Ultrastructural Studies of the Bile Duct in Alcoholic Liver Disease

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Summary: The fine structural characteristics of the bile duct in patients with alcoholic disease are described. Dark cell metamorphosis, edematous microvilli, and increased number of pinocytotic vesicles on the basal wall surface of the duct epithelium were observed. These alterations may be interpreted as evidence of disordered water metabolism, probably reflecting secretion and reabsorption hyperfunction in the duct epithelium. In addition, widened intercellular spaces in the basal half of the epithelium suggested retention of fluid following reverse pinocytosis along the lateral cell surface. Although no alterations of the duct epithelium distinct from those in patients with other liver diseases were apparent in patients with alcoholic liver disease, the basement membrane of the bile duct exhibited unusual duplication with multiple layers and occasional loop-formation in lacunae on the basal surface.

Key words: alcoholic liver disease—dark cell metamorphosis—pinocytosis—duplicated basement membrane—bile duct—ultrastructure

Introduction

It has been suggested theoretically (Andrews, 1955) and shown experimentally (Goldfarb, 1963) that the bile duct is not a simple tube, but has functions which affect the composition of bile during its passage through the biliary ductular system. Alteration of bile may occur under various conditions, which have been considered to be partly implicated in the derangement of bile duct functions in the extrahepatic portions. In alcoholic liver disease, some alterations in bile formation and in bile salt secretion have been described (Neale et al. 1971; Boyer, 1972). However, little attention has been focused on the pathological changes in alcoholic liver disease occurring in the bile duct, the fine structural features of which we describe in this report.

Materials and Methods

Liver biopsy specimens were obtained from 18 patients (11 males and 7 females) with alcoholic liver disease consisting of fatty liver in 4, acute sclerosing hyaline necrosis (Edmondson et al. 1963) in 7, chronic sclerosing hyaline disease (Edmondson et al. 1963) in 6, and cirrhosis in 1 at Liver Unit Pathology, Rancho Los Amigos Hospital of University of
Southern California. These patients had neither history of pancreatitis nor extrahepatic biliary obstruction. Liver biopsy specimens obtained from 2 patients with chronic active hepatitis, 2 with acute hepatitis, 1 with primary biliary cirrhosis, and 1 with normal liver were examined as controls. Clinical data and histological diagnosis for these patients are summarized in Table 1.

Percutaneous liver biopsy was performed using Klatskin or Trucut needle under local anesthesia. A portion of the liver specimen was cut into 1 mm cubes, fixed in 2.5% glutaraldehyde in sodium phosphate buffer (0.01 M, pH 7.4) for two hours, and kept for 1 to 14 days in sodium phosphate buffer containing 8% sucrose. The specimens were post-fixed in 1% osmium tetroxide in sodium phosphate buffer, dehydrated in a graded ethanol series, and embedded in Epon 812. Sections 1 μm thick were cut on an LKB Ultratome and examined after staining with toluidine blue for bile ducts in the portal areas. Ultrathin sections were then double-stained with uranyl acetate and lead citrate and observed with an electron microscope (Hitachi-12). The remaining portion of the specimen was submitted to routine light microscopic observation. The term bile duct used herein refers to the branches of the biliary tree in extralobular locations, and corresponds to the bile ductule and bile ducts defined by Steiner (Steiner et al. 1961). Bile canaliculi, bile preductules (duct of Hering), and areas of proliferation of the ductular structure were excluded from this study. The total number

| TABLE 1. |
|Clinical data and histological diagnosis of patients with alcoholic liver disease|

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/Sex (Years)</th>
<th>Chief complaint</th>
<th>Bil</th>
<th>GOT</th>
<th>GPT</th>
<th>Histological diagnosis</th>
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F: female; M: male; Bil: bilirubin (mg/dl); GOT: glutamate oxaloacetate transaminase (IU); GPT: glutamate pyruvate transaminase (IU); CSHD: chronic sclerosing hyaline disease; ASHN: acute sclerosing hyaline necrosis
of bile ducts examined in this study was 26.

**Results**

Light microscopically, the bile duct was composed of two to several epithelial cells presenting no significant changes.

Electron microscopic observation revealed alterations of the bile duct in most patients with alcoholic liver disease, even though these showed variation in intensity and frequency from one case to another. However, with respect to alterations of the bile duct, no qualitative differences were observed among patients with different types of alcoholic liver disease or between those with cholestasis and those without. The following ultrastructural findings in the bile duct were obtained.

