450)

Oval cells are small nonparenchymal cells found in the liver of carcinogen-treated rats and first described by Opie in 1944.[1]

The term oval cells is coined by Farber[2]; Popper et al[3] called them disorganized ductular cells. Oval cells have been studied extensively *in vivo* and *in vitro* during the early stage of hepatocarcinogenesis in rats.[4–10]

The nature of oval cells remains a controversy.[11] It is believed that they are related to the terminal bile ductules,[4,5] although an origin from a hypothetical liver stem cell has also been proposed.[7,11–13] Other authors[9,14] further postulated that such a cell is in fact a facultative stem cell since it proliferates only in severe liver injury or after exposure to hepatocarcinogens.

Few studies on human liver are on record; these only suggest the possible existence of a facultative stem cell in human liver.[15,16] In cases with hepatitis and liver cirrhosis, alpha-fetoprotein producing cells were described as having the appearance of "oval cells" and transitional cells.[17]

Some studies on human liver using keratin immunohistochemistry have drawn attention to the occurrence of "small cells," rich in cytokeratins, which apparently are related in some way to ductular proliferation, and which are found at some distance from the portal tracts[18,19] (and Desmet V et al, Hepatology 1990, 12:1249–1250).

This study identified similar small cells at the ultrastructural level by analyzing in electron microscopy the proliferation of bile ductules in portal and periportal areas in human liver in several pathologic conditions associated with chronic cholestasis.

Materials and Methods

Thirteen needle liver specimens taken for diagnostic purposes from patients with chronic liver diseases were selected for this study.

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Specimens were received fresh. One part was Bouin-fixed and used for paraffin sections; another part was snapfrozen in liquid-nitrogen–cooled isopentane and stored for later immunohistochemical studies. A third small sample was fixed in 2.5% glutaraldehyde, postfixed in 1% OsO4 and prepared for routine electron microscopy. Tissue blocs containing areas with ductular structures were selected on semithin sections by light microscopy; these were selected for cutting ultrathin sections. The selected tissue specimens correspond to the following histologic and clinical diagnoses:

Five cases with alcoholic hepatitis
Two cases with longstanding extrahepatic obstruction
One case with primary sclerosing cholangitis
Two cases with primary biliary cirrhosis
Two cases with liver cirrhosis (possibly alcoholic)
One case with congenital hepatic fibrosis

Results

Special "small cells" are observed in the periportal liver parenchyma, especially close to the proliferating ductules and around some hepatocytes in acinar arrangement. Three types can be distinguished on account of their morphologic characteristics and position.

The type I cell, approximately 7–10 μm diameter has an oval nucleus (Figures 1–4). The heterochromatin of the nucleus forms a thick marginal waving rim and small clumps dispersed in the nucleoplasm. Sometimes a round nucleolus is seen preferentially located at the nuclear margin. The cytoplasm contains a moderate number of organelles.

The mitochondria, provided with long transverse cristae, are round to oval; the granular endoplasmic reticulum forms well-developed slightly dilated cisternae; the agranular endoplasmic reticulum forms some small ves-

