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Ultrastructural Changes of Bile Duct Epithelium in Primary Biliary Cirrhosis in Relation to Progression of Bile Duct Loss

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Summary. Using wedge liver biopsies from patients with primary biliary cirrhosis (PBC), ultrastructural features of the intrahepatic bile ducts in livers with slight or no bile duct loss were compared with those in livers with advanced bile duct loss and in extrahepatic cholestasis (EHC).

Most changes in the biliary epithelium in PBC were similar to those in EHC. Microvillous loss and bleb formation, mitochondrial damage and increase in endoplasmic reticulum and ribosomes were found in PBC irrespective of the degree of bile duct loss, and also in EHC. These changes were present almost equally at any level of the biliary tree, and are presumed to represent a variety of non-specific lesions of biliary epithelial cells. As the loss of bile ducts in PBC progressed, cytoskeletal filaments and cytophagosomes increased in number and basement membranes were more thickened and reduplicated. These changes were more or less conspicuous in smaller branches of the biliary tree, and were also prominent in EHC. They might be causally related to the bile flow disturbance in the liver. Lateral intercellular spaces were irregularly dilated and contained osmiophilic membranous and/or granular material, similar to that found in duct lumena, within and without the basement membrane, and in the cytoplasm of periductal macrophages. Furthermore, pinocytotic vesicles were increased in the biliary cytoplasm facing periphery. These findings suggest possible alteration of the permeability of biliary epithelial cells, probably in the direction from the lumena to the periductal tissue. Such changes were found in PBC livers with virtual absence of bile duct loss, and the significance of this phenomenon is discussed.

Key words: Primary biliary cirrhosis – Ultrastructure of bile duct – Bile flow disturbance – Intrahepatic biliary tree

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Light microscopic observations of livers with primary biliary cirrhosis (PBC) indicate that the small bile ducts are primary sites of damage (Baggenstoss et al. 1964; Nakanuma and Ohta 1979; Rubin et al. 1965). However, little is known about the ultrastructural changes in these bile ducts in PBC because of the difficulty in finding the bile ducts in needle biopsy specimens under electron microscopy. In particular, no comparative studies have been undertaken of the changes of ultrastructural features of the intrahepatic bile ducts in relation to progression of bile duct loss in PBC livers.

The present investigation, using wedge liver biopsies from 16 patients with varying degrees of bile duct loss (Nakanuma and Ohta 1979), was intended to clarify the ultrastructural features of the intrahepatic bile ducts in the livers with slight bile duct loss, to compare these lesions to those in patients with advanced bile duct loss and also with extrahepatic cholestasis (EHC), and thereafter to evaluate their pathological significance.

Materials and Methods

Wedge liver biopsy specimens were obtained at laparotomy from 16 patients with PBC in our University Hospital, 3 patients with EHC caused by carcinoma of the extrahepatic biliary tree (duration of jaundice $\leqq 3$ months), and two patients with normal liver and gastric ulcer. Each biopsy specimen was divided into two parts; one was fixed in 10% formalin and about 200 serial sections of each paraffin block were cut, and every fourth was stained with haematoxylin and eosin. The other was fixed in 2.5% cold buffered glutaraldehyde (pH 7.4), postfixed in 1% osmium tetraoxide (pH 7.4) and embedded in EPON 812 for electron microscopy.

The 16 patients with a clinical and histological diagnosis of PBC (29–68 y of age, 14 females and 2 males) had not been treated with D-penicillamine or Azathioprine before laparotomy. Pruritus and/or jaundice were present in 3 cases and absent in the remaining 13. Mitochondrial antibodies (AMA) were found in 14 of 16 patients (87.5%). The two AMA-negative patients were diagnosed as PBC on the basis of clinical, laboratory and histological findings. PBC was staged on the basis of histological changes, as previously described (Nakanuma and Ohta 1979): livers showing only destructive cholangitis without periportal fibrosis were designated as showing the non-fibrotic stage; slight to severe fibrosis with ductular proliferation and destruction as the fibrotic stage; and regenerative nodules throughout the liver as the cirrhotic stage.

Ultrathin sections from about 30 to 70 blocks per case were stained with uranylacetate-lead citrate. Ultrastructural lesions of a) small vesicles in the cytoplasm, b) dilatation of the lateral intercellular spaces, and c) thickening and/or multilayering of the basement membrane, were judged according to the grades of severity (none; mild; moderate; marked).

