

Lectin Binding of Intrahepatic Bile Ducts and Peribiliary Glands in Normal Livers and Hepatolithiasis

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SAITO, K. and NAKANUMA, Y. *Lectin Binding of Intrahepatic Bile Ducts and Peribiliary Glands in Normal Livers and Hepatolithiasis.* Tohoku J. Exp. Med., 1990, 160 (1), 81-92 — Histochemical features of the intrahepatic bile ducts and peribiliary glands were examined with five biotinylated lectins in 12 normal human livers and 12 livers with hepatolithiasis. There was a marked proliferation of peribiliary glands, especially mucous cells, in the stone-containing bile ducts. Based on the carbohydrate binding specificity for each lectin, it was found in both the normal and hepatolithiasis livers, that N-acetylglucosamine, N-acetylgalactosamine, D-galactose and L-fucose were abundant in the mucous acinar cells of the intrahepatic peribiliary glands, and mannose (or glucose) was present exclusively in the serous acinar cells and surface lining epithelium. Considerable case to case and cell to cell variations, however, were noted in the expression of lectin binding activity. It has been shown that major monosaccharide components of biliary glycoproteins are N-acetylglucosamine, galactose and fucose. All of these carbohydrates were detected in the peribiliary glands, especially mucous acinar cells, in this study. Therefore, the peribiliary mucous cells are mainly responsible for the secretion of the glycoproteins with these carbohydrates into the hepatic bile, and may play a role in the calculi formation in the intrahepatic biliary tree. ——— lectin histochemistry; hepatolithiasis; peribiliary glands

Our previous study (Terada et al. 1987; Ishida et al. 1989) disclosed that there are peribiliary glands along the intrahepatic large bile ducts near the hepatic hilus and these glands drain into the intrahepatic biliary lumen directly or via their own conduits. These peribiliary glands are speculated to secrete several substances such as mucous glycoproteins and secretory IgA into the bile ductal lumen thereby modifying the composition and characteristics of bile and also playing a role in the local mucosal immunity of the biliary tree (Sugiura and Nakanuma 1989). The mucous glycoproteins in bile may also protect the biliary surface epithelium. Pathologically, the biliary mucous glycoproteins are probably involved in several disease processes of the hepatobiliary system, especially

Received December 4, 1989; revision accepted for publication December 25, 1989.

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in cholelithiasis (Soloway et al. 1977 ; Bouchier et al. 1979 ; Lee et al. 1979 ; Lee 1981 ; Pearson et al. 1982 ; LaMont et al. 1983 ; Lee and Nicholls 1986). Our previous study disclosed that there were multiple concentric layers of mucous substances alternated with bilirubin pigment layers in the brown pigment stones, and these mucous substances might have been secreted from the proliferated peribiliary glands in the calcuous bile ducts (Nakanuma et al. 1988 ; Terada and Nakanuma 1988).

Structurally, the constituents of mucous glycoproteins comprise a backbone polypeptide chain to which carbohydrate moieties are attached (Goldstein and Hayes 1978 ; Sato 1984). There have been several analytical studies on the carbohydrate moieties of the mucous glycoproteins in bile. For example, Bouchier et al. (1979), Lee et al. (1979) and Lee and Nicholls (1986) analyzed the carbohydrate moieties of the mucus in the gallbladder and hepatic bile by gas-liquid chromatography and demonstrated that biliary glycoproteins contained 55-75% of carbohydrates, and 80-85% of the monosaccharide components were galactose, fucose and N-acetylglucosamine (Bouchier et al. 1979 ; Lee et al. 1979 ; Lee and Nicholls 1986). The exact sites of the biliary mucous glycoprotein production with these carbohydrate moieties, however, remain to be determined. Carbohydrate moieties of glycoproteins in tissues can be localized by a very sensitive, specific and simple technique, using a biotin-labeled lectin followed by an avidin-biotin-peroxidase complex (Hsu and Raine 1982). Several investigators have demonstrated pathologic changes of the mucus in carcinogenesis and during development using lectin histochemistry (Goldstein and Hayes 1978 ; Hsu and Raine 1982 ; Geleff and Bosk 1984 ; Sato 1984). The present study was undertaken to explore the carbohydrate moieties of the intrahepatic lining and glandular biliary epithelium in normal livers and hepatolithiasis with use of five biotinylated lectins in combination with the conventional mucous histochemistry.