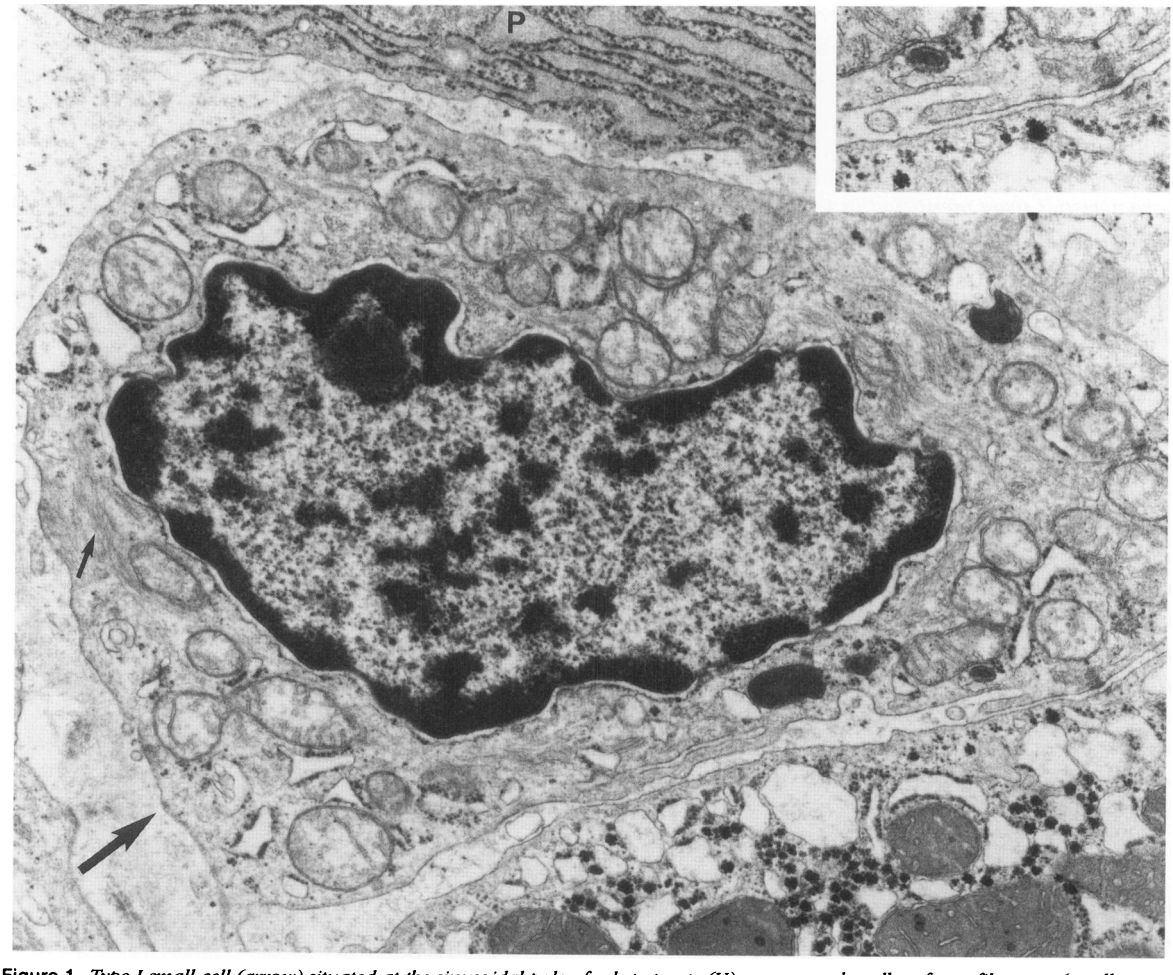

Figure 1. *Type I small cell (arrow) situated at the sinusoidal pole of a hepatocyte (H); numerous bundles of tonofilaments (small arrow); plasma cell (P). Magnification, ×23000. Inset: Detail of dense-cored vesicle and developing desmosomal junction. Magnification, ×36800.*

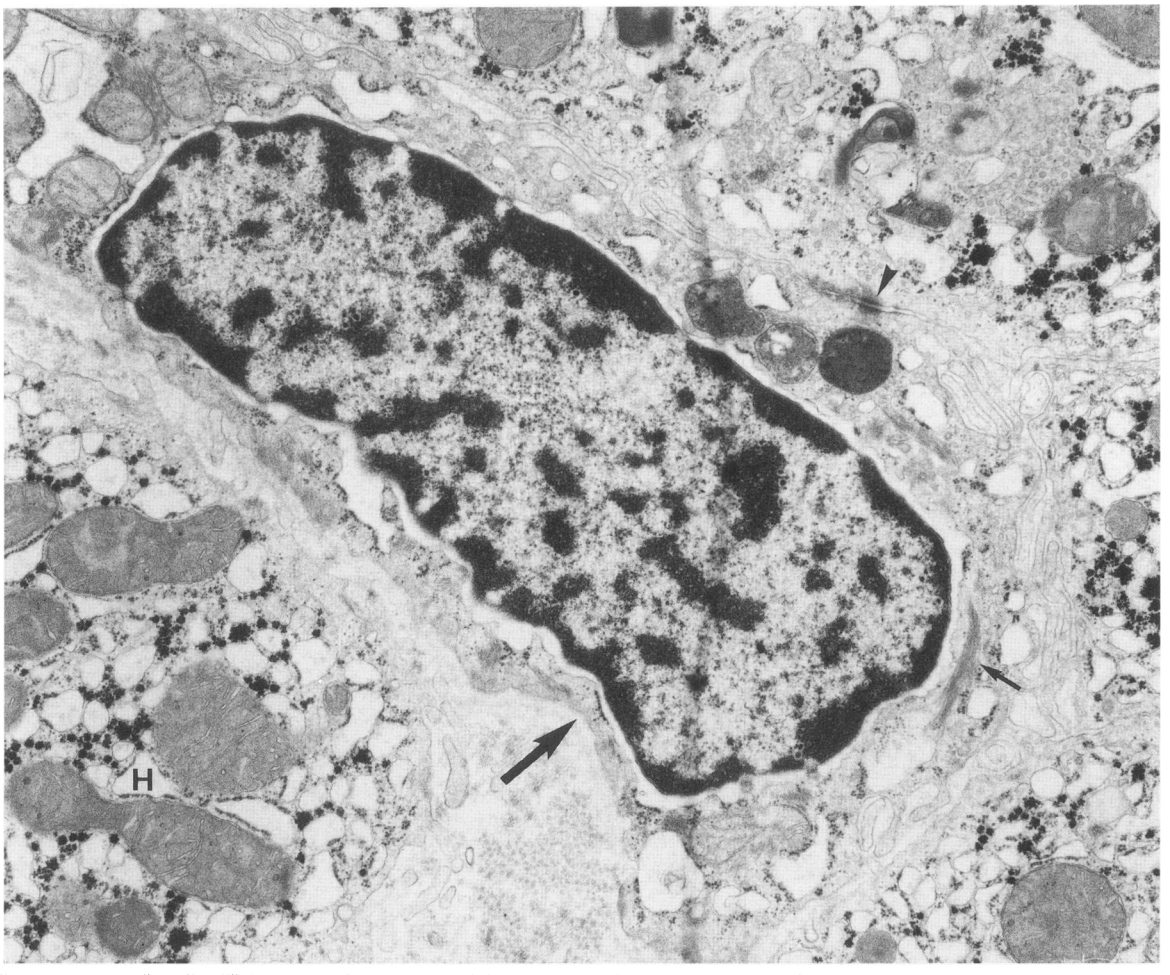

Figure 2. *Type I "small cell" (arrow) at the perisinusoidal recessus near hepatocytes (H); tonofilaments (small arrow); desmosome (arrowhead). Magnification, ×18400.*

icles and the Golgi apparatus is moderately developed; there are a few prominent lysosomes. Numerous bundles of tonofilaments are always obvious (Figures 1–4); a dense-cored vesicle of the type observed in neuroendocrine cells is occasionally found (Figure 1, inset). This type I "small cell" is situated at the sinusoidal pole of hepatocytes and seems to penetrate in the perisinusoidal recessus; occasionally this "small cell" is wedged between adjacent hepatocytes (Figure 3).