Using the histometric method previously described (Nakanuma and Ohta 1979) the proportion of the number of arteries (external caliber $=35$–54 μm) accompanied by a parallel-running bile duct to all arteries (external caliber $=35$–54 μm) present in three sections, selected at random from many serial sections of each case, was calculated. Examination of this parallelism was carried out for the smaller interlobular bile ducts (external caliber $=20$–50 μm) (Nakanuma et al. 1981). The parallelism was relatively constant (70–80%) in control livers (Nakanuma and Ohta 1979). Thus, the result reflects the degree of bile duct disappearance in PBC livers; the more the rate of parallelism decreased, the more the bile ducts had disappeared. The degree of bile duct disappearance was graded as follows: 1) no or slight degree of bile duct disappearance (rate of parallelism $>50\%$) (group A), 2) moderate degree ($25\% \leqq$ rate of parallelism $\leqq 50\%$) (group B), and 3) marked degree (rate of parallelism $<25\%$) (group C).

The intrahepatic biliary tree was classified into bile ductules, interlobular bile ducts and septal bile ducts (Nakanuma and Ohta 1979). Bile ductules were defined as tubular structures with not more than 5 nuclei (Hopwood and Nyfors 1977; Sasaki et al. 1967; Schaffner and Popper 1961) unless cut longitudinally. Interlobular bile ducts were divided into small ones with 5–10 nuclei and medium-sized ones with more than 11 nuclei. Septal bile ducts were excluded from the study.

Results

Six asymptomatic cases were in group A, seven cases in group B and the remaining three cases in group C. Two cases of group A failed to reveal any bile duct disappearance by serial section observation (no bile duct disappearance group), but the remaining four cases of group A showed a variable though slight degree of bile duct disappearance. Twelve of the 13 asymptomatic cases belonged to the non-fibrotic stage, and the remaining one to the fibrotic stage. All symptomatic cases showed the fibrotic stage.

The number of bile ductules, small and medium-sized interlobular bile ducts examined were 12.2 ± 7.4, 13.2 ± 8.2 and 6.2 ± 1.9 (mean $\pm$ SD), respectively, in group A (6 cases), 8.9 ± 2.1, 12.9 ± 5.7 and 6.4 ± 4.4 in group B (7 cases), 16.3 ± 7.7, 5.7 ± 1.2 and 3.0 ± 2.4 in group C (3 cases), and 12.3 ± 3.9, 11.3 ± 1.2 and 3.3 ± 2.5 in EHC (3 cases), respectively.

The fine structural features of intrahepatic bile ducts, which varied in severity and distribution at different levels of the biliary tree and according to the degree of bile duct loss, will be shown as below.

1. Ultrastructural Changes of the Biliary Epithelium in Group A (PBC)

Dark cells and variability of cytoplasmic density (Fig. 1) were found more frequently in all sizes of ducts examined when compared with normal livers. The following changes were found almost equally in all parts of the biliary tree: complete absence or partial loss of microvilli, microvillous bleb formation, increased numbers of pinocytotic vesicles in the peripheral cytoplasm (Figs. 2–4), increase in the number and size of Golgi apparatus, increase of free ribosomes, dilatation of the cisterna of the endoplasmic reticulum (Figs. 2–4), and abundant secondary lysosomes. Membrane whorls and cytophagosomes, containing many osmiophilic membranes and granular or irregularly shaped materials suggesting bile components were occasionally present in some bile ducts. Cytophagosomes were also found in the periductal macrophages (Fig. 5). Increased number of mitochondria, increase in the density of mitochondrial matrices, accumulation of such dense mitochondria in some portions of the cytoplasm were consistently or frequently noted at any level of the biliary tree (Figs. 2, 4, 6). A considerable number of mitochondria revealed swelling, disorganization of their cristae and membrane whorl formation. Vacuoles, 1 to 3 μm or less in diameter, containing a fine particulate or amorphous material of moderate density with occasional smaller electron dense cores similar to secretory droplet (Howpood et al. 1979) were rather prominent in the supranuclear portions of cells of medium-sized interlobular bile ducts. Lipidlike materials were also seen (Fig. 4b).

Cytoskeletal filaments were increased in two forms: 1) dense filamentous networks beneath the luminal surface with loss of microvilli and reduced numbers of pinocytotic vesicles (Figs. 3, 6), and 2) bundles of filaments, especially around the nuclei (Figs. 2, 3, 6). The former linked with desmo-