MATERIALS AND METHODS

Intrahepatic biliary tree was defined as the biliary tree proximal to the hepatic duct confluence, and was classified into the right and left hepatic ducts, segmental ducts (the first major branches of each hepatic duct), area ducts (the first major branches of each segmental duct), and their finer branches according to Healey and Schroy (1953). The hepatic, segmental and area ducts were collectively termed as "intrahepatic large bile ducts" in the present study. The wall of these ducts consisted of a hypocellular collagenous band covered with lining epithelium. The periductal tissue was a loose fibrous connective tissue around the bile duct wall. Peribiliary glands consist of the intramural and extramural glands (Terada et al. 1987 ; Ishida et al. 1989). The former is a tubular gland with no or few branchings within the ductal walls, and the latter is arranged as a lobule consisting of several acini in the periductal fibrous connective tissue, and is located at both sides of the bile ducts and parallel to the hepatic parenchymal edge. These acini are composed of the serous and mucous cells. Serous acinar cells have scanty cytoplasm and the nucleus is located centrally. On the other hand, mucinous cells have clear and ample cytoplasm (mucus) with basally situated nuclei. Bile ductules, interlobular bile ducts and septal bile ducts, are finer branches of the area ducts and are only identified microscopically

(Nakanuma and Ohta 1979). The interlobular and septal bile ducts are collectively termed as "small bile duct" in the present study, and they are in the peripheral zone of the portal tracts and have a lumen larger than 20 μm . Bile ductules are considered tubular structures in the peripheral zone of the portal tract with a lumen smaller than 20 μm .

The liver specimens were obtained from 12 patients with hepatolithiasis (all were adults; 10 surgical and 2 autopsy specimens) and 12 normal human livers (all were adults; obtained all autopsy specimens). A diagnosis of hepatolithiasis was made when stones were found in the right and/or left hepatic ducts and/or their tributaries, irrespective of coexistence of choledocholithiasis. All of these materials were immediately fixed in 10% neutral formalin, and then were embedded in paraffin.

About 30 serial sections, 5 μm -thick, were cut from each paraffin block, and they were stained with hematoxylin and eosin (H & E), and double staining with a periodic-acid Schiff's solution after diastase digestion and alcian blue dye at pH 2.5 (d-PAS/AB pH 2.5). The neutral mucus was shown by PAS stain, and acid mucus was by alcian-blue stain.

The remaining sections were used for lectin immunohistochemistry using the avidin-biotin-peroxidase complex method (ABC method) (Hsu and Raine 1982). Five biotinylated lectins shown in Table 1 were purchased from E-Y Lab. Inc. (San Mateo, CA, USA). These lectins represent five groups of lectins showing characteristic carbohydrate-binding specificity (Goldstein and Hayes 1978; Tsuji and Oosawa 1983). Con-A (concanavalin A) represents a D-mannose or D-glucose-binding lectin, WGA (wheat germ agglutinin) a N-acetylglucosamine-binding lectin, HPA (*Helix pomatia* agglutinin) a N-acetylgalactosamine-binding lectin, RCA-I (*Ricinus communis* agglutinin I) as a D-galactose-binding lectin, and UEA-I (*Ulex europeaus* agglutinin I) a L-fucose-binding lectin. Briefly, deparaffinized sections were pretreated with 0.3% H_2O_2 for 20 min and then 1% normal bovine serum albumin (diluted in phosphate buffered saline (PBS)) for 60 min. The sections were then incubated with 10 $\mu\text{g}/\text{ml}$ of a solution of biotinylated lectins (diluted in PBS) for 30 min at room temperature. Then, freshly prepared avidin-biotin-peroxidase complex (25 μl of avidin DH 25 μl of biotinylated peroxidase + 5 ml of PBS) (Vector Lab., CA, USA) was applied for 30 min at room temperature. The histochemical reaction for peroxidase was carried out using a 0.01% H_2O_2 -0.2 mg/ml 3, 3'-diaminobenzidine (DAB) (Sigma Chemical Co., St. Louis, MO, USA) solution. After washing with PBS, the sections were lightly counterstained with Meyer's hematoxylin. Staining patterns of these lectins were then correlated with those of mucus histochemistry.