1. **Dark cell metamorphosis**

   One of the frequently observed changes in the duct epithelium was dark cell metamorphosis. Dark cells contained more electron-dense cytoplasm than did their neighbors (Fig. 1). Most commonly only one or two dark cells were observed in a cross-section of a bile duct, but occasionally the entire epithelium of a bile duct cross-section was made of dark cells. The bile ducts in which dark cells were found presented in general a well-preserved lumen, but, occasionally, disarray of the epithelium due to variation in cell size was also observed. The dark cells showed nuclear and cytoplasmic shrinkage and varying degrees of condensation, depending on the extent of advance of the process of metamorphosis. The nuclei varied considerably in size and shape, and frequently showed widened perinuclear space (Fig. 2). In many dark cells, advanced shrinkage was accompanied by flattening of the luminal surface in association with decreased number of microvilli. Furthermore, the endoplasmic reticulum had become dilated, forming

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*Fig. 1. Patient 3. A bile duct with dark cell metamorphosis. Two dark cells (arrows) present electron-dense and shrunken cytoplasm. One dark cell (large arrow) contains vacuolated cytoplasm containing a large lysosome. Bar=1μm.*
multiple vacuolizations in the cytoplasm, while other organelles were obscure and decreased in number. In the most advanced stage, dark cells had retracted from their neighbors, and their basal borders had become detached from the basement membrane (Fig. 3).

Fig. 2. Patient 6. Dark cells in a disarrayed area of the duct epithelium. The nuclei are irregular in shape with widened perinuclear spaces. Bar=1μm.

Fig. 3. Patient 7. The most advanced phase of dark cell metamorphosis. The cell has retracted from its neighbors and detached from the basement membrane. Multiple vacuolizations are observed in the cytoplasm. BM: basement membrane, Bar=1μm.
II. Changes in the cell surface

The luminal surface of the biliary epithelial cells presented a normal number of microvilli, but, in some bile ducts, there was frequent edema of microvilli (Fig. 4). Such microvilli were usually broad-based and projected into the lumen, occasionally causing narrowing of the lumen. However, total occlusion of the lumen was not observed. The matrix was less electron-lucent than the cytoplasm, contained no organelles except for a few ribonucleic acid granules, and was abruptly demarcated from the subjacent matrix of the cytoplasm. No luminal recesses were observed. Cilia were rarely observed. The lateral surfaces of the duct epithelial cells were sometimes curved and in the basal halves of the cells, interdigitated with one another (Fig. 5). Widened intercellular spaces were often found, and there were several hyaloplasmic processes of varying length projecting from the lateral cell surface. These projections failed to interlock with the adjacent epithelial cells when the intercellular space was widened (Fig. 6). However, terminal bars and desmosomes were fairly intact in the luminal half, even when the intercellular space was markedly widened toward the base.

III. Changes in the organelles

Mitochondria were not altered in size or number, although they were, rarely, distorted in shape. Rough endoplasmic reticulum profiles were few in number, and were short and flattened. Smooth endoplasmic reticulum was also sparse except in dark cells. Free ribosomes, lying singly or arranged in small rosettes, varied in number from cell to cell, although these were generally not abundant. Golgi bodies were generally elaborate and sometimes somewhat enlarged, with occasional dilation of the cisternae.

Fig. 4. Patient 5. The luminal aspect of the bile duct contains edematous microvilli of varying sizes. A large edematous microvillus presenting amorphous hyaloplasm devoid of organelles is fairly well demarcated from the subadjacent matrix of the cytoplasm. Two cilia (arrows) are present in the lumen. Bar=1μm.
Fig. 5. Patient 8. Widened intercellular spaces at the basal half of the duct epithelium. The basement membrane is markedly duplicated (arrows). Bar=1 μm.

Fig. 6. Patient 8. Hyaloplasmic projections from the lateral surface of duct epithelial cells project into the widened intercellular space.
Fig. 7. Patient 2. (A) A bile duct with complexes of the basement membrane. (B) An enlarged view of the area in the rectangle in (A). Pinocytotic vesicles on the basal surface of a duct epithelial cell. The basement membrane is markedly distorted in a reticular fashion. Bar = 1 μm.
Lysosomes of various sizes were seen, usually near the Golgi bodies, some of which were enlarged to 1.7 μm in diameter and contained electron-dense material (Figs 1 and 5). Numerous fine intracytoplasmic filaments were found scattered randomly throughout the cytoplasm. Pinocytic vesicles, measuring 5 to 20 nm in diameter, were found near the apical, lateral and basal surfaces of the duct epithelial cells. The pinocytic vesicles were often increased in number on the lateral surface along the widened intercellular spaces and sometimes on the basal surface.

IV. Changes in the basement membrane

The basement membrane was continuous around the entire ductular structure, but showed considerable variation in thickness. There were also frequent complexes of basement membranes due to an unusual degree of branching (Figs 3, 5, and 7). The complex, branching structure of the basement membrane tended to be pronounced around the areas where more pinocytic vesicles were noted on the basal surface (Fig. 7).