Rudimentary (Figures 1, 4) or well-developed junctional complexes of the desmosomal type (Figure 2) join the "small cell" with neighboring hepatocytes.

Sometimes a discontinuous basement membrane is found to partly surround the small cell (Figure 4).

The type II "small cell" presents the same general characteristics as type I but deviates from it by the following features. As to their position, type II "small cells" are located in the neighborhood of ductular proliferations where they tend to lie next to ductular cells composing small bile ductules (Figures 5a, 6a). Their apical cell membrane forms microvillous-like extensions; their lateral cell membrane often forms interdigitations with adjacent cells as well as typical tight junctions (Figure 5b). The basal part of the cell often shows some pinocytic invaginations (Figure 6b) and is surrounded by a distinct basement membrane (Figures 5b, 6b).

The type III "small cell" approximately 10–15 μm diameter also has the same overall features of type I cell but presents in addition characteristics of hepatocytes. The nucleus is more prominent; the cytoplasm is more voluminous with more numerous organelles and glycogen rosettes. The type III "small cell" is a polarized cell forming an intercellular canaliculus with the neighboring hepatocytes, as well as intercellular junctions at their lateral membranes (Figures 7, 9). Some bundles of tonofilaments are apparent (Figure 7). Cell types that are intermediate between type I and type III may occur (Figure 8).

As far as can be judged on the topography of acinar

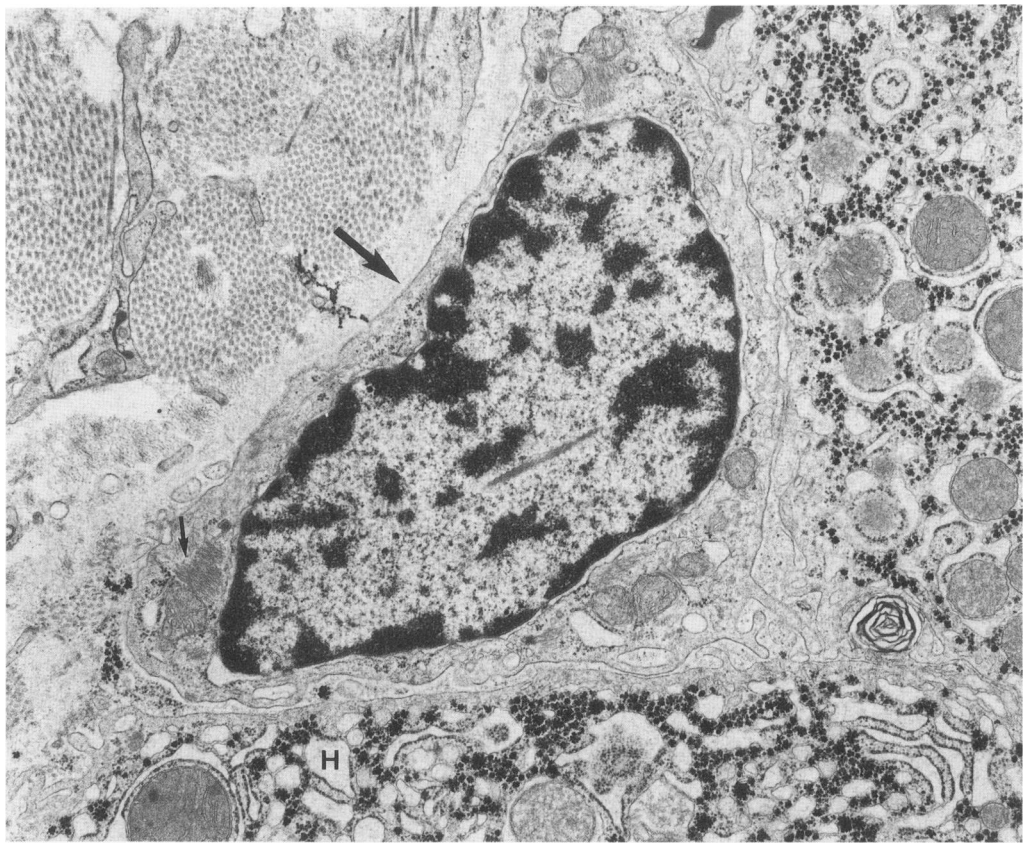

Figure 3. *Type I "small cell" (arrow) wedged between two hepatocytes (H); tonofilaments (small arrow). Magnification, ×18400.*

units in small ultrathin sections, the three types of "small cells" are found in periportal areas. As to the frequence of these "small cells" they are not numerous, varying between none and three to four per ultrathin section and variable from specimen to specimen; it is sometimes hard to detect them at all.