The following staining and specificity controls were performed. No positive stain was obtained when H_2O_2 without DAB or DAB without H_2O_2 was applied. Positive stain was abolished when PBS or nonimmune serum was used as the first layer. The specificity controls for lectin binding were done by incubating deparaffinized sections with mixtures of lectins in PBS together with the appropriate sugars in concentration of 0.2 to 0.4 M. The following sugars were obtained from E-Y Lab. Inc.: methyl-mannopyranoside for Con A; N-acetyl-D-glucosamine for WGA; N-acetyl-D-galactosamine for HPA; D-galactose for

TABLE 1. Carbohydrate-binding specificity of five lectins

Lectin	Carbohydrate-binding specificity
Con-A (Concanavalin A)	α -D-mannose > α -D-glucose
WGA (Wheat germ agglutinin)	{ β (1 \rightarrow 4)-D-N-acetylglucosamine} ₂ , D-N-acetylglucosamine
HPA (<i>Helix pomatia</i>)	α -D-N-acetylgalactosamine, α -D-N-acetylglucosamine
RCA-I (<i>Ricinus communis</i> agglutinin-I)	β -D-galactose > α -D-galactose
UEA-I (<i>Ulex europeaus</i> agglutinin-I)	α -L-fucose

RCA-I; and L-fucose for UEA-I.

RESULT

In normal livers, intrahepatic small and large bile ducts were lined by single-layered and occasionally, multi-layered columnar epithelium. In addition, there were intramural and extramural glands (Fig. 1). On the contrary, in hepatolithiasis, the biliary lining epithelium was hyperplastic and multilayered, although some of these lining epithelia were artificially dropped out. Furthermore, both extramural and intramural glands markedly increased in the fibrously thickened ductal walls (Fig. 2). Proliferated intramural glands were predominantly composed of mucous acinar cells. Mucous and serous acinar cells were intermingled at variable proportions in the extramural glands of normal livers and hepatolithiasis, while the proportion of mucous acinar cells increased in he-

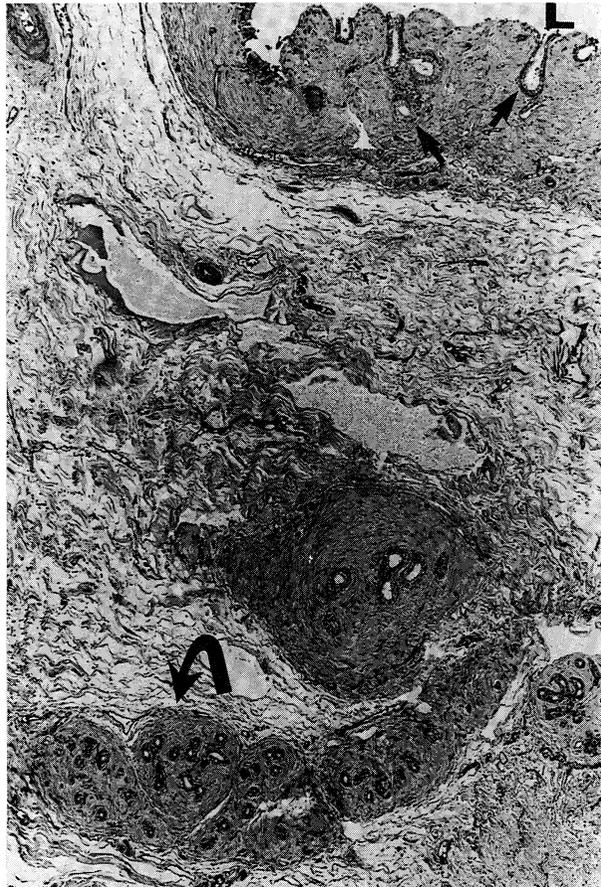


Fig. 1. Intramural (straight arrows) and extramural (curved arrow) peribiliary glands in the intrahepatic large bile duct of normal liver. L: bile duct lumen. HE., $\times 80$.

