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Electron Microscopic Study of Human Cholestasis.* (25091)

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Pathogenesis of jaundice associated with intrahepatic cholestasis is not established despite extensive research for many years. Jaundice with clinical and laboratory findings suggestive of biliary obstruction and without indications of injury of liver cells or hemolysis is present in the absence of a demonstrable obstacle in intrahepatic or extrahepatic bile ducts. On the basis of clinical, functional and light microscopic investigations, the alteration was localized into different sites, such as liver cells(1,2), Kupffer cells(3), cholangioles or ductules(4) as well as into lining of bile canaliculi(5,6). To throw light on this phenomenon, the ultrastructure of liver in human extrahepatic biliary obstruction, intrahepatic cholestasis and other types of hepatic jaundice were studied under the electron microscope. For comparison, liver of rats with experimental injuries from ethionine(7) and experimental extrahepatic biliary obstruction were studied. Previous electron microscopic observations have indicated abnormal communication between dilated bile canaliculae and perisinusoidal tissue spaces(8).

Material and methods. Biopsy specimens of liver of patients with hepatic injury and jaundice following intake of chlorpromazine, chlorpropamide, norethandrolone, and with primary biliary cirrhosis served as examples of intrahepatic cholestasis. Extrahepatic biliary obstruction was represented by carcinoma of the pancreas, jaundice associated with liver cell injury by viral hepatitis, and one case of chronic idiopathic jaundice was included. Small pieces of these as well as of livers of experimental animals mentioned above were fixed in 2% buffered ice-cold osmium tetroxide and thin sections examined with Philips model EM 100 B electron microscope. Other parts of tissue were routinely fixed in formalin and examined by standard light microscopic technics, which included particularly the periodic acid Schiff reaction after glycogen removal with diastase for demonstration of carbohydrate-bound protein.

Results. The bile canaliculus of normal human beings and rats appeared under the electron microscope as a round or elliptical lumen approximately one μ in diameter into which fine fingerlike projections extended (microvilli) (Fig. 1). It was separated from nearby extensions of the perisinusoidal tissue spaces by closely approximated straight cell borders. Liver cells projected as less regular microvilli into tissue spaces including their intercellular narrow extensions referred to above. Under the light microscope tissue spaces and some of the extensions gave a faint PAS reaction. Each liver cell usually had more than one bile canaliculus on its borders. The inner border of ductules was also lined by microvilli which were shorter and further apart than in liver cells.

In cases of intrahepatic biliary obstruction studied, the bile canaliculi appeared less numerous (Fig. 2). A few were the same size as normal canaliculi whereas others were dilated to a maximal diameter of 4 μ; in the latter, electron opaque debris was noted. In all canaliculi the microvilli were either entirely absent or further apart than normal and shortened, stunted or irregularly shaped. In a few instances a communication between dilated microvilli and extension of tissue spaces was

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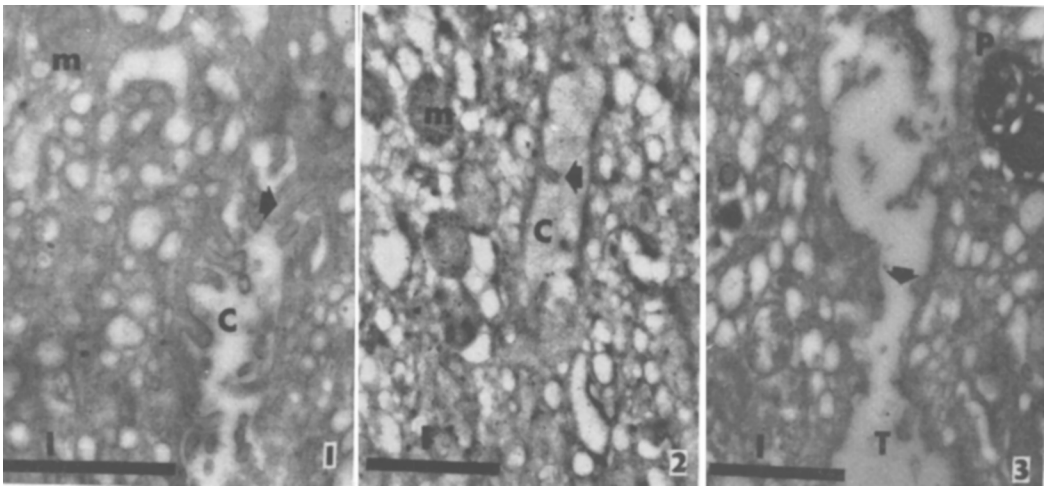

FIG. 1. Normal human bile canaliculus (c) between 2 liver cells showing long delicate microvilli (arrow). A mitochondrion (m) is in left upper corner. The round cystic structures are part of endoplasmic reticulum. Heavy line in left lower corner is one micron. (× 21,334).