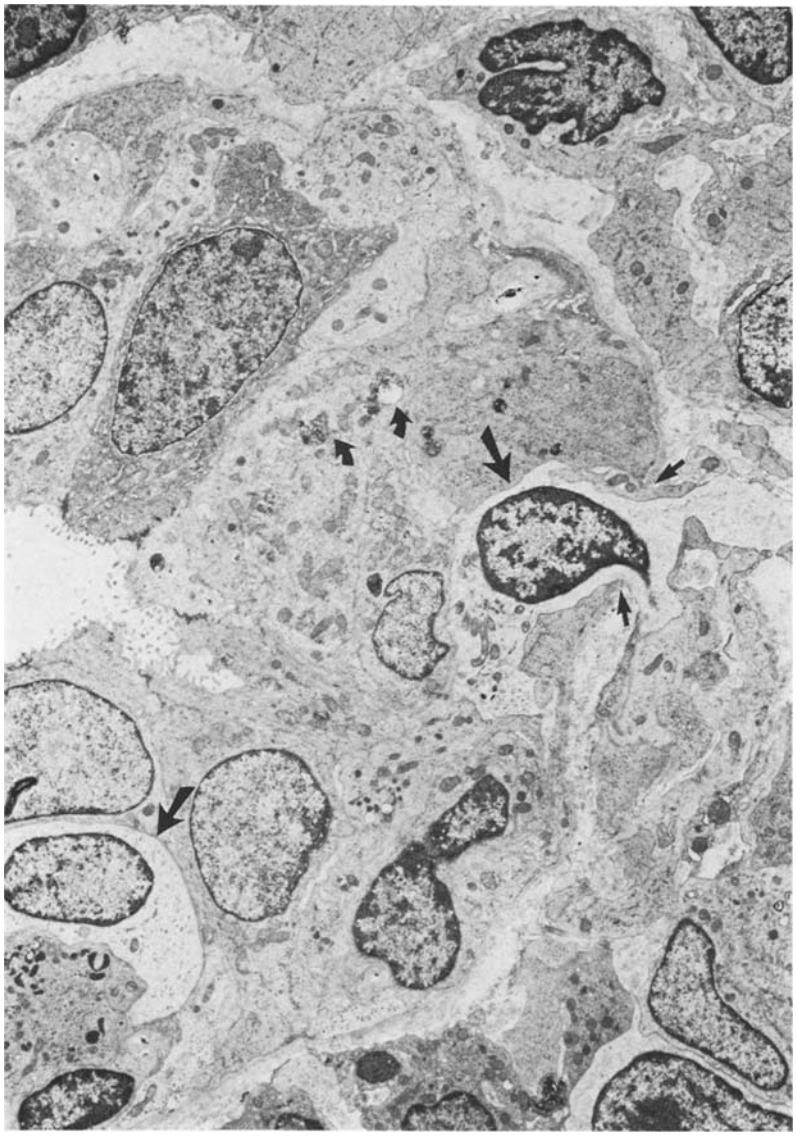

Fig. 1. Dark cells (D) and migrating lymphocytes (➡) in a medium-sized interlobular bile duct surrounded by many inflammatory cells. One lymphocyte has just migrated through the duct, with rupture of the basement membrane (➡). *L*: lumen, ➡: secondary lysosomes. Wedge liver biopsy in asymptomatic primary biliary cirrhosis (non-fibrotic stage) with little or no bile duct loss. ×5,000

somes which were increased in number and size, and showed foci of higher density (Fig. 3) suggesting the presence of actin elements. The networks and bundles usually linked and bundles were attached to or surrounded cell organelles. An increase in the number of both types of filaments was noted in some bile ducts, especially smaller ones, in many examples of group A.

The digitated lateral cell membranes were separated and variably sized dilated intercellular spaces contained finely flocculent, vesicular and particular (Fig. 2), and osmiophilic membranous and granular materials (Figs. 3,