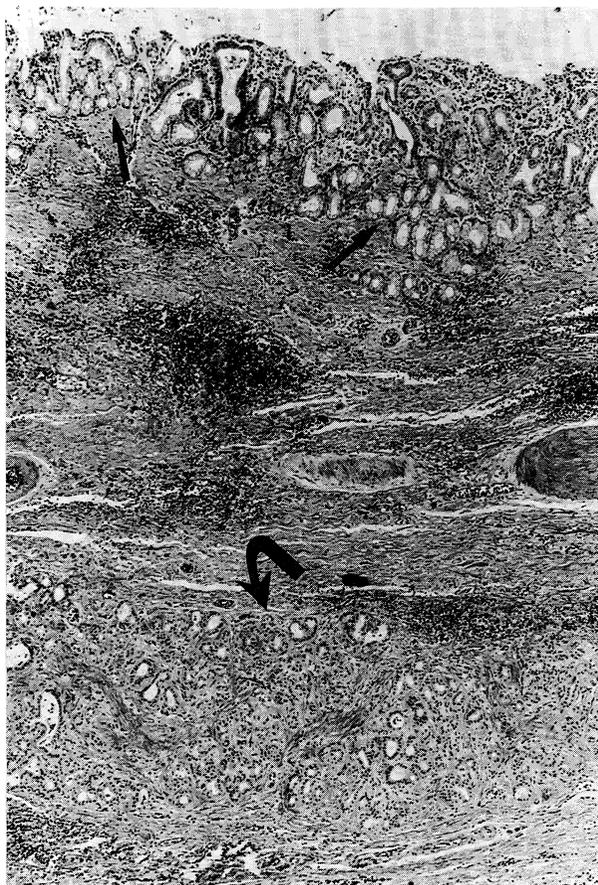


Fig. 2. Stone-containing bile duct showing marked proliferation of intramural (straight arrows) and extramural glands (curved arrow). HE., $\times 80$.

patolithiasis compared to that in normal livers.

Histochemistry and lectin binding patterns of the peribiliary glands

Mucous acinar cells in the intramural glands were usually positive for d-PAS but negative for alcian blue, whereas the mucous cells in the extramural glands were positive for alcian blue and/or d-PAS (Fig. 3). A few mucous granules which were positive for alcian blue staining, were frequently found in the serous acinar cells in the extramural glands, and their luminal borders were also positive for alcian blue staining (Fig. 4). There were a few acinar cells showing intermediate histochemical features between mucous and serous cells in the peribiliary glands.

Serous acinar cells of the peribiliary glands were diffusely or focally positive for Con-A (Fig. 5). However, mucous granules and luminal borders of these