FIG. 2. Bile canaliculus (c) between 2 liver cells in a patient with cholestasis following administration of chlorpropamide showing few and abnormal microvilli (arrow). Heavy line in left lower corner is one micron. (× 15,334).

FIG. 3. Bile canaliculus (c) in a patient with extrahepatic biliary obstruction due to carcinoma of the pancreas showing communication (arrow) with perisinusoidal space (T) and virtual absence of microvilli. Dark body (P) in the right upper corner is lipofuscin. Heavy line in left lower corner is one micron. (× 16,000).

noted by separation of borders of neighboring liver cells. The only alteration of the cytoplasmic detail of the liver cell was in the endoplasmic reticulum. Under the light microscope the PAS reaction in the perisinusoidal area was increased and in the liver cells PAS positive granules were found in increased numbers, some of them bile-pigmented. Moreover, bile canaliculi contained PAS positive material in addition to bile. Bile ductules were not recognized in specimens studied so far. In extrahepatic biliary obstruction, similar changes were found except that fewer bile canaliculi were noted particularly in rats 3 weeks after ligation of the bile duct. Some bile canaliculi were conspicuously dilated up to 10 μ in diameter, contained much radiopaque debris, apparently bile plugs. Many canaliculi were formed by several surrounding liver cells. Also, communications with tissue spaces were readily apparent (Fig. 3). The microvilli in all bile canaliculi were absent or considerably distorted. The cytoplasmic and the light microscopic changes were similar to those in intrahepatic cholestasis. In viral hepatitis or in rats with ethionine intoxication despite conspicuous alteration of cytoplasmic detail including mitochondria and endoplasmic reticulum, the bile canaliculi and microvilli appeared unaltered as a rule. Only in some instances of viral hepatitis slight dilatation with alteration of microvilli and radiopaque intraluminal precipitates were noted. Also in chronic idiopathic jaundice with heavy cytoplasmic pigment deposition, normal bile canaliculi and microvilli were noted.

Discussion. The electron microscopic examination indicated a characteristic alteration of the canalicular border of the liver cell with abolition or distortion of the normally present microvilli. The latter can be considered as a structural expression of interphase or membrane processes on the biliary border of the liver cell. In extrahepatic biliary obstruction, these alterations are presumably the result of increased hydrostatic pressure which is also reflected in dilatation of the canaliculi and their subsequent communication with the widened tissue spaces. This regurgitation previously described in experimental animals(8) may account for at least some of the hyperbilirubinemia. Moreover, alteration of microvilli

interferes with recognition of bile canaliculi which appeared reduced in number. The precipitation of bile and presence of protein-bound carbohydrates in the biliary passage suggests an alteration of the bile which may produce an intrahepatic component of the cholestasis in extrahepatic biliary obstruction.

In intrahepatic cholestasis exemplified by a variety of drug-induced alterations(9), the same disturbance of microvilli exists. This is presumably primary, although a hydromechanical factor is reflected in the dilatation of the canaliculi. However, the latter is far less conspicuous than in extrahepatic obstruction and possibly is the result of the inspissation of bile. In both intrahepatic cholestasis and extrahepatic obstruction, the fine structure of liver cell cytoplasm does not suggest a significant functional involvement of the interior of the cell. The basic similarities despite quantitative differences in the picture of extrahepatic biliary obstruction and intrahepatic cholestasis explains why the morphologic picture does not assist in differential diagnosis of both conditions. In other types of hepatic jaundice such as in chronic idiopathic jaundice and viral hepatitis, the bile canaliculi are not necessarily changed. The focal alterations in viral hepatitis might reflect the known intrahepatic cholestatic component occurring in this condition which sometimes is in the foreground of the clinical picture. Involvement of the ductules or cholangioles in both types of cholestasis has not been demonstrated and therefore at this time neither support for nor opposition to the concept of involvement of these structures reflected in the conventional name "cholangiolitis" can be offered.

Summary. In both extrahepatic biliary obstruction and intrahepatic cholestasis, alteration of the bile canalicular wall of liver cells and distortion of normally present microvilli can be demonstrated by electron microscopy. The functional defect is localized into membrane of liver cells, the more so since cytoplasm of liver cells does not necessarily show significant alterations. It is assumed that the lesion in extrahepatic biliary obstruction results from increased hydrostatic pressure while it cannot be decided whether in intrahepatic cholestasis it is primary or also secondary to increased pressure. Microvillous changes are not found in chronic idiopathic jaundice and only focally in viral hepatitis with liver cell injury possibly as the result of intrahepatic cholestatic component. In both types of cholestasis, communication between dilated bile canaliculi and perisinusoidal tissue spaces may account by regurgitation for at least part of the jaundice.

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