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Analysis of proliferating biliary epithelial cells in human liver disease using a monoclonal antibody against DNA polymerase α

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Abstract. The proliferative activity and ultrastructural characteristics of proliferating biliary epithelial cells were analysed immunohistocytochemically in 39 biopsied liver specimens from patients with acute viral hepatitis, chronic hepatitis and liver cirrhosis using a monoclonal antibody against DNA polymerase α (DNA-PA). In acute viral hepatitis with perivenular confluent necrosis, proliferation of typical bile ducts was found frequently in portal areas. In chronic aggressive hepatitis and cirrhosis, ductular proliferation of both typical and atypical forms was found in enlarged portal and periportal areas and in confluent necrotic areas. The number of proliferating biliary epithelial cells that stained positive for DNA-PA was small. There were very few positively stained cells in atypical bile ducts in confluent necrotic areas of cirrhosis. Atypical bile ducts seen in chronic aggressive hepatitis, cirrhosis and acute hepatitis with confluent necrosis were positively stained for both cytokeratins 8 and 19. In cirrhosis, the number of stained biliary epithelial cells in typical bile ducts was larger than the number of such cells in atypical bile ducts ($P < 0.01$). By electron microscopy, the cells positively stained for DNA-PA were mostly so-called clear cells with irregular nuclei containing coarse nucleoplasm, and a few small cells with scanty cytoplasm and few organelles.

Key words: Biliary epithelial cells – DNA polymerase α – Ductular proliferation – Immunohistocytochemistry

Introduction

The morphological reaction of the liver to damage of any kind includes degeneration and necrosis of hepatocytes, an inflammatory reaction followed by regeneration, fibrosis and proliferation of bile ductules (Buyssens 1965). When hepatocytes do not completely repair injured areas, fibrosis and bile duct proliferation are found. In human liver disease and in animal experiments two forms of bile duct proliferation, typical and atypical, have been defined. The former refers to the hyperplasia associated predominantly with acute liver injury and the newly formed bile ductules have well-defined lumina and are confined to the portal tracts. This proliferation seems to involve replication of pre-existing biliary epithelial cells rather than differentiation of hepatocytes (Schaffner and Popper 1961; Steiner and Carruthers 1963; Masuko et al. 1963; Popper 1986; Slott et al. 1990). Atypical bile duct proliferation is observed mainly in chronic liver disease. These bile ductules have an abnormal structure, and are thought to be derived from metaplasia of hepatocytes (Uchida and Peters 1983; Desmet 1987; Eyken et al. 1987). However, it is not clear which type of cell gives rise to the newly formed typical and atypical bile ductules. Apart from their origin, the proliferative activity of epithelial cells in human bile ducts, including proliferating bile ductules, and the ultrastructural characteristics of the proliferating biliary epithelial cells have not been studied in detail (Alpini et al. 1989; Slott et al. 1990). The present study was undertaken to investigate the proliferative activity and morphological characteristics of biliary epithelial cells in human liver diseases immunohistochemically using monoclonal antibodies against DNA polymerase α (DNA-PA) and cytokeratins 8 and 19. Tissue preparations were examined by light (LM) and electron microscopy (EM).

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Materials and methods

The subjects of this report were 39 patients with liver disease diagnosed on the basis of clinical and histological examination (Table 1). Liver biopsy specimens were obtained with a Silverman needle and peritoneoscopy from 9 patients with acute viral hepatitis (AVH, 3 with classical type and 6 with perivenular zonal necrosis or confluent necrosis; Bianchi et al. 1979), 3 patients with chronic persistent hepatitis (CPH), 16 patients with chronic aggressive hepatitis (CAH, 6 with CAH 2A and 10 with CAH 2B) and 11 with

Table 1. Clinical data on 39 patients with various liver diseases

Acute viral hepatitis (AVH, $n=9$)		
A)	Classical AVH ($n=3$)	
	Mean age:	40.3 years (range: 26–66)
	Male/female ratio	2/1
	Causative agents:	HAV, 1; HBV, 1; HCV, 1
B)	AVH with confluent necrosis ($n=6$)	
	Mean age:	43.8 years (range: 23–74)
	Male/female ratio:	3/3
	Causative agents:	HBV, 4; unknown, 2
Chronic hepatitis ($n=19$)		
A)	Chronic persistent hepatitis ($n=3$)	
	Mean age:	40.6 years (range: 35–56)
	Male/female ratio:	2/1
	Causative agents:	HBV, 2; unknown, 1
B)	Chronic aggressive hepatitis 2A ($n=6$)	
	Mean age:	38.0 years (range: 33–55)
	Male/female ratio:	5/1
	Causative agents:	HBV, 3; HCV, 2; unknown, 1
C)	Chronic aggressive hepatitis 2B ($n=10$)	
	Mean age:	42.9 years (range: 32–63)
	Male/female ratio:	7/3
	Causative agents:	HBV, 6; HCV, 4
Liver cirrhosis ($n=11$)		
	Mean age:	50.0 years (range: 38–67)
	Male/female ratio:	11/0
	Causative agents:	HBV, 3; HCV, 8

AVH, Acute viral hepatitis; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus

liver cirrhosis (LC). The biopsy specimens were divided into two unequal portions. The large portion was used for histological diagnosis. The other portion was used for this study. All biopsies were performed during convalescence. Before the biopsies, each patient and a family member gave their informed consent.

For EM the specimens were fixed with 3% glutaraldehyde and 2% osmium tetroxide, dehydrated in a series of graded ethanol solutions, and embedded in Epon 812. Ultra-thin sections were double-stained with uranyl acetate and lead citrate; they were examined by EM (300B, Hitachi, Tokyo, Japan; or 1200X, JEOL, Tokyo, Japan).