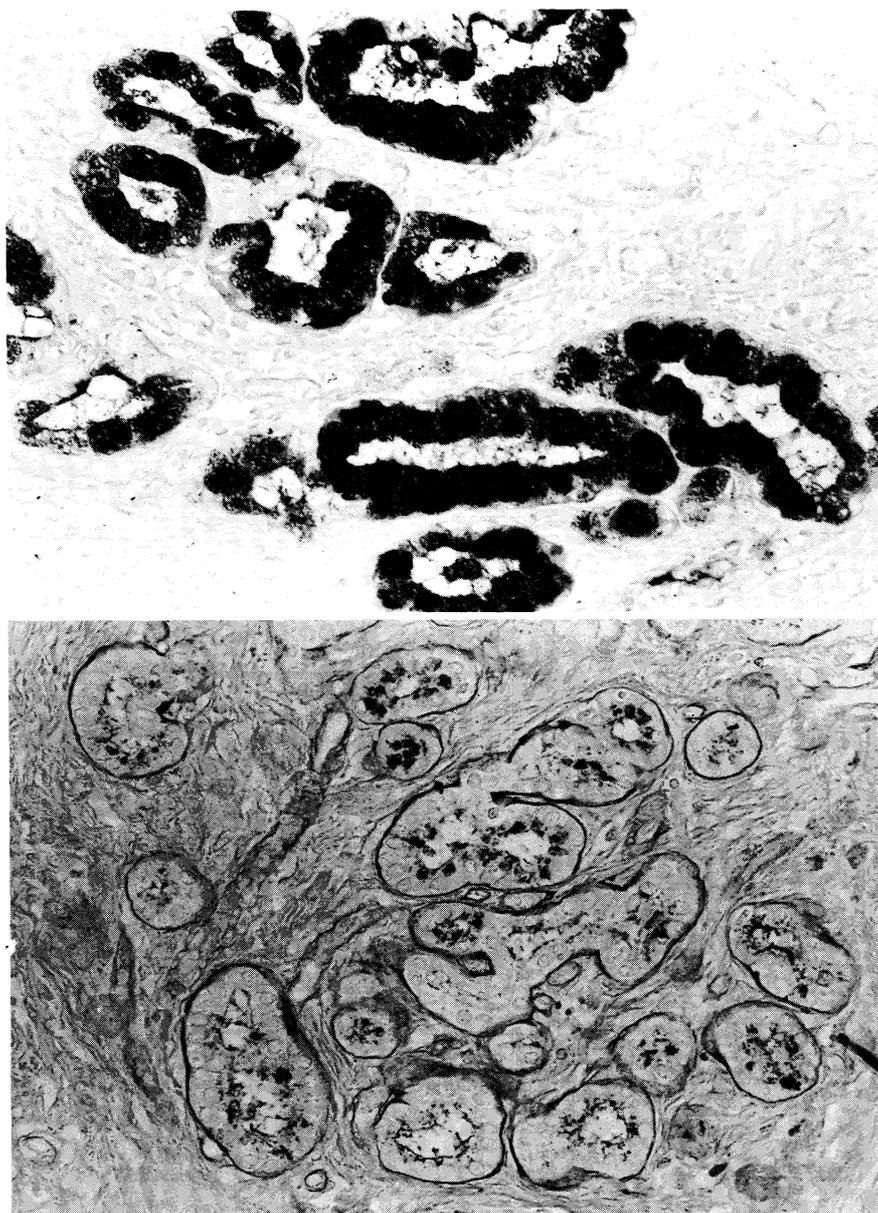


Fig. 3 (upper). Mucous acinar epithelial cells of extramural glands of the stone-containing bile ducts showing abundant acid and neutral mucus in their cytoplasm. d-PAS/AB pH 2.5 stain, $\times 200$.

Fig. 4 (lower). Extramural glands of the stone-containing bile duct of hepatolithiasis. There is a little amount of acid mucus in the luminal border and apical cytoplasm of the serous acinar cells. Basement membrane is also stained. d-PAS/AB pH 2.5 stain, $\times 200$.