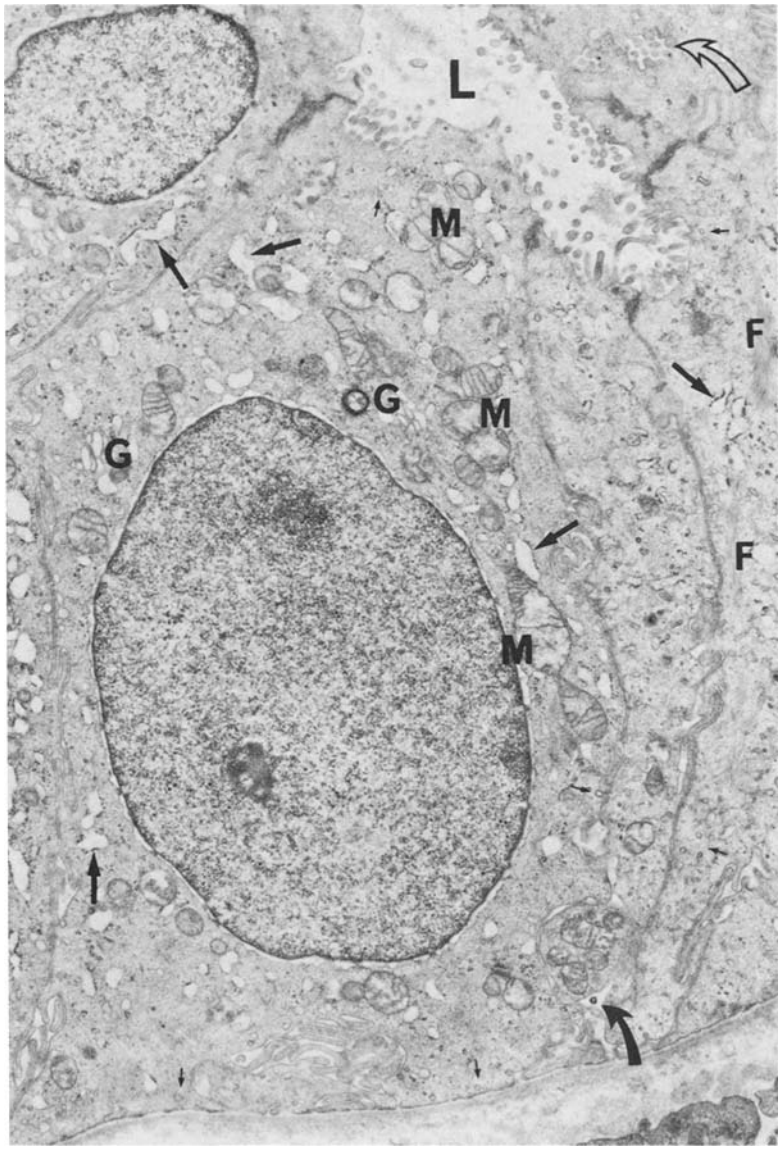

Fig. 2. Mitochondrial (*M*) damage is seen in the form of swelling, disorganization of the cristae and formation of membrane whorls. Cisternae of rough endoplasmic reticulum are dilated (➞). Bundles of cytoskeletal filaments (*F*), prominent desmosomes and increase in small vesicles (→) associated with occasional multivesicular bodies are shown, and lateral intercellular spaces are focally dilated (➥). *L*: lumen, *G*: Golgi apparatus. ⇨: cytoplasmic diverticulum. Wedge liver biopsy in asymptomatic primary biliary cirrhosis (non-fibrotic stage) with little or no bile duct loss. ×7,000