For immunostaining for DNA-PA fresh liver specimens were fixed for 6 h in a mixture of periodate, lysine, and 3% paraformaldehyde; immersed for 6–12 h in 0.1 mol/l phosphate buffer (pH 7.4) containing 8.5% sucrose, 15% sucrose and 20% sucrose; embedded in OCT compound (Miles Scientific, Naperville, Ill., USA); and frozen in liquid nitrogen. Before the staining, frozen sections 6 μm thick were prepared and washed in 0.01 mol/l phosphate-buffered saline (PBS). Next, the sections were treated with 10% normal goat serum for 20 min at room temperature, covered with a drop of a solution of mouse monoclonal antibody against DNA-PA [Medical Biological Laboratories (MBL), Nagoya, Japan] or with 0.1% normal mouse serum without the mouse monoclonal antibody against DNA-PA (control), and left overnight at 4° C. Then, to inhibit endogenous enzyme activity, the sections were incubated with methyl alcohol containing 0.03% hydrogen peroxide, rinsed with PBS five times for 5 min each time, and treated with goat polyclonal antibody against mouse IgG (MBL) at 4° C for 6 h. The peroxidase-antiperoxidase (PAP) complex was allowed to react with goat antibody, and the sections were stained with 3,3′-diaminobenzidine tetrahydrochloride (DAB) containing 0.03% hydrogen peroxide and washed in PBS for 10 min. The sections were counterstained to demonstrate the nuclei with 10% methyl green and mounted with mounting medium. Specimens from 23 patients were stained for DNA-PA (AVH, 5; CPH, 2; CAH 2A, 3; CAH 2B, 5; and LC, 8). Nuclei that reacted with the monoclonal antibody against DNA-PA were stained brown under LM.

For EM, after treatment by the PAP method, the sections were washed with PBS containing 10% sucrose at 4° C for 20 min. The sections were then immersed in DAB solution without hydrogen peroxide for 30 min and then in DAB containing 0.03% hydrogen peroxide. The sections were fixed again with 1–2% osmium tetroxide, washed with PBS, dehydrated with graded concentrations of ethanol, and embedded in epoxy resin 812. Ultra-thin sections were examined by EM without being stained.

Cytokeratins were examined in liver specimens fixed in 10% formaldehyde, embedded in paraffin and cut at 6 μm. After deparaffinization through xylene and ethanol, the sections were treated with 0.1% trypsin [10 ml 0.05 M TRIS-buffer solution, 10 mg trypsin (Sigma type I), 10 mg calcium chloride] at 37° C for 2 min and rinsed with distilled water and PBS. They were then treated with 10% normal rabbit serum at room temperature for 20 min and covered with 100 μl of a solution of a monoclonal antibody against cytokeratins 8 and 19 purchased from Boehringer Mannheim (Mannheim, Germany; concentration of antibody 5 μg/ml) or normal mouse serum (control) at 4° C overnight. To inhibit endogenous tissue enzyme activity, the sections were incubated with methyl alcohol containing 0.3% hydrogen peroxide, and then rinsed with PBS five times. Next, a Histofine peroxidase antiperoxidase kit (Nichirei, Tokyo) was used. In brief, the sections were treated with rabbit polyclonal antibody against mouse IgG, IgA and IgM at room temperature for 1 h. The PAP complex was allowed to react with rabbit antibody and the sections were stained with DAB containing 0.03% hydrogen peroxide, and then washed in PBS for 10 min. Next, the sections were counterstained to demonstrate the nuclei with 10% methyl green and mounted with mounting medium. Specimens from 16 patients were stained for cytokeratins 8 and 19 (AVH, 4; CPH, 1; CAH 2A, 3; CAH 2B, 5; and LC, 3). Cells that reacted with the monoclonal antibodies against cytokeratins 8 and 19 were stained brown.