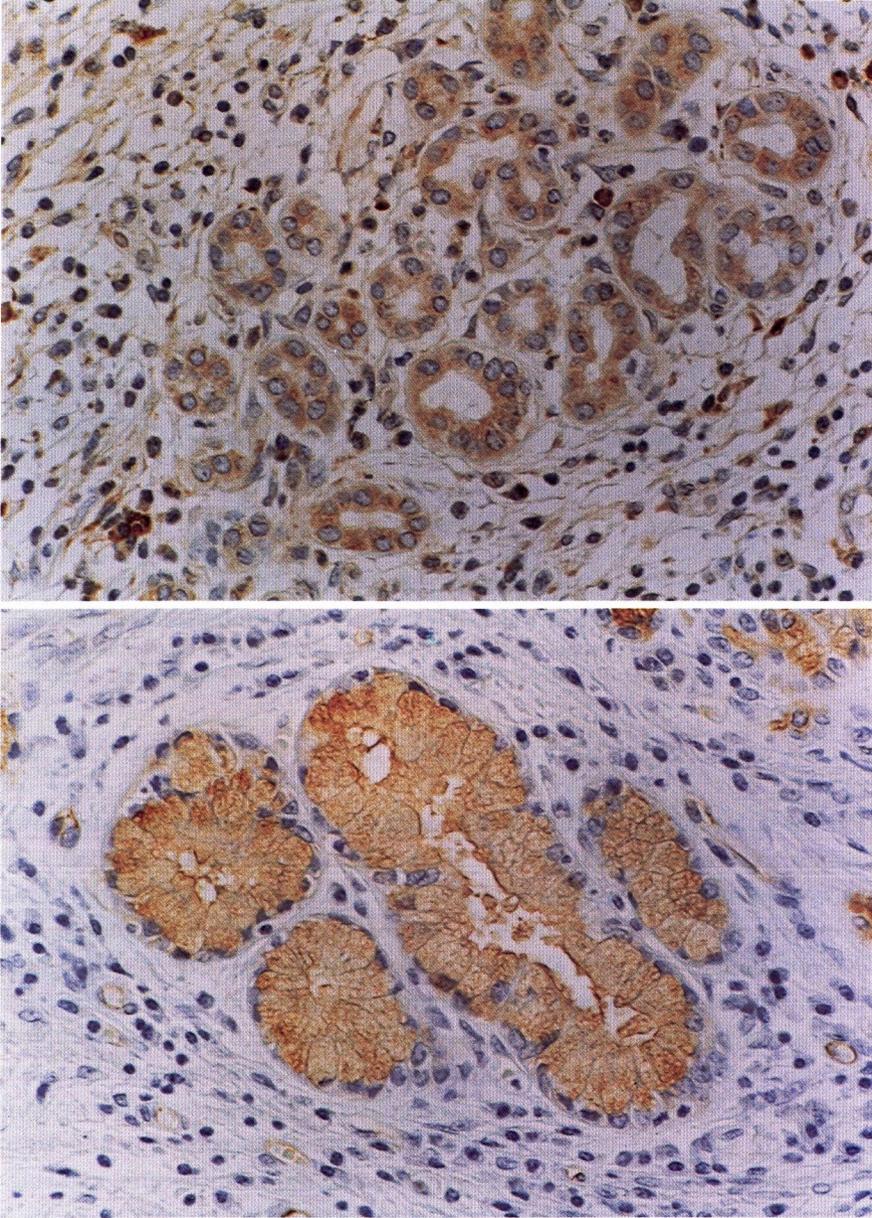


Fig. 5 (upper). Serous acinar cells whose cytoplasm reacted well with Con-A. Luminal borders and apical mucous granules are negative. The extramural glands of the stone-containing bile duct of hepatolithiasis. Con-A (ABC method) and hematoxylin, $\times 200$.

Fig. 6 (lower). Mucous acinar cells in which UEA-1 bond well with intracytoplasmic abundant mucin. The extramural glands of stone-containing bile duct of hepatolithiasis. UEA-1 (ABC method) and hematoxylin, $\times 200$

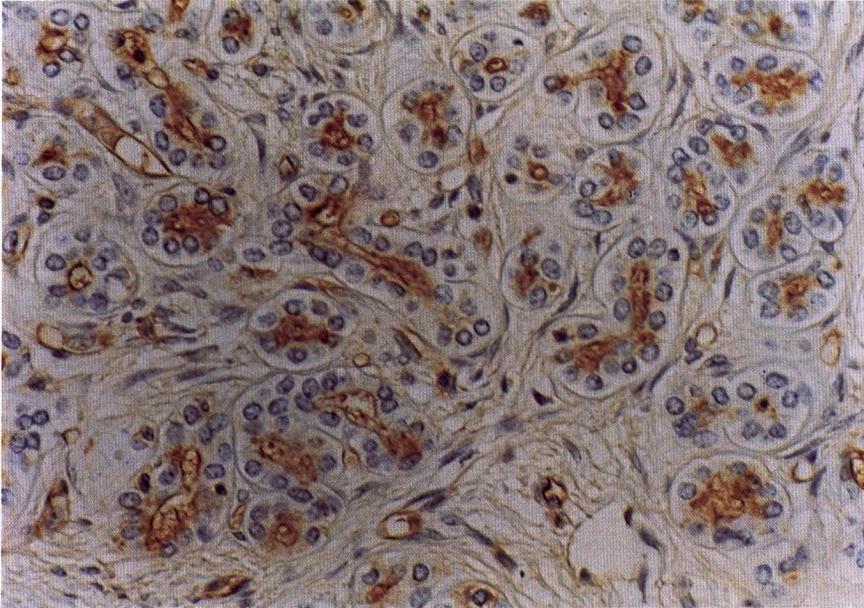


Fig. 7. Serous acinar cells in which WGA reacted with mucin-positive luminal border and apical cytoplasm. The extramural glands of the stone-containing bile duct of hepatolithiasis. WGA (ABC method) and hematoxylin, $\times 200$.

serous cells were negative for Con-A. This lectin was also negative in mucous acinar cells.