However in three cases with chronic alcoholic liver disease in which marked ductular reaction was apparent,

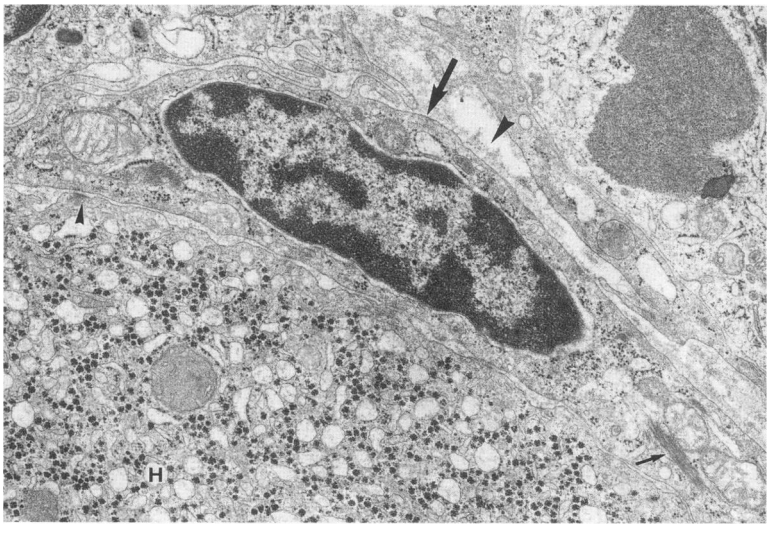

Figure 4. *Type I "small cell" (arrow) situated at the sinusoidal pole of a hepatocyte (H); tonofilaments (small arrow); basement membrane (arrowhead); desmosome in early stage of development (small arrowhead). Magnification, ×23000.*

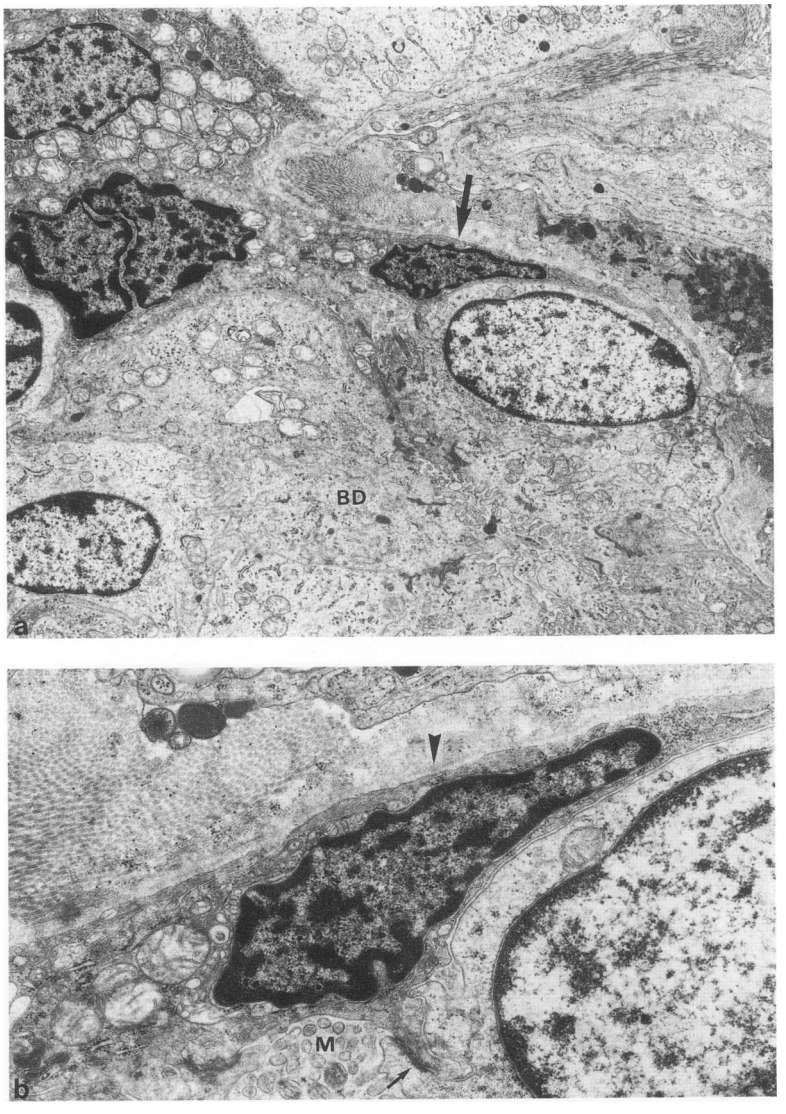

Figure 5. *Electron micrograph of liver biopsy from a patient with longstanding extrahepatic obstruction.* **a**: *Type II small cell (arrow) close to proliferated ductule (BD). Magnification, ×7250.* **b**: *Higher magnification of* (**a**) *showing microvilli (M); tight junctions (small arrow); basement membrane (arrowhead). Magnification, ×23000.*

these "small cells" are easily found. In the same tissue specimens, all possible combinations of one type, two types, or three types of "small cells" can be found.

Discussion

Our ultrastructural findings on the presence and appearance of "small cells" in pathologic human liver specimens with chronic ductular reaction are intriguing and have not yet been previously described.