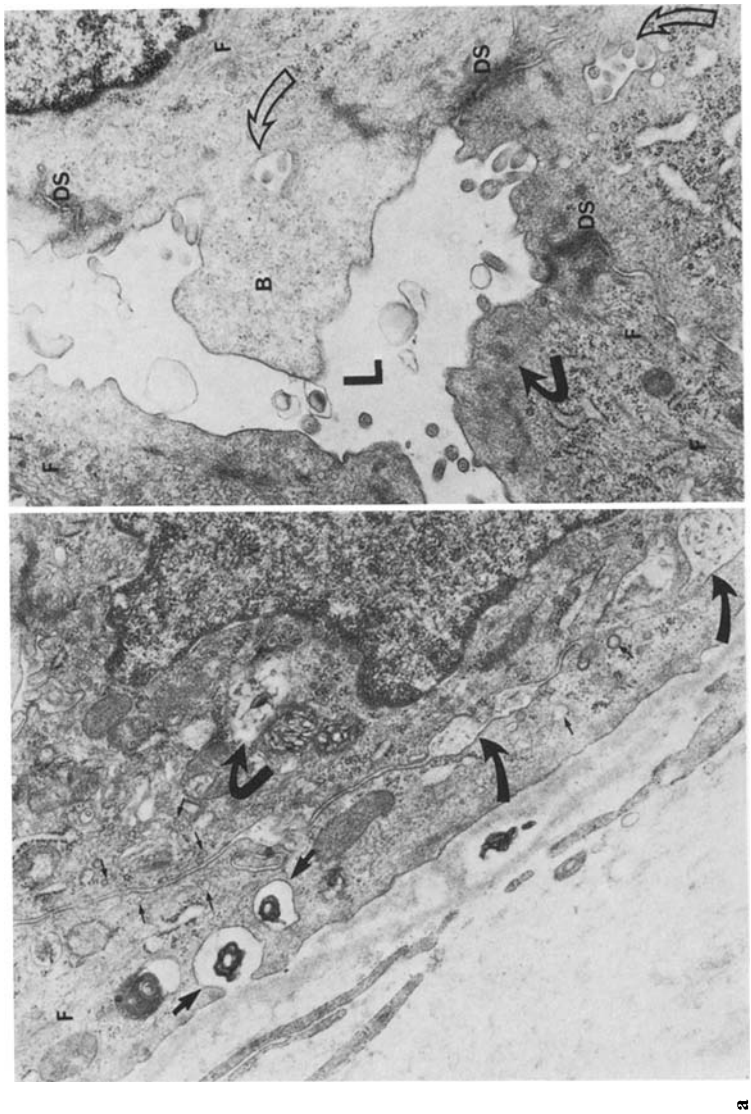

Fig. 3. (a) Osmiophilic membrane or other irregularly shaped materials are seen within a layer of duplicated basement membrane. There are vacuoles consisting osmiophilic material near or at the basal surface (→) of the small interlobular bile duct. Lateral intercellular surfaces are focally dilated and the intercellular space contains osmiophilic granular material (→). Small vesicles (→) are found around the lateral intercellular spaces. Part of the cytoplasm is undergoing degeneration, but is not walled off (↝). **(b)** Bleb formation (*B*), intracytoplasmic diverticulum (⇦), thickened cytoskeletal filamentous networks with focal density (↝), loss of microvilli with few pinocytotic vesicles and prominent desmosomes (*DS*) are seen in a small interlobular bile duct. *L*: lumen, **a**, b: Wedge liver biopsy in symptomatic primary biliary cirrhosis (fibrotic stage) with marked bile duct loss. **(a, b)** ×7,000

5, 6). Dilatation of intercellular spaces was usually mild and focal in degree and extent in most bile ducts of all size, and only occasionally moderate or severe (Figs. 3, 5, 6).

Focal and mild multilayering and/or thickening of the basement membrane was present in a considerable number of bile ducts of all sizes in group A as well as in some normal livers. These features (Figs. 3, 6) were rarely moderate or marked in some cases of group A, and were usually associated with tortuosity of the basal epithelial borders. Ruptures of the basement membrane were absent except at the sites of penetration of leukocytes (Fig. 1). Granular, membranous and irregularly shaped osmiophilic

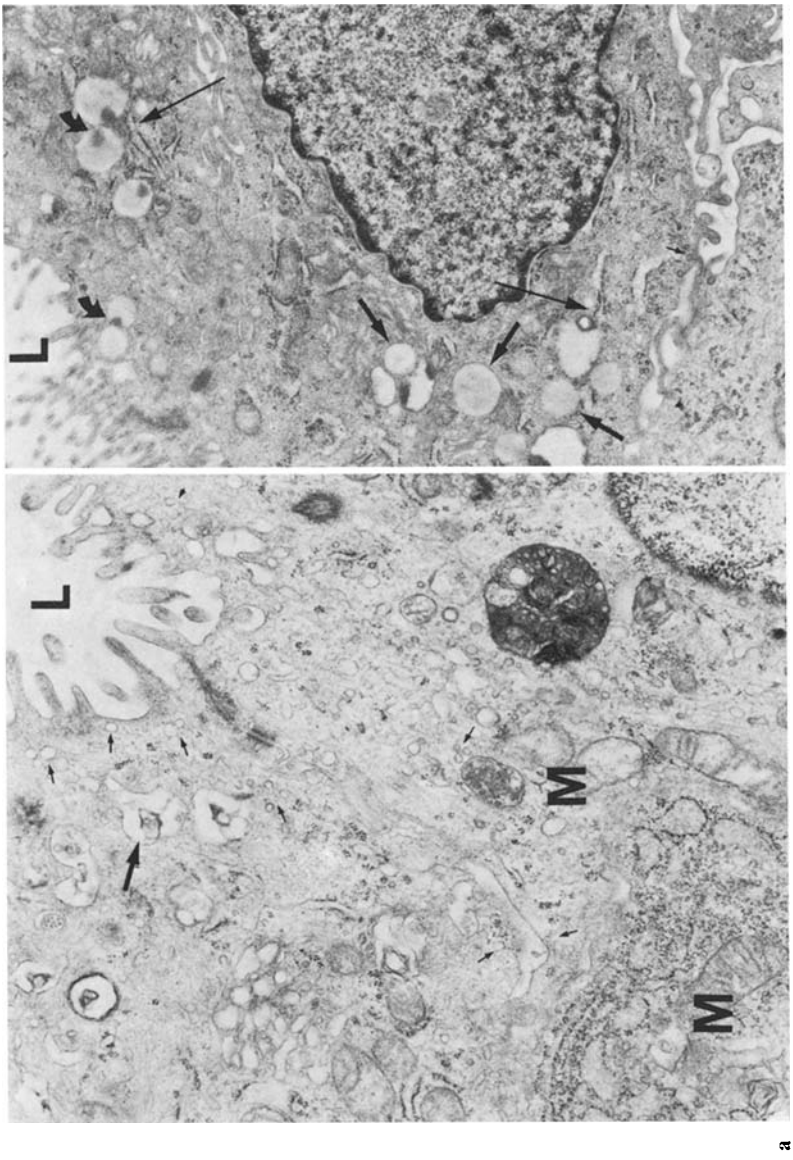

Fig. 4a, b. Vacuoles (➡) containing moderately electron-dense particular or amorphous (lipid-like) material are seen in the supranuclear cytoplasm of a small interlobular bile duct (**a**) and medium-sized interlobular bile duct (**b**). Smaller electron-dense foci (➡) are occasionally found within them, simulating secretory droplets. Some are also associated with dilated endoplasmic reticulum (→). *L*: lumen *M*: mitochondria, →: small vesicles, G: Golgi apparatus, C: cytophagosomes. **(a, b)** Wedge liver biopsy in asymptomatic primary biliary cirrhosis (non-fibrotic stage) with little or no bile duct loss, **(a, b)** × 5,000