Fisher's exact probability test was used to determine the statisti-

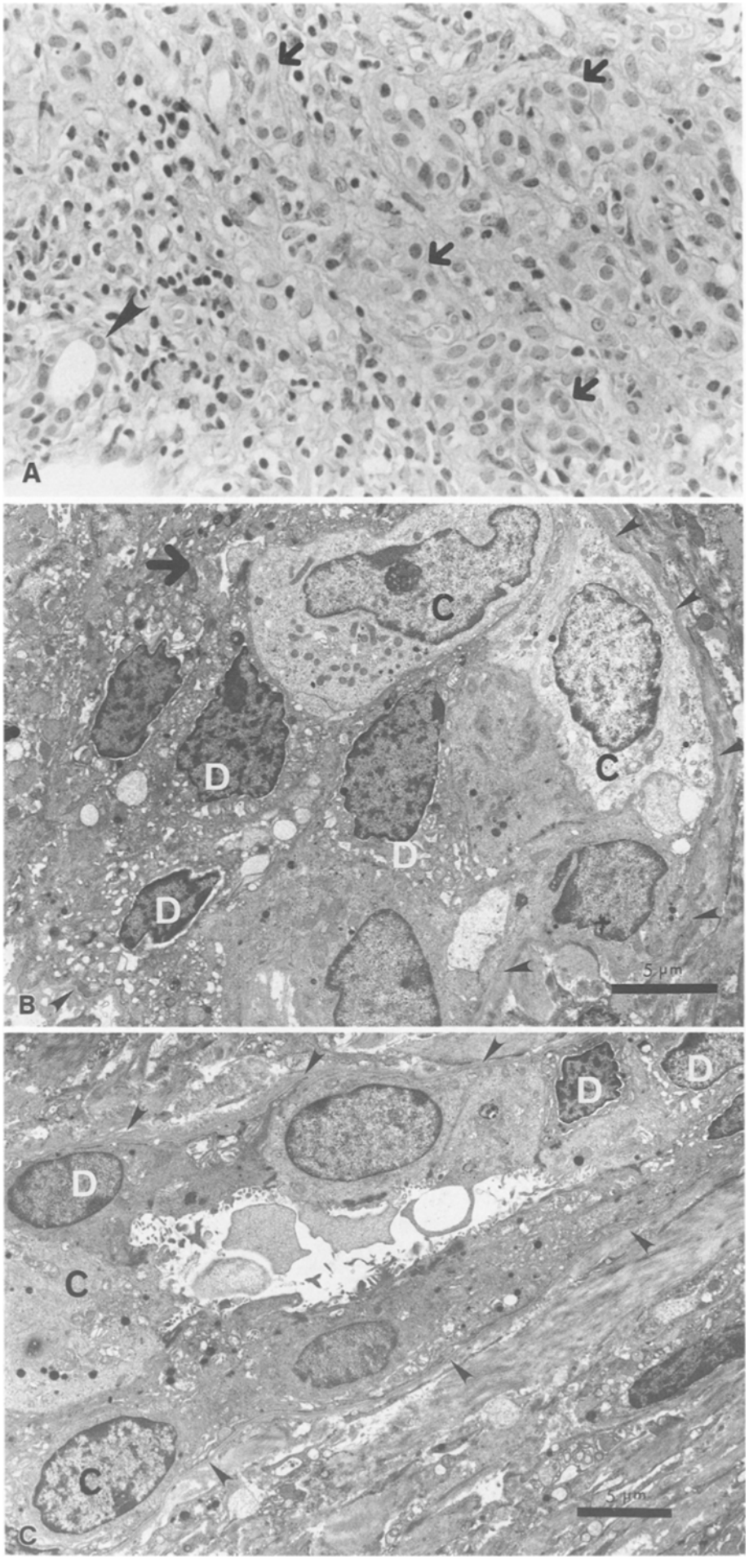


Fig. 1A–C. Typical bile ducts and atypical bile ducts. **A** Light micrograph of typical bile duct (*arrowhead*) in portal triad and proliferating atypical bile ducts (*arrows*) in the periportal area after confluent hepatocytic necrosis. Atypical bile ducts have small lumina and an abnormal configuration. Haematoxylin and eosin staining, ×340. **B** Electron micrograph of a proliferating atypical bile duct. This bile duct contains some clear (*C*) and dark cells (*D*). Bile canaliculi (*arrow*) are lined with microvilli of dark cells and blebs of clear cell. The junctional complex is well preserved. A basement membrane (*arrowheads*) surrounds bile duct. ×4800. **C** Electron micrograph of a typical bile duct. This bile duct has a clear lumen, which is lined with many microvilli and blebs, and contains some clear (*C*) and dark cells (*D*). *Arrowheads* indicate a basement membrane. ×4300

Table 2. Number of bile ducts, total ductal cells and ductal cells that stained positive for DNA polymerase α in acute viral hepatitis (mean ± SD, n = number of subjects)

		Number of bile ducts	Number of stained ductal cells Number of total ductal cells
Bile ducts that consisted of cuboidal cells			
A.	Classical type ($n=3$)		
	Typical ducts	4.7 ± 2.9 (14)[a]	0.3 ± 0.5 (1)[b] 50.7 ± 26.1 (152)[c]
	Atypical ducts	1.7 ± 1.3 (5)	0 11.3 ± 8.7 (44)
B.	Confluent necrosis type ($n=2$)		
	Typical ducts	2.5 ± 0.5 (5)	3.5 ± 2.5 (7) 18.5 ± 6.5 (27)
	Atypical ducts	1.0 ± 1.0 (2)	0 5.5 ± 5.5 (11)
Bile ducts that consisted of columnar cells			
A.	Classical type	not found	
B.	Confluent necrosis type	not found	

[a] Number of ducts seen in all sections
[b] Total number of stained cells seen in all sections
[c] Total number of ductal cells seen in all sections

cal significance of the data, and $P<0.05$ was regarded as significant.

Results

In the biopsy specimens studied by LM, we found two kinds of bile ducts: those lined by columnar biliary epithelial cells and ductules composed of cuboidal epithelial cells. These cuboidal-celled ducts could be further classified into the typical, with well-defined lumina, and atypical, with ill-defined lumina and an abnormal structure (Fig. 1 A). Bile ducts made up of columnar cells and typical bile ducts were found mainly in the portal tracts. Most atypical bile ducts were found in enlarged portal and periportal areas and in confluent necrotic areas where many hepatocytes had disappeared. In EM, the atypical bile ducts contained some clear cells and dark cells and had small lumina (Fig. 1 B). Small lumina were lined with microvilli, blebs and junctional complexes in EM. Clear cells had irregular nuclei and clear cytoplasm that contained mitochondria with high electron density, Golgi complexes, polysomes and cytoplasmic tonofilaments. Dark cells had irregular nuclei with a large amount of condensed chromatin, mitochondria with low electron density, dilated endoplasmic reticulum and many lysosomes. Atypical bile ducts formed an irregular configuration and had an irregular basement membrane. Typical bile ducts had clear lumina in portal tracts and contained some clear cells and some dark cells (Fig. 1 C). Clear, large lumina were lined with many microvilli and some blebs. The junctional complexes were well preserved. Typical bile ducts were also surrounded by basement membrane and some collagen bundles.

The distribution of proliferating biliary epithelial cells positive for DNA-PA was analysed on frozen sections under LM. DNA-PA was detected in the nucleus and was stained light or dark brown. In control specimens, no cell nuclei were stained. We counted the number of bile ducts, total biliary epithelial cells and biliary cells positive for DNA-PA LM in three appropriate areas for each case (Tables 2–4). There were no stained columnar biliary epithelial cells in LC. There were no bile ducts lined with columnar cells in specimens from patients with AVH, CPH or CAH. In LC, there were a few bile ducts with flat epithelium and some cuboidal epithelial cells in typical and atypical bile ductules stained positive for DNA-PA (Fig. 2 A, B). There were more stained epithelial cells in advanced chronic liver disease (LC, CAH 2B and CAH 2A) than in AVH of the classical type or CPH (Tables 2–4). The number of positively stained cuboidal cells of typical bile ducts was larger than that of positively stained cells of atypical ducts in LC, CAH 2B and CAH 2A. In LC, of 391 biliary epithelial cells examined in typical ducts, 8 were positively stained; and of 1913 epithelial cells in atypical ducts inspected, 5 were positively stained. There were significantly more positively stained biliary cells in typical bile ducts than in atypical bile ducts ($P<0.01$, Fisher's exact test). In AVH with confluent hepatic necrosis, some cuboidal epithelial cells of typical bile ducts in portal tracts were positively stained. In confluent necrotic areas of LC, however, many atypical bile ductules composed of cuboidal or flat cells were found, but very few positively stained epithelial cells were found. Some hepatocytes still surviving in these confluent necrotic areas were positively stained and formed rosettes (Fig. 3 A–E).