Three types of "small cells" can be distinguished in the periportal liver parenchyma. They all share common characteristics: small size, oval nucleus, early or established formation of junctional complexes with adjacent cells, a full assortment of cytoplasmic organelles, well-developed rough endoplasmic reticulum cisternae and mitochondria, and some prominent lysosomes and bundles of tonofilaments.

In addition, the type II cell presents peripheral membrane specializations: lateral interdigitations, apical microvilli, well-established junctional complexes, basal pinocytotic vacuoles, and well-developed basement membrane. It is a polarized cell that clearly presents characteristics of the neighboring bile duct cells.

In turn, the type III cell is a more voluminous cell with a more prominent nucleus. It is a polarized cell that forms a hemicanaliculus, provided with glycogen rosettes in the cytoplasm. This cell type shares differentiation characteristics with hepatocytes.

The type I cell can be considered the less-differentiated cell type. To our knowledge, this type I

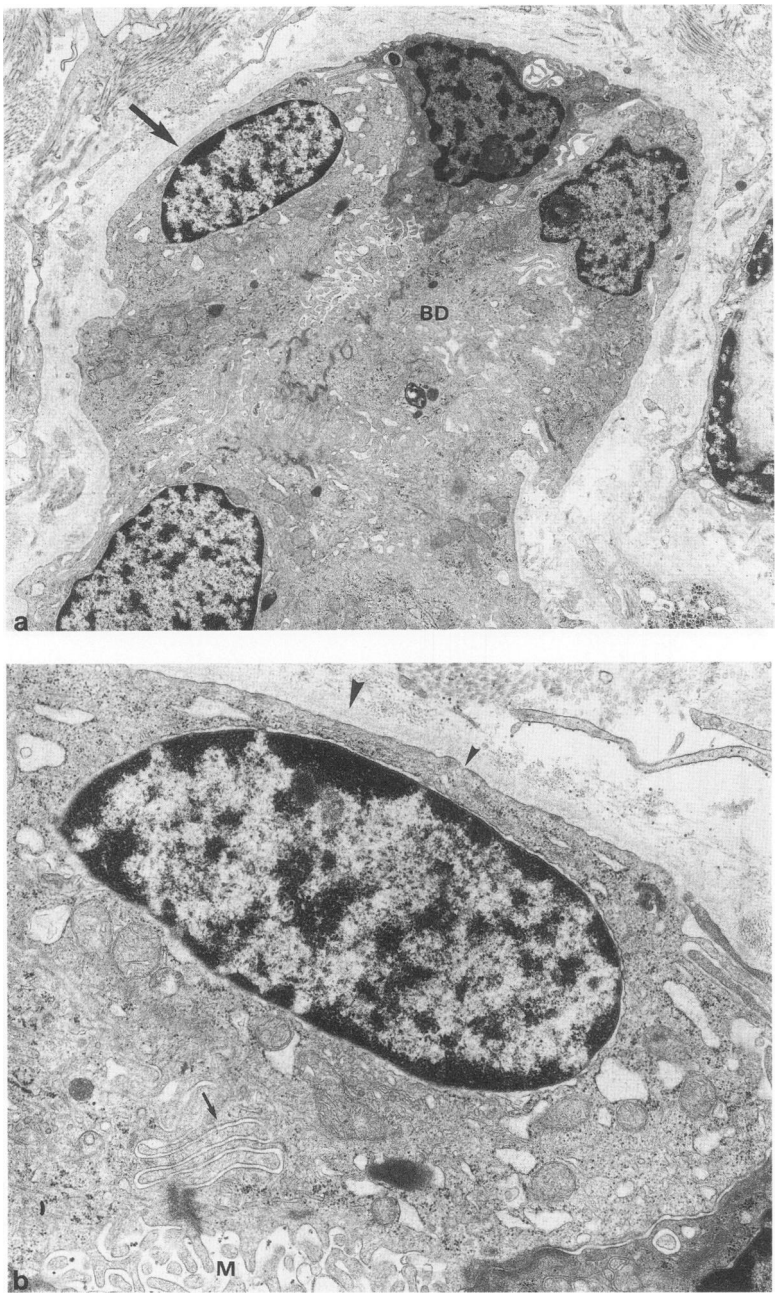

Figure 6. *Electron micrograph of liver biopsy from patient with alcoholic hepatitis.* **a**: *Type II "small cell" (arrow) next to bile ductular cells (BD). Magnification, ×7250.* **b**: *Higher magnification from* (**a**) *showing microvilli (M); interdigitations of lateral cell membrane (small arrow); pinocytotic invaginations (small arrowhead); basement membrane (arrowhead). Magnification, ×23000.*

"small cell" has not been described in human liver. The objection may arise that this cell is a mesenchymal cell, e.g., fat-storing cell. Fat-storing cells are usually localized in the Disse space close to the endothelial barrier but occasionally they can occur between two hepatocytes and they may lack fat droplets. However fat-storing cells usually do not establish different types of junctional structures with adjacent cells in contrast to the "small cells" described.