substances with morphological similarity to those seen in duct lumina, widened intercellular spaces and cytophagosomes, were deposited within and outside the basement membranes, and also within the cytoplasm of periductal macrophages (Fig. 5). Some vacuoles appeared to extrude these osmiophilic substances towards the outside of basal or lateral cell border (Fig. 4). The osmiophilic substances were always surrounded by clear electron lucent areas within the basement membranes, and seemed to differ from electron dense immune complexes (Heptinstall 1974; Kalderon and Bogass 1977).

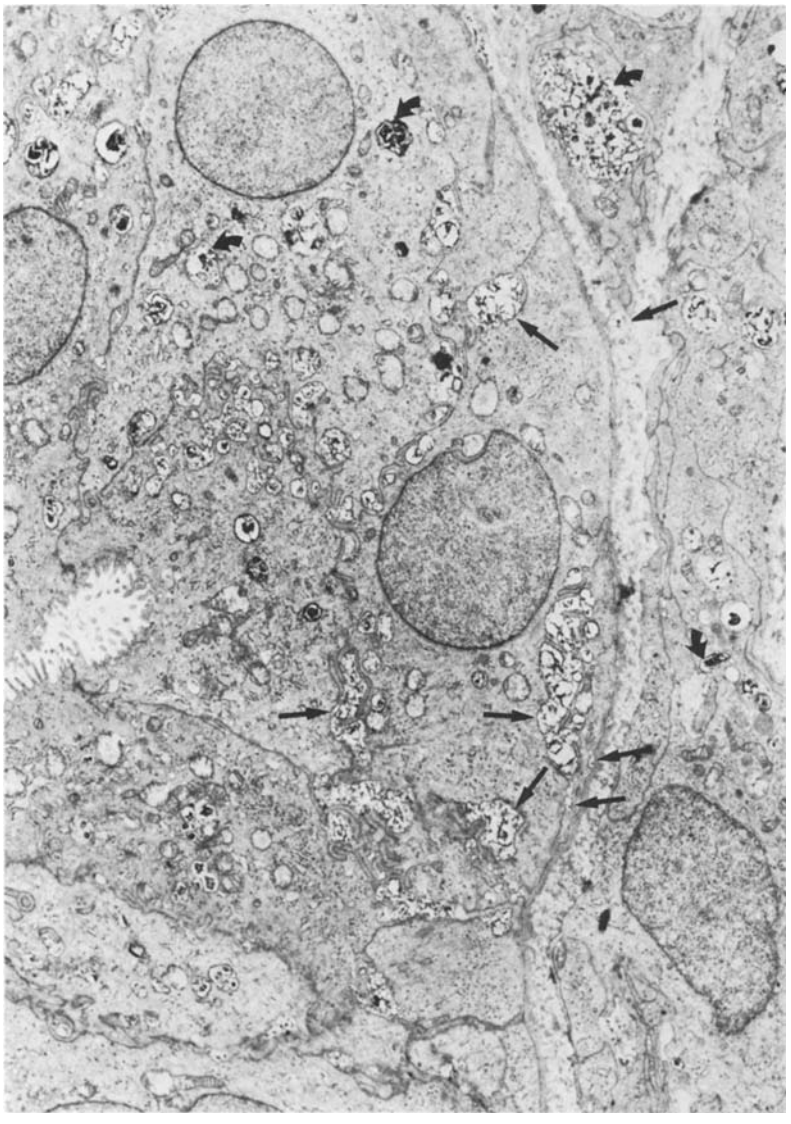

Fig. 5. Many cytophagosomes with osmiophilic material are found within epithelial cells of a medium-sized interlobular bile duct (↑) as well as in periductal macrophages (↑). Widened intercellular spaces and basement membranes contain osmiophilic materials (→). M: mitochondria, L: lumen. Wedge liver biopsy in asymptomatic primary biliary cirrhosis (non-fibrotic stage) with a moderate degree of bile duct loss. ×3,000

Migrating cells, mainly lymphocytes and occasionally neutrophils, were frequently seen in the biliary epithelial layer (Fig. 1) of medium-sized interlobular bile ducts, but their number was less in the smaller branches of biliary tree.