Table 3. Number of bile ducts, total ductal cells and ductal cells that stained positive for DNA polymerase α in chronic hepatitis (mean ± SD, n = number of subjects)

		Number of bile ducts	Number of stained ductal cells Number of total ductal cells
Bile ducts that consisted of cuboidal cells			
A.	Chronic persistent hepatitis ($n=2$)		
	Typical ducts	3.0 ± 1.0 (6)	0 26.5 ± 18.5 (53)
	Atypical ducts	6.5 ± 4.5 (13)	0 48.0 ± 23.0 (96)
B.	Chronic aggressive hepatitis 2A ($n=3$)		
	Typical ducts	3.7 ± 1.3 (11)	0.3 ± 0.5 (1) 29.0 ± 3.8 (87)
	Atypical ducts	12.7 ± 5.4 (38)	0 76.3 ± 28.6 (229)
C.	Chronic aggressive hepatitis 2B ($n=5$)		
	Typical ducts	2.6 ± 1.7 (13)[a]	0.8 ± 1.0 (4)[b] 51.4 ± 17.5 (247)[c]
	Atypical ducts	13.7 ± 12. (68)	1.0 ± 2.0 (5) 108.6 ± 94.2 (541)
Bile ducts that consisted of columnar cells			
A.	Chronic persistent hepatitis	not found	
B.	Chronic aggressive hepatitis 2A	not found	
C.	Chronic aggressive hepatitis 2B	not found	

[a] Number of ducts seen in all sections
[b] Total number of stained cells seen in all sections
[c] Total number of ductal cells seen in all sections

Table 4. Number of bile ducts, total ductal cells and ductal cells that stained positive for DNA polymerase α in liver cirrhosis (mean ± SD, n = number of subjects)

	Number of bile ducts	Number of stained ductal cells Number of total ductal cells	
Bile ducts that consisted of cuboidal cells ($n=8$)			
Typical ducts	6.8 ± 3.2 (48)[a]	1.0 ± 1.3 (8)[b] 48.9 ± 26.6 (391)[c]	*
Atypical ducts	27.6 ± 17.3 (221)[c]	0.6 ± 1.7 (5) 239.2 ± 178.6 (1913)	*
Bile ducts that consisted of columnar cells ($n=3$)			
Bile ducts	1.3 ± 0.5 (4)	0 55.7 ± 18.7 (167)	

* $P<0.01$, Fisher's exact probability test
[a] Number of ducts seen in all sections
[b] Total number of stained cells seen in all sections
[c] Total number of ductal cells seen in all sections

In EM, various numbers of granules stained for DNA-PA were found in the nuclei of cuboidal biliary epithelial cells of both the typical and atypical bile ducts (Fig. 4A–D). In AVH with confluent necrotic areas, we occasionally found typical bile ducts composed of biliary cells, some of which were positively stained for DNA-PA in variably inflamed portal areas. Some positively stained epithelial cells had lost the usual complex interdigitation of their adjoining lateral membranes or had injured desmosomes in their apical zones. Spaces between these cells were dilated, and plicated lateral membranes were sometimes found in the dilated intercellular spaces.