Intercellular junctions of the "adherens type" between fat-storing cells and hepatocytes have been described in alcoholic liver injury in baboons.[20] In view of the "adherens type" of junction and the more actinlike appearance of the perijunctional filamentous material illustrated in this article,[20] and the lack of cytokeratin filaments in fat-storing cells, it appears that the type of junction described between fat-storing cells and parenchymal cells does not correspond to a "macula adhaerens" or "desmosome" in its strict definition. Furthermore, the cytoskeleton of fat-storing cells has been shown by immunohistochemical analyses to comprise actin, vimentin, and desmin, but not cytokeratin.[21] However, tonofilaments are a constant finding in the "small cells."

Several features like tonofilaments, junctional com-

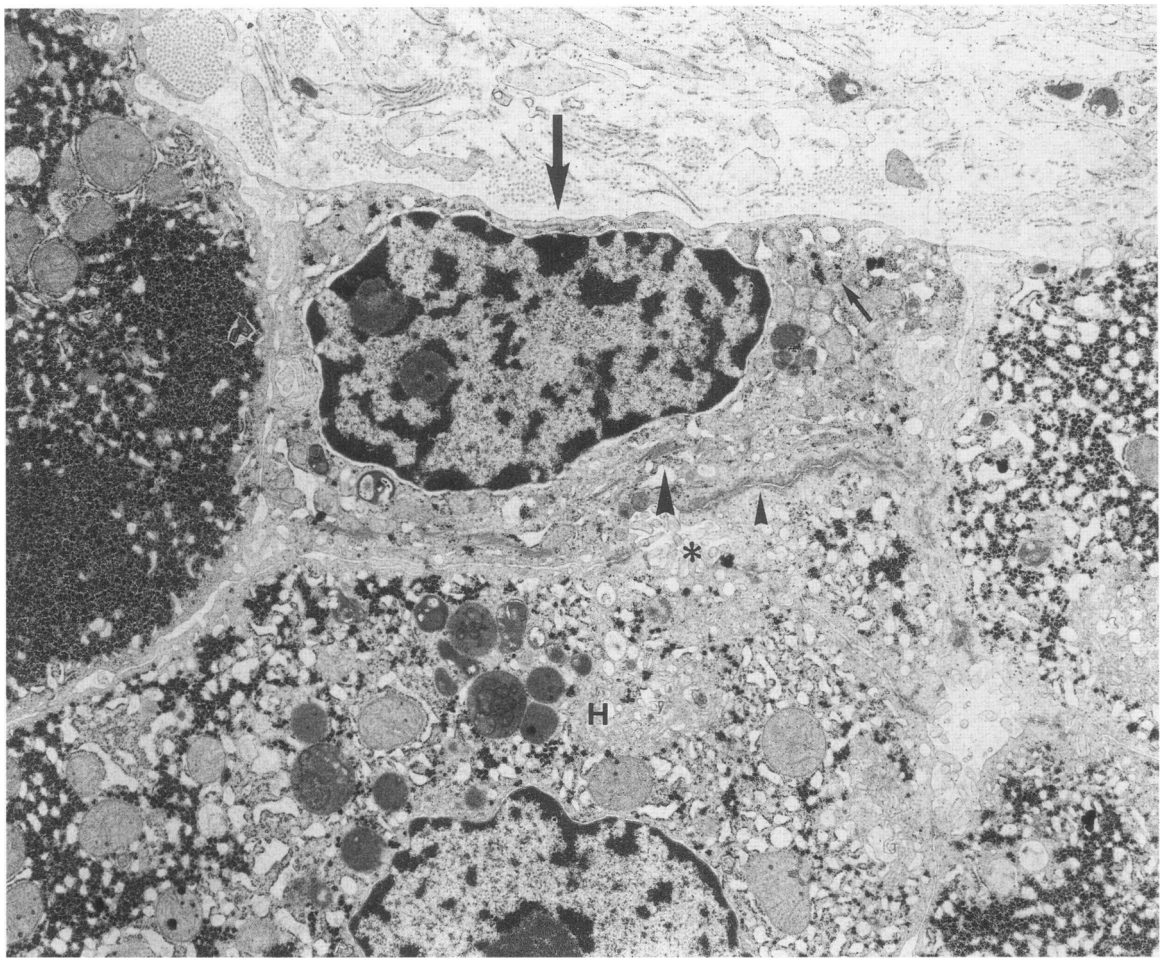

Figure 7. *Electron micrograph of liver biopsy from patient with alcoholic hepatitis. Type III "small cell" (arrow) situated next to hepatocytes (H); tonofilaments (arrowhead); canalicular microvilli (asterisk); glycogen (small arrow); intercellular junctions (small arrowhead). Magnification, ×9200.*

plexes, and basement membrane speak in favor of the epithelial nature of the "small cells."

On the base of their morphologic characteristics, type II and type III "small cells" appear to be related to the type I cells. They present the same overall features but show in addition characteristics of bile duct cells for type II and characteristics of hepatocytes for type III.