There was no clear difference in the degree and extent of the above-mentioned changes between cases with no bile duct disappearance and cases with a slight degree of bile duct disappearance.

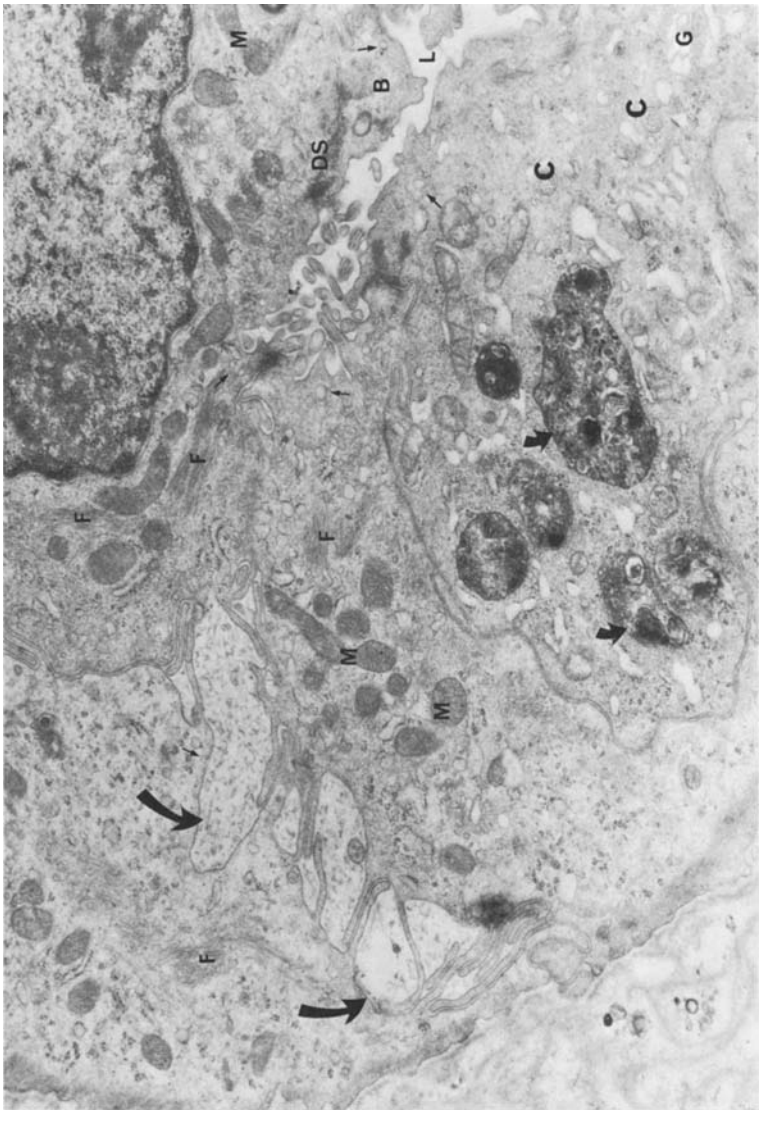

Fig. 6. Cytophagosomes (➔) with osmiophilic material are seen in a biliary epithelial cell, and cytoskeletal filaments are prominent (*F*). Laicral intercellular spaces (➔) are dilated and contain granular or flocculent material. The density of mitochondrial matrices is increased (*M*). *B*: bleb formation, *C*: centriole, *G*: Golgi apparatus, →: small vesicles. Wedge liver biopsy in asymptomatic primary biliary cirrhosis (non-fibrotic stage) with a moderate degree of bile duct loss. A small interlobular bile duct. ×5,000

2. Ultrastructural Changes of Biliary Epithelium in Groups B and C (PBC)

The changes in group B and C were quite similar to those in group A, comprising presence of dark cells, variability of cytoplasmic density, changes of luminal surfaces, increase in the number of pinocytotic vesicles, Golgi apparatus and free ribosomes, dilatation of RER, vacuoles in the supranuclear cytoplasm, mitochondrial changes, a variable dilatation of lateral

spaces (rather prominent in group B), and migrating cells in the biliary epithelial layer (especially in medium-sized interlobular bile ducts).

On the other hand, the following changes were conspicuous and widespread in group B and even more in group C as compared with group A: increase in the number of secondary lysosomes, cytophagosomes, increase in cytoskeletal filaments in the two forms described, and thickening and/or multilayering of the basement membrane with osmiophilic deposits and/or tortuosity of the basal epithelial borders (more frequent in smaller levels of the biliary tree). There were occasionally several findings suggestive of duct epithelial necrosis in both groups and in group A. This is described in detail in the another paper (Nakanuma and Ohta, in preparation).