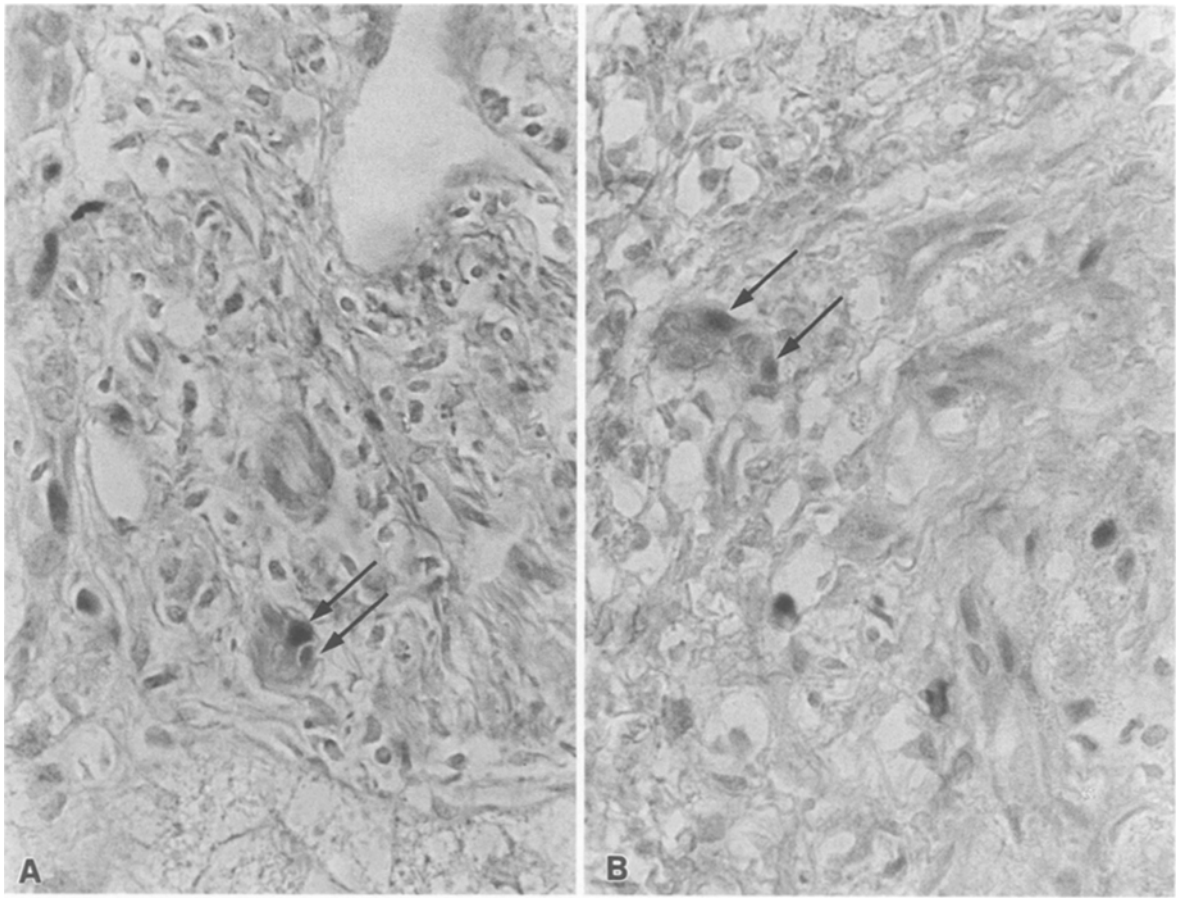
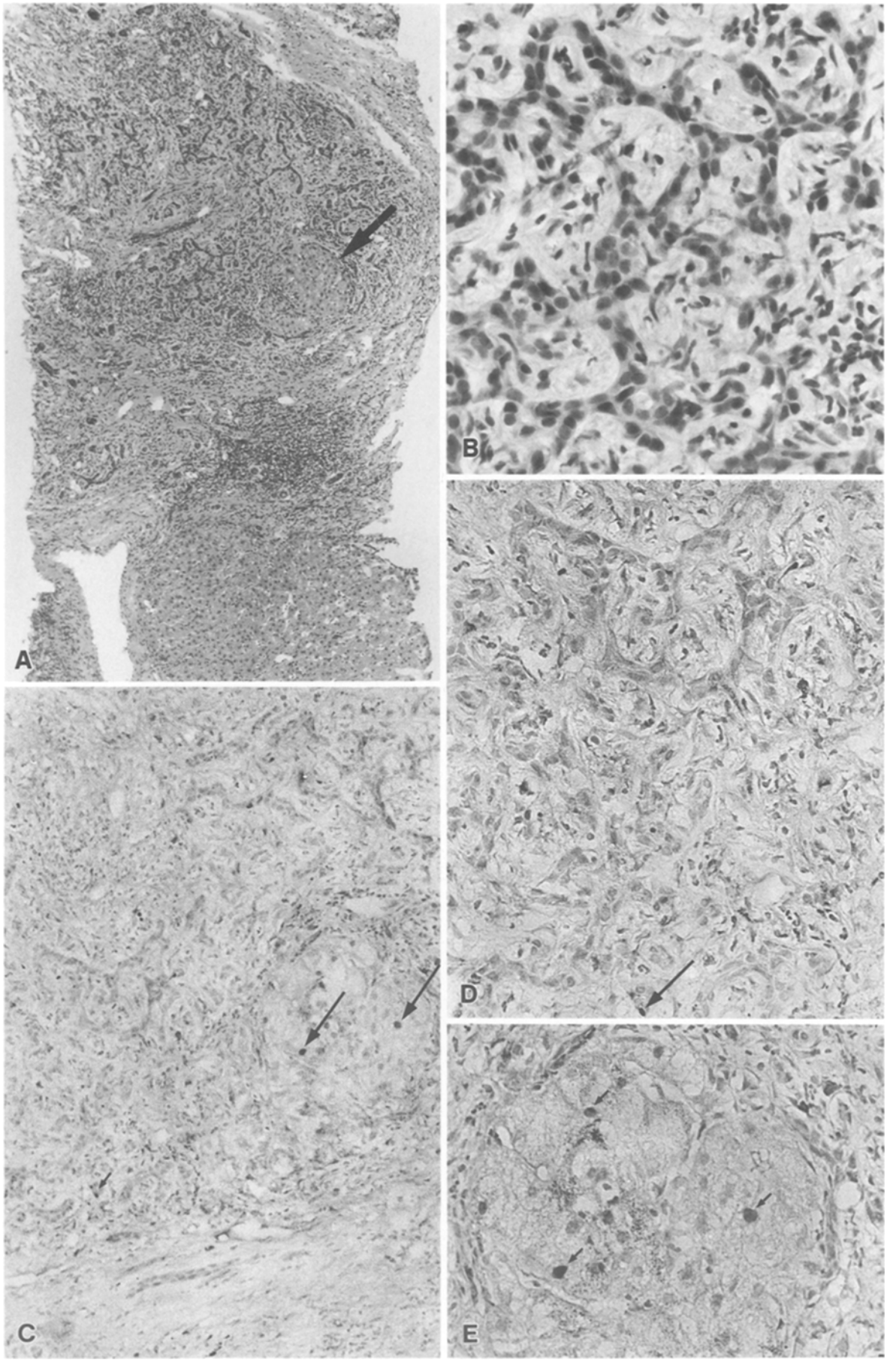


Fig. 2A, B. Biliary epithelial cells positively stained for DNA polymerase α (DNA-PA). **A** Positive biliary cells (*arrows*) in a typical bile duct within a portal tract of a patient with acute viral hepatitis with central confluent necrosis. Oedematous change is seen in this portal tract. ×690. **B** Positive biliary cells (*arrows*) in an atypical bile ductule in the enlarged portal area of a patient with chronic aggressive hepatitis 2B. ×690. Fixed with PLP, PAP method