On the other hand, mucous acinar cells of the peribiliary glands showed a diffuse positive reaction with WGA, HPA, RCA-I and UEA-I (Fig. 6). These lectins were positive for both the acid and neutral mucin, and both types of mucus in the cytoplasm. Mucous granules in the apical cytoplasm and luminal borders of the serous acinar cells were also positive for these lectins (Fig. 7). Non-mucous cytoplasmic parts of these cells were generally negative for these lectins.

These two patterns of lectin reactivity in relation to mucous staining were similar in both the normal and hepatolithiasis livers. This was also the case with the biliary surface lining cells of the intrahepatic bile ducts (see below). Considerable case to case and cell to cell variations in the expression and staining activity of individual lectins were noted in the glandular epithelium, and this was also the case in the bile duct lining epithelium (see below).

Histochemistry and lectin binding patterns of the bile duct lining epithelium

The lining epithelium of large bile ducts contained a small to moderate amount of mucus (predominantly acid type) along the luminal border and in apical cytoplasm (Fig. 8). Small bile ducts, especially septal bile ducts, also showed such mucous granules in the apical cytoplasm as well as the luminal border. Con-A diffusely reacted with the cytoplasm of duct lining epithelial cells

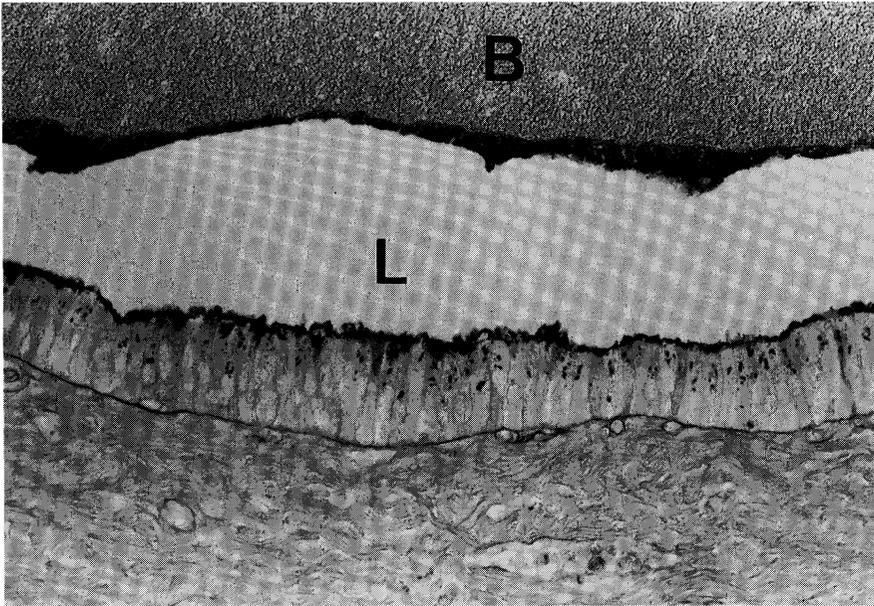


Fig. 8. Acid-mucus positive granules in the apical cytoplasm and along the luminal border of the bile duct lining epithelium. The intrahepatic large bile duct of normal liver. Luminal content (B) is barium injected for cholangiography at autopsy. L, ductal lumen. d-PAS/AB pH 2.5, $\times 160$.

of the intrahepatic large and small bile ducts though mucin-positive luminal border was generally spared. On the contrary, WGA, HPA, RCA-I and UEA-I were positive in the mucous areas or granules of the surface-lining epithelium. The luminal border and apical mucous cytoplasm of serous acinar cells were also similarly positive for these lectins.

DISCUSSION

It is well known that there is a considerable amount of glycoproteins in the bile (Soloway et al. 1977; Bouchier et al. 1979; Lee et al. 1979; Lee 1981; Pearson et al. 1982; LaMont et al. 1983; Lee and Nicholls 1986). Their classification, biochemical characters, origin in the hepatobiliary system and pathogenetic significance in the development of hepatobiliary diseases remain, however, unsettled.