3. Ultrastructural Changes of Biliary Epithelial Cells in EHC

The changes in EHC appeared similar to those of group C of PBC except that severe dilatation of lateral cell spaces was absent in EHC, and migrating cells in the epithelial layers were scanty, especially in medium-sized interlobular bile ducts.

Discussion

Occurrence of dark cells, loss of microvilli and bleb formation, increased number of and damage to mitochondria, hyperplasia of Golgi apparatus and increase in RER, were found equally in PBC (group A, B and C) and EHC, irrespective of duct size. Similar changes have been reported in the biliary epithelium in several other human diseases as well as in experimental animals with or without cholestasis (Hopwood and Nyfors 1977; Johannessen 1979; Popper et al. 1962; Sasaki 1965; Sasaki et al. 1967; Seki 1975; Steiner and Carruthers 1962; Steiner et al. 1962). Thus, these represent a variety of non-specific lesions of bile ducts, which may be found as the initial ultrastructural lesions in PBC.

On the other hand, increase of cytoskeletal filaments and secondary lysosomes, cytophagosomes, and multilayering and thickening of the basement membrane with frequent osmiophilic deposition, known to be common in cholestatic liver diseases of any aetiology (Adler et al. 1980; Chedid et al. 1974; Popper et al. 1962; Sasaki 1965; Sasaki et al. 1967; Steiner and Carruthers 1962), were found to increase with progression of bile duct loss in PBC. Thus, it is possible that these features in PBC develop in close association with bile flow disturbance. It was very striking that such lesions were found in some biliary epithelium of group A, which showed only florid duct lesions but no or only slight bile duct loss in a given wedge biopsy specimen. It seems likely, therefore, that bile flow disturbance in PBC livers might not necessarily result from mechanical obliteration of intrahepatic bile ducts, but might be initiated by segmental florid duct lesions.

There were many findings in biliary epithelium suggesting an increased

or altered permeability from the duct lumena to the periductal tissue at any level of the biliary tree in PBC, unrelated to cholestasis or duct loss, as well as in EHC: Such findings include many pinocytotic and exocytotic changes (Schaffner and Popper 1968; Sasaki 1965; Sasaki et al. 1967), dilatation of lateral cell spaces (Diamond et al. 1966; Dobbins 1971; Kaye et al. 1966), and osmiophilic substances within the spaces and deposition of these substances within and without the basement membranes and in the periductal macrophages. Furthermore, blood plasma proteins were frequently found in the biliary epithelial cytoplasm in PBC and EHC (Nakanuma and Ohta, unpublished data), suggesting absorption of these macromolecular proteins from bile fluid. Such a process has been suspected for a long time in PBC (Nakanuma and Ohta 1979; Rubin et al. 1965; Sasaki 1965; Schaffner 1968). Large molecules in bile such as bile specific antigen(s), to which many PBC patients and a considerable numbers of EHC patients show sensitization (McFarlane et al. 1979), might easily leak into the periductal tissue and form immune complexes, excess amounts of which might spill over into the general circulation (Sherlock 1981). Large sized, complement fixing complexes might exert cytotoxic effects on the biliary cells. Leakage into the periductal tissue of bile accompanying fragments of altered biliary epithelial cells, might participate in the production of cholangitis and even granuloma formation, as suggested by Popper (personal communication), and also in the portal inflammation and fibrotic process (Popper et al. 1962; Sasaki 1965; Sasaki et al. 1967).

The cause of this increasing transepithelial transport of bile elements might be an increased pressure of bile flow in EHC, associated with an alteration of permeability of the duct epithelium. However, similar mechanism may not operate in PBC livers, especially in cases with little or no bile duct loss. Primary alteration of membrane traffic of the duct epithelium may be related to the aetiopathogenesis of PBC, and may be followed by a multiplicity of secondary immunological responses which may develop only in the patients susceptible to PBC.

Several common histological, serological and clinical features between PBC and chronic graft-versus-host disease (GVHD) after bone marrow transplantation have recently been discussed (Epstein et al. 1981; Sherlock 1981). Bernuau et al. (1981) have reported electron microscopic studies showing that cytolysosomes (=focal cell death), abnormalities of the basement membrane and apoptosis were important in bile duct destruction in both PBC and GVHD. Apoptosis, however, appears to be neither prominent nor increased in bile ducts in PBC compared to EHC livers (Nakanuma and Ohta, in preparation), and rather rare in the biliary tree. Furthermore, cytolysosomes and abnormalities of the basement membrane were infrequent in PBC livers with a slight degree of bile duct loss, but prominent in PBC livers with advanced bile duct loss as well as in EHC livers in the present study. These observations suggest that such lesions are neither specific nor significant for the bile duct damage and destruction characteristic of PBC, and many of them may merely reflect disturbance of bile flow.

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