Some cells had lost their apical microvilli. Binucleated biliary cells were occasionally found near the basement membrane. Most positively staining biliary cells had irregular nuclei with coarse nucleoplasm, less heterochromatin than normal and scanty cytoplasm with few organelles. They did not have electron-dense mitochondria or electron-dense nuclei, but had the usual number of pinocytic vesicles, Golgi apparatus, mitochondria and free polyribosomes. The DNA-PA-positive biliary cells were similar to normal biliary epithelial cells in terms of organelle content. In serial sections stained for DNA-PA (usually double-stained with uranyl acetate and lead citrate), most of the biliary cells that stained positive were the so-called clear cells that Phillips et al. (1987) have described (Fig. 5). Dark cells were seldom found in this study, and did not stain positive for DNA-PA. Stained biliary cells in atypical ducts were found in confluent necrotic areas of LC and in the enlarged portal and periportal areas, but the number of positive cells was small, though the total number of biliary cells in the atypical ducts was large (Figs. 2–4, Tables 2–4). Most of the atypical bile ducts in these areas were composed of clear cells. These showed well-developed interdigitation of the lateral membranes, with normal intercellular spaces forming part of small biliary lumina with many microvilli. A few of these clear cells were positively stained for DNA-PA. The stained cells had small nuclei with coarse nucleoplasm and scanty cytoplasm with few organelles. In enlarged portal and periportal areas in CAH 2B, we frequently found rosette formation that sometimes contained Hering ductules; the rosettes rarely contained hepatocytes positive for DNA-PA (Fig. 6). We did not find biliary cells positive for this enzyme. In these areas, as demonstrated by LM, hepatocytes were

Fig. 3A–E. Atypical bile ducts found in a confluent necrotic area in a patient with liver cirrhosis. **A** Many atypical bile ducts (ductules) are newly formed after confluent hepatocytic necrosis. In one small area, some hepatocytes (*arrow*) survived. Fixed with PLP, haematoxylin and eosin staining, ×70. **B** Higher magnification of an area shown in **A**. Atypical bile ducts composed of cuboidal cells and a few flat cells without visible lumina and forming abnormal structures can be seen. ×340. **C** Staining for DNA-PA. Some hepatocytes (*long arrows*) and one biliary epithelial cell (*short arrow*) in atypical bile ductules are positively stained for this enzyme. Fixed with PLP, PAP method, ×170. **D** Higher magnification of an area shown in **C**. Biliary cells in atypical ducts do not stain positive for this enzyme, with one exception (*arrow*). ×340. **E** Higher magnification of stained hepatocytes (*arrows*). This small nodule contained some hepatocytes arranged in a rosette. ×340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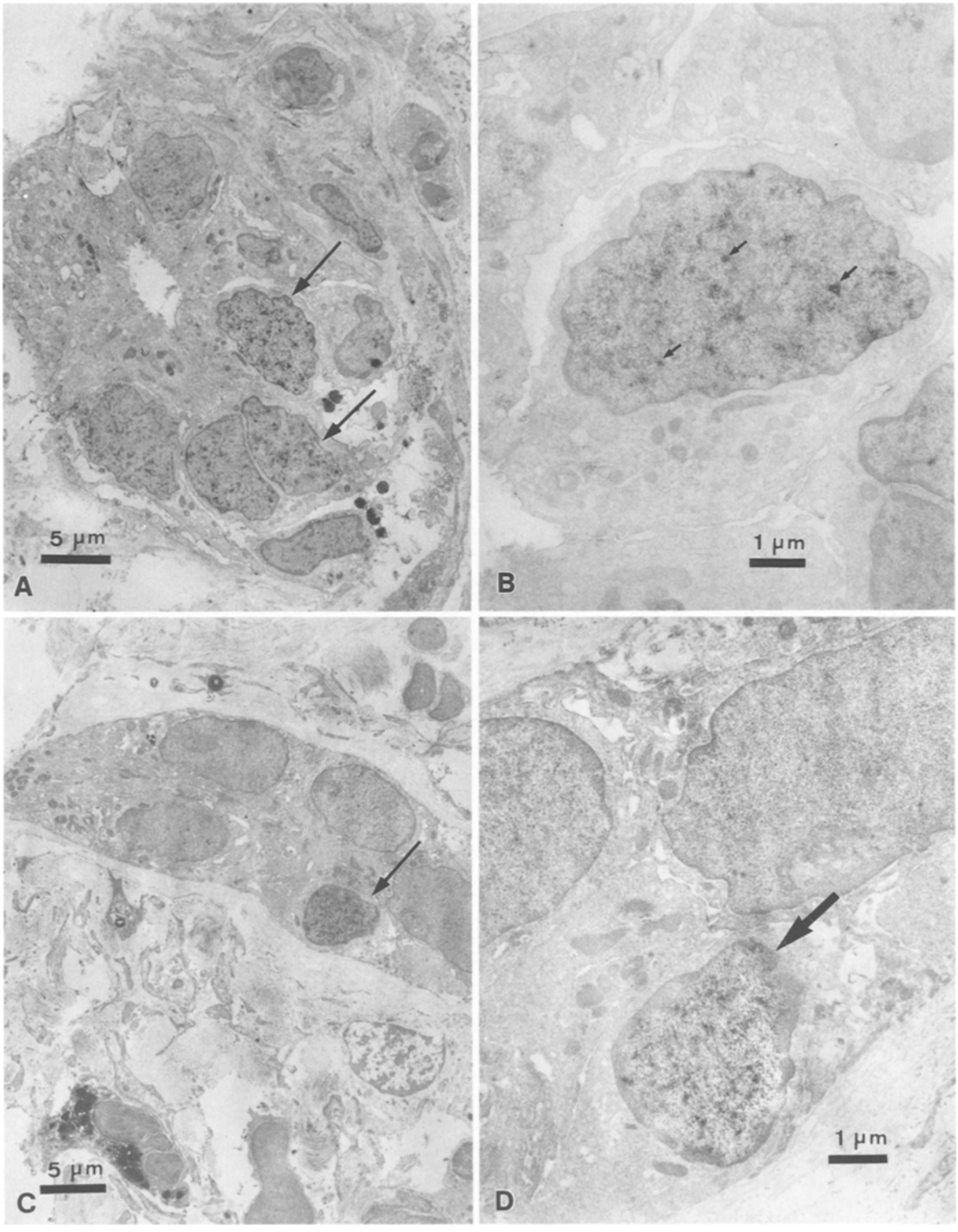