These "small cells" are clearly different from the cubic proliferated bile ductular cells in alcoholic liver disease described by Uchida et al.[22]

It is tempting to compare these "small cells" with oval cells, a variant of bile ductular reaction,[3] most extensively studied *in vivo* and *in vitro* during the early stage of hepatocarcinogenesis in rats.[4–10] Oval cells, as described during rat liver carcinogenesis, have morphologic characteristics comparable to those of the "small cells" observed in this study; they form intercellular junctions and express cytokeratins.[23] The well-developed endoplasmic reticulum in the "small cells" is also found in oval cells in rat liver where it may indicate an important physiologic function as a source of major serum protein when hepatocytes are unable to synthesize these proteins.[24] Several studies interpreted the oval cell compartment as the source of pluripotent stem cell in the liver having the capability to differentiate into bile ducts and into hepatocytes.[7,8,11–13,18]

Furthermore, recent studies on cell kinetics in the normal rat liver indicate that there is a continuous production of hepatocytes[25,26] and bile duct cells.[27] These newly formed hepatocytes arise in the periportal zones and stream gradually towards the central zones accompanied by littoral cells[28] where the old cells are apparently eliminated by apoptosis.[29] This concept implies the existence of a progenitor or stem cell compartment in the normal adult liver.

We speculate that the type I "small cell", the less-differentiated type, may correspond to a primitive type of cell, possibly a progenitor cell.

The dense-cored vesicles occurring in some of the "small cells" may reflect an endocrine function and may

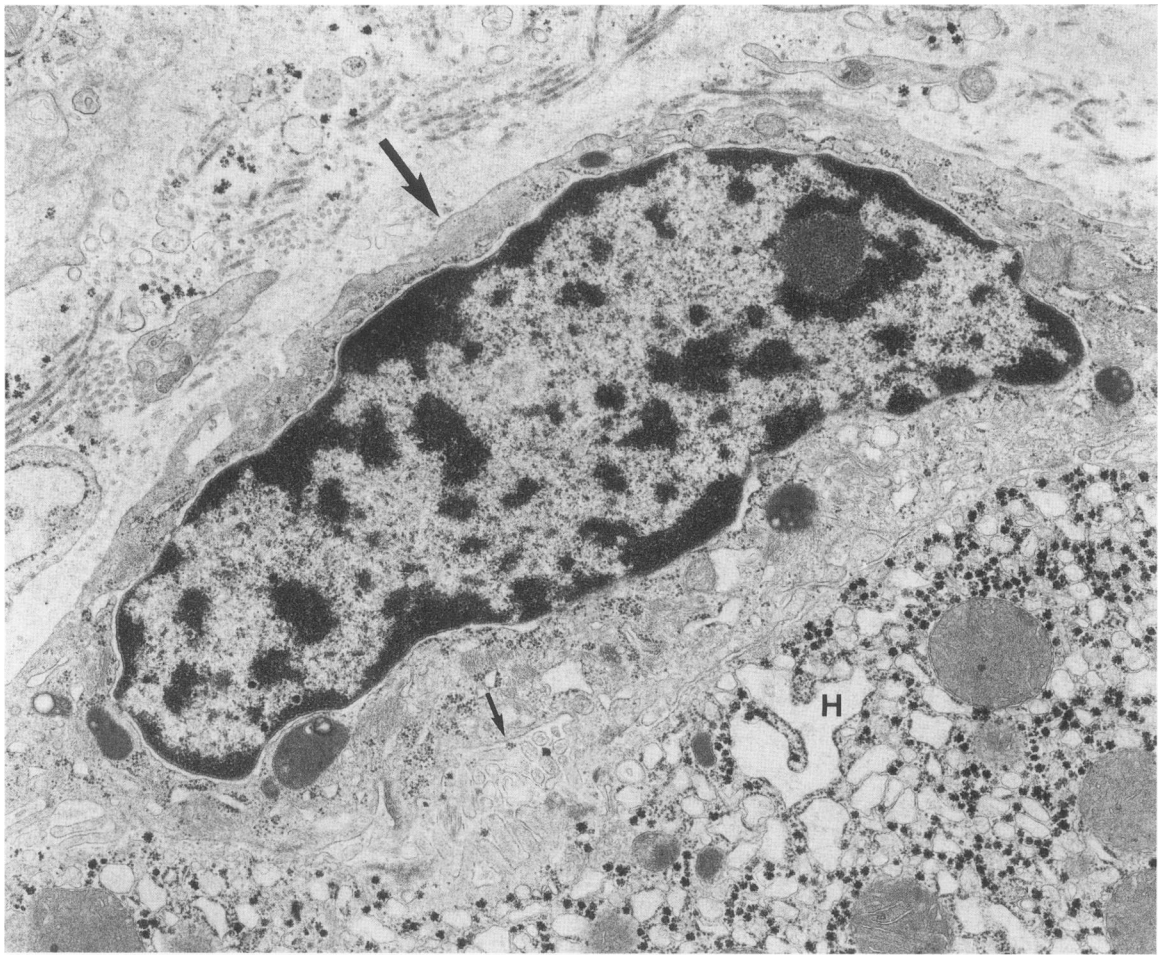

Figure 8. *Electron micrograph of liver biopsy from patient with alcoholic hepatitis. Type III "small cell" (rather intermediate between type I and type III) (arrow) situated next to a hepatocyte (H); canalicular microvilli (small arrow). Magnification, ×18400.*

be involved in growth and metabolic processes. Neuroendocrine features of reactive bile ductules in cholestatic liver diseases have been demonstrated.[30]

The type II "small cell" is a more differentiated cell with bile ductular features. It is comparable to an oval bile-duct cell precursor found in rat liver as demonstrated by monoclonal antibodies.[31]

The type III "small cell" in contrast presents features of hepatocytes. Several studies reported biochemical findings in favor of nonbiliary cell markers in oval cells like alpha-fetoprotein and albumin and alpha-fetoprotein mRNAs[9,12,14,17,32,33]; hybrid forms of certain hepatic isozymes[34] and glucose-6-phosphatase.[35]

An important indication of the origin and nature of stem cells in the liver can be derived from the studies of Rao[36] on the development of pancreatic hepatocytes in adult rats maintained on a copper-deficient diet. Under such conditions pancreatic parenchymal and interstitial cells resembling hepatic oval cells are capable of transforming into pancreatic hepatocytes; these cells may be considered as stem cell equivalents.