Occasionally, looped branches projected from the basement membrane into the lacunae on the basal surface (Fig. 8). Intertwined fibrils were usually seen along the outside of the duplicated basement membranes, while the basal space revealed no similar fibrils. The fibrils were unevenly distributed and showed no periodicity. Occasionally, aggregations of the fibrils were found between the basement membrane and bundles of collagen fibers. There were a few mesenchymal cells present in close proximity to the basement membrane, but none were identified in the basal space. Inflammatory cell infiltration into the duct epithelium was rarely seen.

Fig. 8. Patient 4. The basement membrane of a bile duct projecting a looped branch into a lacuna of epithelial cells. Aggregates of fibrils and collagen fibers are present beneath the basement membrane. Bar = 1 μm.
Discussion

One frequently observed alteration in the duct epithelium in alcoholic liver disease is dark cell metamorphosis. This alteration has been described in patients with various types of liver disease (Steiner and Carruthers, 1961, 1962; Steiner et al. 1962; Seki, 1975), in α-naphthyl isothiocyanate-fed rats (Steiner and Carruthers, 1963), and in experimental biliary obstruction (Steiner et al. 1963), but its significance has not been fully clarified. However, Seki (1975) described the process of disintegration of dark cells in the bile ducts, and concluded that dark cell metamorphosis is an alteration leading to duct epithelial necrosis. The reduction of cell size and the increase in cytoplasmic and nuclear density characteristic of the dark cells are suggestive of a coagulative process. Similar changes have been described in goblet cells of the gut (Elorey et al. 1962), and such changes are known to arise as a result of massive discharge of secretory products. Although our present observations revealed no ductular cells containing mucinous droplets, such changes as reduced pinocytotic vesicles and loss of hyaloplasmic projections from the lateral cell surface may reflect an exhaustion or emptying phenomenon, resulting from a discharge of secretion or loss of water from the cytoplasm.

Edematous microvilli in the duct epithelium have been found under various conditions (Steiner and Carruthers, 1961; Steiner et al. 1962, 1963), and are occasionally seen even in normal duct epithelium (Schaffner and Popper, 1961). The presence of edematous microvilli may indicate altered water balance in these cells, although it is still unclear whether this is the result of a disturbance of cell membrane permeability, leading to abnormal imbibition or abnormal retention of fluid in the microvilli, or of a disturbance of fluid distribution in the duct epithelial cells (Steiner et al. 1963). In addition, well-developed interdigitations on the lateral cell surface with frequent hyaloplasmic projections similar to those in the gall bladder (Hayward, 1962) may also reflect water transport across the cell membrane in this area. Another interesting finding for the basal surface is the increased number of pinocytotic vesicles, which were sometimes seen together with widened intercellular space in the basal half. It can be inferred that the intercellular spaces may have resulted from retention of fluid following reverse pinocytosis along the lateral cell surface. Sasaki et al. (1967) have suggested that, in cholestasis, pinocytotic vesicles may move from the luminal surface to the lateral surface in a basal direction. In animal experiments, particles of mercuric sulfide injected into the ligated bile duct were observed to localize within intracytoplastic vacuoles, in the intercellular spaces, and scattered within the connective tissues around the basement membrane (Steiner et al. 1962).

The duplicated branching of the basement membrane with multiple layers, which was frequently observed in our materials, seems to be less common in other conditions (Caralli et al. 1971), with the exceptions of extrahepatic biliary obstruction (Sasaki et al. 1967) and in α-naphthyl isothiocyanate-fed rats (Steiner and Carruthers, 1963). As to the nature of the basement membrane, it is postulated that the epithelium bordering the basement membrane is not of mesenchymal origin (Midgley and Pierce, 1963). On the basis of this hypothesis, it has been suggested that the duplicated basement membrane of the bile duct may be derived from a material which is discharged from the duct epithelium by reverse pinocytosis and transported towards the basement membrane (Sasaki et al. 1967). Our observations would support
this speculation, since pinocytotic vesicles were at times increased in number at the sites where duplication of the basement membranes was prominent and where there was formation of loops with density similar to that of the basement membrane in lacunae of the duct epithelial cells or in the intracellular space. The pathological significance of the duplicated basement membrane is unknown. Although our present observations do not clarify this question, it is possible that the alteration could represent not only the simple accumulation of excretion from the duct epithelium but also an adaptive phenomenon to prevent extensive macromolecular diffusion from the bile duct.

Our investigation of the bile duct in patients with alcoholic liver disease revealed neither the specific nor the striking changes usually seen in biliary obstruction other than markedly duplicated basement membrane. However, such alterations as dark cell metamorphosis, edematous microvilli, and increased pinocytotic vesicles in duct epithelium can be interpreted as morphological expressions of deranged water and electrolyte balance, probably resulting from the hyper-secretive and -absorptive function of these cells. The main goal of our future studies will be to determine how these alterations affect the composition of bile or the flow of bile in alcoholic liver disease.

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References


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