Fig. 4A–D. Immunoelectron micrographs of staining for DNA-PA. **A** Typical bile duct in a portal area. Some biliary cells (*arrows*) are positively stained for DNA-PA. Some intercellular spaces are dilated. Oedematous change is present. ×2900. **B** Higher magnification of a positively stained biliary cell. Many granules positively stained for DNA-PA (*arrows*) are found in the nucleus. This cell has an irregular nucleus with coarse nucleoplasm and a few organelles. The intercellular spaces are dilated. ×9500. **C** Atypical bile duct in a patient with cirrhosis, containing a cell positively stained for DNA-PA (*arrow*). Intercellular spaces are narrow. ×2200. **D** Higher magnification of the positively stained biliary cell (*arrow*). DNA-PA-positive granules are found in nucleus. This cell is small; it has a nucleus with coarse nucleoplasm, and has scanty cytoplasm with few organelles. ×8400

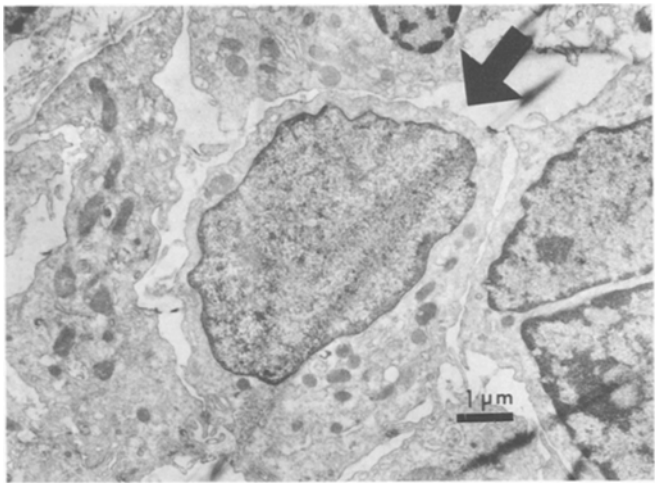

Fig. 5. Electron micrograph showing a different section of the biliary epithelial cell shown in Fig. 4B and which stained positively for DNA-PA (*arrow*). This section was double-stained with uranyl acetate and lead citrate. This cell is a clear cell according to the description of Phillips et al. (1987). ×9500

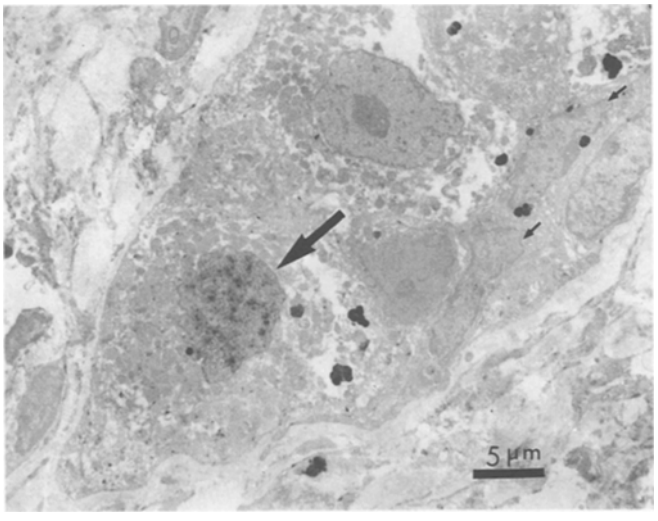

Fig. 6. A rosette comprising some hepatocytes and biliary cells was found in an enlarged portal area in chronic aggressive hepatitis 2B. A hepatocyte positively stained for DNA-PA can be seen (*large arrow*), but the flat biliary epithelial cells (*small arrows*) are not stained. ×2200

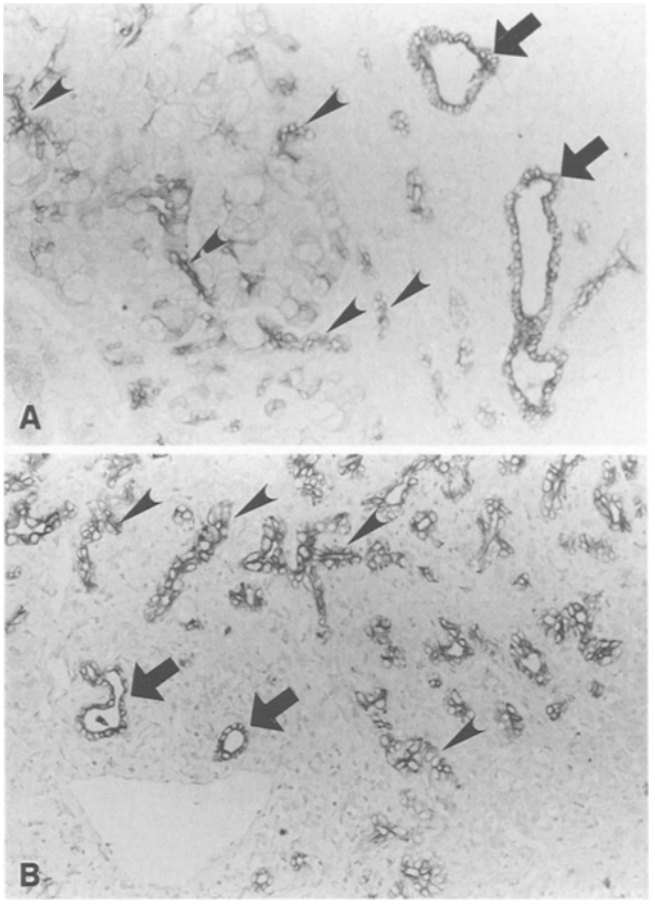

Fig. 7A, B. Paraffin sections stained with mouse monoclonal antibodies against cytokeratins 8 and 19. **A** Staining for cytokeratin 8. Typical bile ducts (*arrows*), atypical bile ducts (*arrowheads*) and some hepatocytes were positively stained for cytokeratin 8 in liver cirrhosis. PAP method, counterstained with methyl green, ×170. **B** Staining for cytokeratin 19. Typical bile ducts (*arrows*) and proliferating atypical bile ducts (*arrowheads*) were positively stained for cytokeratin 19 in acute hepatitis with confluent necrosis. PAP method, counterstained with methyl green, ×170

often stained, but biliary epithelial cells were seldom stained.