The present study using lectin immunohistochemistry showed that there was a characteristic combination of binding pattern of the lectins at the mucous and serous acinar cells of the peribiliary glands and also at the lining epithelial cells of the bile ducts. That is, WGA, HPA, RCA-I and UEA-I diffusely labeled the mucous acinar cells of the peribiliary glands and also mucous granules of the lining epithelium and serous acinar cells. A combination of these lectins suggests the presence of N-acetylglucosamine, N-acetylgalactosamine, D-galactose and

L-fucose (Goldstein and Hayes 1978; Tsuji and Oosawa 1983) in these biliary glandular and lining epithelium. It is of interest that these carbohydrate moieties are largely identical to the major monosaccharide components of the soluble glycoproteins of human gallbladder and hepatic bile, and the gallstones (cholesterol and pigment stones), that is, galactose, fucose and N-acetylglucosamine (Bouchier et al. 1979; Lee et al. 1979; Lee and Nicholls 1986). N-acetylgalactosamine is also known to be present in the biliary glycoprotein (Bouchier et al. 1979; Lee et al. 1979; Lee and Nicholls 1986). Thus, the biliary glycoproteins with these carbohydrate moieties, at least in the hepatic bile, may be mainly produced in the peribiliary glands with these carbohydrate moieties and then be secreted into the bile.

Bouchier et al. (1979) and Lee et al. (1979 and 1986) pointed out that there were neither obvious nor consistent differences in the proportions of monosaccharides in glycoproteins between the bile with or without gallstones. Their data seem compatible with our present study, that is, there were basically no differences in the lectin binding patterns in the intrahepatic biliary tree and peribiliary glands between normal and hepatolithiasis livers. There was, however, the considerable case to case and cell to cell variations of expression as well as staining intensity of these lectins, and this phenomenon may be related to poorly understood individual determinants.

Because there were no differences in the lectin binding patterns between acid-mucus and neutral-mucus in extramural as well as intramural glands, these mucus seem to share common carbohydrate moieties. However, the present study using histochemical reaction was not, however, enough to analyze the quantities of carbohydrate moieties and/or substances reactive for alcian blue dye or periodic-acid Schiff's solution. Furthermore, the characterization of glycoproteins by five lectins was limited, so that the acid and neutral mucus in the biliary lining and glandular epithelium might be different in their three configurational structures. Further analysis of the carbohydrate moieties of biliary mucus seems mandatory to resolve this problem.

In the stone-containing gallbladder, glycoproteins increased in the bile as well as in the mucosa when compared with those in normal livers. Histological studies also showed that the calculous gallbladder mucosa contained more mucous-secreting cells than the normal mucosa (Lee and Nicholls 1986). These findings and the evidence from experimental lithogenesis in animals, indicating that glycoproteins or mucous production increased before stones developed (Pemsingh et al. 1987), suggest that the glycoproteins were secreted by the gallbladder mucosa into bile and were then incorporated into the gall stones. The similarity of carbohydrate moieties found in the mucosa, bile and gallstones in cholecystolithiasis (Lee and Nicholls 1986), supports the above-mentioned suggestion. The present study also showed that mucous glycoproteins with the carbohydrates moieties identical to those of the biliary glycoproteins in the hepatic and gallblad-

der bile were found in the stone-containing bile ducts, especially in the mucous acinar cells. It seems, therefore, possible that a large amount of mucus with these carbohydrate moieties might be produced in the peribiliary glands and then secreted into the biliary lumina, and thereafter may be incorporated into brown pigment stones in the intrahepatic biliary tree. However, further biochemical analysis of the hepatic bile from the stone-containing bile ducts seems mandatory.

Non-mucinous cytoplasm of serous acinar cells of the peribiliary glands and biliary surface epithelial cells contained Con-A, so that carbohydrate moieties with D-Mannose (or D-glucose) were plentiful in these biliary cells. These carbohydrates were small in their amount in the glycoproteins in the hepatic bile (Bouchier et al. 1979; Lee et al. 1979; Lee and Nicholls 1986). The physiological and pathological significance of the glycoproteins with these carbohydrate moieties in the hepatobiliary system is unclear at present.

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