Only a few studies suggest the possible presence of a facultative stem cell in human liver.[15–17]

We believe that the ultrastructural findings presented in this study add arguments in favor of the existence of a bipotential progenitor epithelial cell in the human liver.

The fact that this cell type has not yet been reported may be related to its small size and inconspicuous appearance. Recent immunocytochemical studies revealing the presence of "small cells" positive on immunostaining for bile-duct type cytokeratins, in the periportal areas of pathologic human liver tissue[18,19] as well as recent studies elucidating further characteristics of oval cells in rat liver[9] prompted the present ultrastructural search for these cells.

Although small in size and number, their existence,

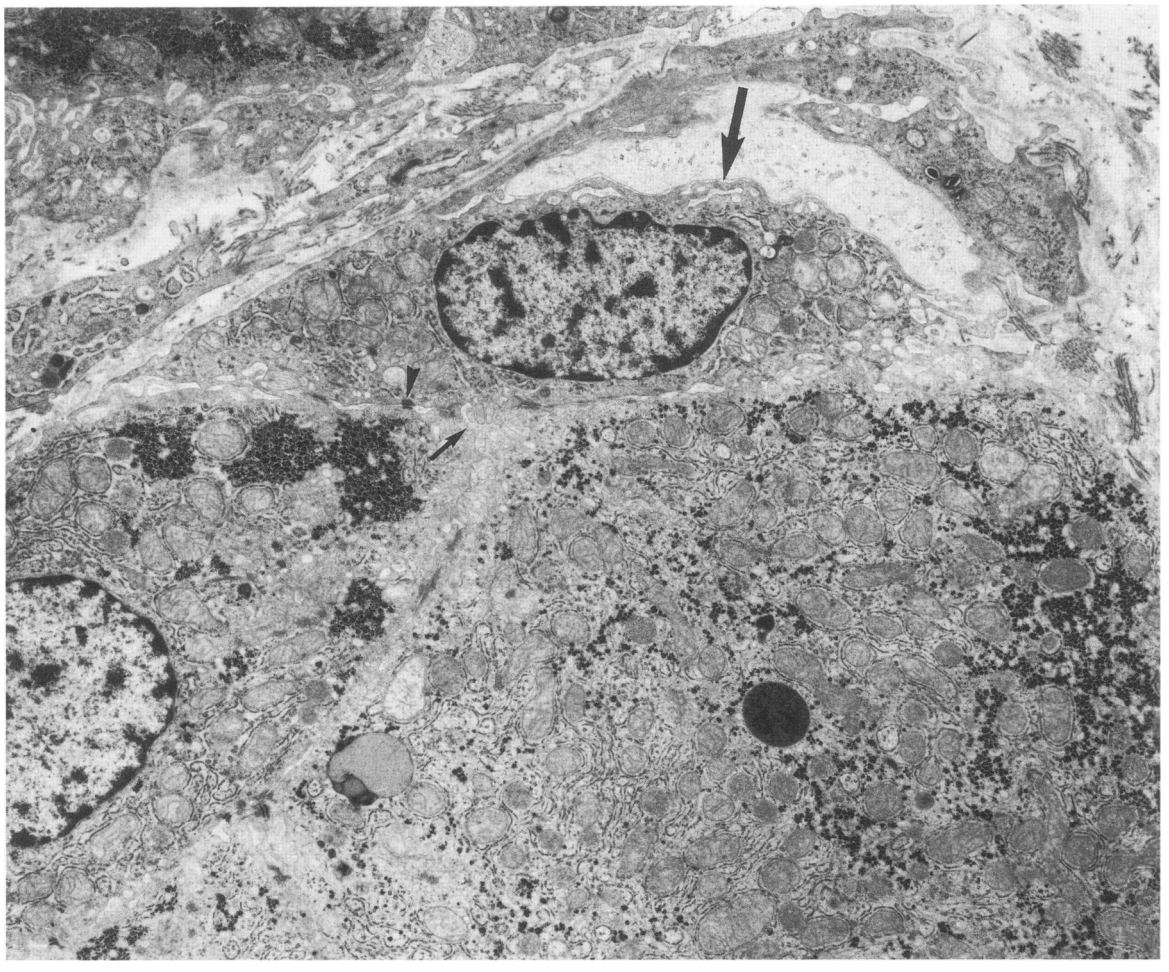

Figure 9. *Electron micrograph of liver biopsy from patient with longstanding extrahepatic obstruction. Type III small cell (arrow); canalicular microvilli (small arrow); intercellular junctions (arrowhead). Magnification, ×7250.*

epithelial nature, and variable differentiation characteristics can be demonstrated at the electron microscopic level.

Further studies including the use of specific immunohistochemical markers are needed to identify the origin, nature, and significance of the peculiar "small cells" in human liver.

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