With cytokeratin 8, many hepatocytes were stained light-brown and ductal cells composing typical and atypical bile ducts were all stained dark-brown in AVH, CPH, CAH and LC (Fig. 7A). Positive atypical bile ducts in the periportal areas were found to be connected to hepatocytes surviving injury. Many inflammatory cells infiltrated these areas. With cytokeratin 19, hepatocytes were not stained, but biliary cells of atypical and typical ducts were stained dark-brown in AVH with confluent necrosis, CAH 2B and LC with active inflammation (Fig. 7B). In controls no cells were stained. Atypical bile ducts found in AVH with confluent necrosis, CAH 2B and LC with active inflammation contained cytokeratins 8 and 19.

Discussion

It has not been determined why bile duct (ductule) proliferation occurs after necrosis of hepatocytes in some human liver diseases. The proliferative activity of biliary epithelial cells and the ultrastructural characteristics of these cells in humans have not been described in detail. One reason for this is that there have not been reliable markers of cell proliferation until recently, but the production of monoclonal antibodies against bromodeoxyuridine (Gratzner 1982; Shimizu et al. 1988), Ki-67 antigen (Gerdes et al. 1984; Braun et al. 1988), proliferating cell nuclear antigen (Garcia et al. 1989; Kawakita et al. 1992) and DNA-PA has made it possible to detect proliferating cells using immunohistocytochemical techniques. We selected the antibody agianst DNA-PA for

this study because we have used it before. DNA-PA has a broad spectrum as a marker of cell proliferation, and the specificity of staining has been already demonstrated (Bensch et al. 1982; Masaki et al. 1982; Tanaka et al. 1982; Nakamura et al. 1984; Namikawa et al. 1987; Mushika et al. 1988; Seki et al. 1990, 1991). Here, in diseased livers, we found typical and atypical bile ducts in addition to large bile ducts lined with columnar cells. In the large bile ducts, we did not find columnar cells that stained for DNA-PA. In bile ducts and ductules lined with cuboidal cells, some cells were positively stained for DNA-PA. By EM, ultrastructural characteristics of biliary epithelial cells in newly formed atypical bile ducts were similar to those of pre-existing biliary epithelial cells in typical bile ducts. Most of them were clear cells; there were only a few dark cells. However, most of these clear cells did not stain positive for DNA-PA, with the exception of a few small cells. In the same sections, some hepatocytes were positively stained for DNA-PA. In rosettes, which included Hering ductules, hepatocytes were occasionally stained in enlarged portal or periportal areas of CAH 2B or LC, but no biliary cells were stained.

In previous studies, it has been suggested that the biliary cells in newly formed atypical ducts are derived from transformation or modulation of hepatic cords; these studies used immunohistocytochemical techniques, cytokeratins and glucose-6-phosphatase (Uchida and Peters 1983; Stosiek et al. 1985; Desmet 1987; Eyken et al. 1987, 1989). However, Schaffner and Popper (1961), using EM observations, reported that atypical bile ducts may arise by proliferation of pre-existing ducts. In our study of cytokeratins, atypical bile ducts were positive for both cytokeratins 8 and 19. It has been reported that anti-cytokeratin 8 decorates both epithelial cells of bile ducts and hepatocytes in liver, and that anti-cytokeratin 19 decorates only epithelial cells of bile ducts but not hepatocytes in normal liver (Stosiek et al. 1985; Desmet 1987; Eyken et al. 1987, 1989). Our results suggest that atypical bile ducts have characteristics similar to those of bile ducts, not hepatocytes. These data do not clarify the origin of these atypical bile ducts, but the results of DNA-PA staining suggest that there is a difference in proliferative activity between surviving hepatocytes in areas of confluent necrosis and the clear cells of newly formed atypical bile ducts. In a previous study (Seki et al. 1990), we found that the number of hepatocytes positively stained for DNA-PA per 1000 hepatocytic nuclei was 52 ± 56 (mean $\pm$ SD) in chronic hepatitis and 38 ± 41 in LC. If biliary epithelial cells in atypical bile ducts derive from hepatocytes by transformation or differentiation, then our results suggest that these biliary cells are less active than the original hepatocytes. Their origin remains undefined. We also found typical ductular proliferation in portal tracts of patients with AVH with confluent necrosis. In these ducts, some cells were positively stained for DNA-PA. Ultrastructurally, they were different from dark cells, they had a small amount of rough and smooth endoplasmic reticulum and fewer mitochondria, all of uniform size (Grishman and Porta 1964; Sasaki et al. 1967; Phillips et al. 1987). Most of them were clear cells with irregular nuclei containing coarse nucleoplasm and the intercellular spaces were dilated, as previously reported (Takino et al. 1976). These findings may indicate that in typical bile ducts, clear cells play a predominant role in ductular proliferation and that the dilatation of intercellular spaces may be a trigger for the initiation of ductular and hepatocyte proliferation (Nakamura et al. 1983). However, this dilatation may also be nothing but evidence of injury. Typical and atypical bile ducts were composed of similar kinds of biliary cells (mainly clear cells) but there may be differences in their proliferative activity and pattern of proliferation. In LC, the proliferative activity of biliary cells was greater in typical bile ducts than in atypical bile ducts ($P < 0.01$). This statistically significant difference may have arisen from the finding of many atypical bile ducts in two cases of LC (Fig. 3) and from the few biliary cells of atypical bile ducts positively stained for DNA-PA.

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