Virchows Archiv A Pathol Anat (1988) 413:167–176

Virchows Archiv A
Pathological Anatomy and Histopathology
© Springer-Verlag 1988

Morphological examination of intrahepatic bile ducts in hepatolithiasis

Tadashi Terada and Yasuni Nakanuma
Second Department of Pathology, School of Medicine, Kanazawa University, Kanazawa 920, Japan

Summary. Numerous glandular elements are characteristically found within and around the intrahepatic bile duct walls in hepatolithiasis. These glandular elements were studied by reconstruction of serial sections and mucus histochemistry. The glands were of two types: glands within the thickened ductal wall (intramural) and those outside the wall (extramural). The former were mucous glands arranged in tubular pattern and the latter seromucous glands arranged in tubuloalveolar pattern. Mucous acini of both glands were rich in neutral, carboxylated and sulfated mucus glycoproteins. Serial section observations showed that the intramural glands communicated with bile duct lumina directly, and the extramural glands with ductal lumina via their own conduits. The intramural glands were usually continuous with the epithelia lining bile ducts, suggesting that they were derived from an invagination and subsequently proliferating epithelium lining bile ducts. The extramural glands may have arisen from a proliferation of the pre-existing peribiliary glands. Hypersecreted mucus from the intramural and extramural glands might be causally related to the development and growth of calculi in the intrahepatic biliary tree.

Key words: Hepatolithiasis – Intrahepatic Peribiliary Glands – Mucus

Introduction

One of the characteristic pathological features in hepatolithiasis, prevalent in the Far East, is a presence of considerable amounts of glandular tissues around stone-containing bile ducts (Nakanuma et al. 1981; Yamamoto 1982; Ohta et al. 1984). These glands are known to secrete much mucus into the ductal lumen and Yamamoto divided them into intra- and extramural; the former were within the thickened duct wall and the latter outside it (Yamamoto 1982). The morphogenesis of these glandular tissues and their pathological significance in calculi formation are, however, still speculative. Some Japanese surgeons have insisted that these glands in hepatolithiasis are anomalous ectopic tissue, although there has been no objective evidence. Furthermore, the relationship between these glands in hepatolithiasis and the peribiliary glands which are present physiologically in normal livers (Terada et al. 1987) remains unclear.

The aims of the present paper are to describe the detailed morphology of these glands in hepatolithiasis and to know the origin of these glandular tissues with a help of serial sections. Mucus histochemistry of the glandular tissue was also examined.

Offprint requests to: T. Terada

Materials and methods

A total of 18 livers with hepatolithiasis (8 autopsy and 10 surgically-resected liver specimens), were used. Age of the patients ranged from 22 to 91 years (62 year mean). The male to female ratio was 10:8. Percutaneous transhepatic cholangiography and/or endoscopic retrograde cholangiography were carried out in the 10 surgical cases. In the 8 autopsy cases, the identification of the stone-bearing bile ducts was made macroscopically, and two autopsy cases were subjected to postmortem cholangiography to determine the exact location of the calculi (Fig. 1). Many tissue blocks containing stone-bearing bile ducts were obtained with reference to the cholangiogram in each case, and were fixed in 10% neutral formalin and embedded in paraffin. Gall stones, which were embedded in paraffin together with bile ducts, could be cut off relatively easily by an ordinary microtome. Ten serial sections (5 μm in thickness) from each block were stained with the following procedures: haematoxylin and eosin, periodic acid-Schiff with or without prior diastase digestion, Alcian blue at pH 1.0 and 2.5, colloidal iron, combined Alcian blue at pH 2.5 and periodic acid-Schiff (Mowry 1963),

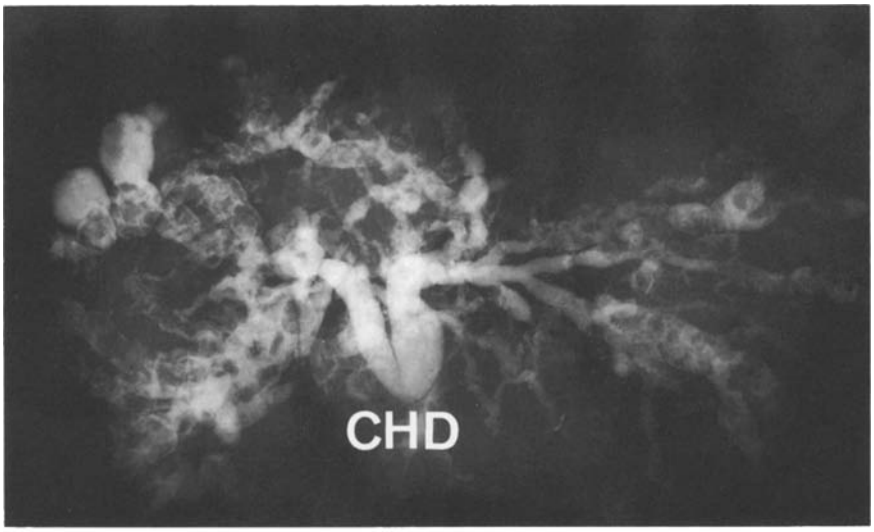
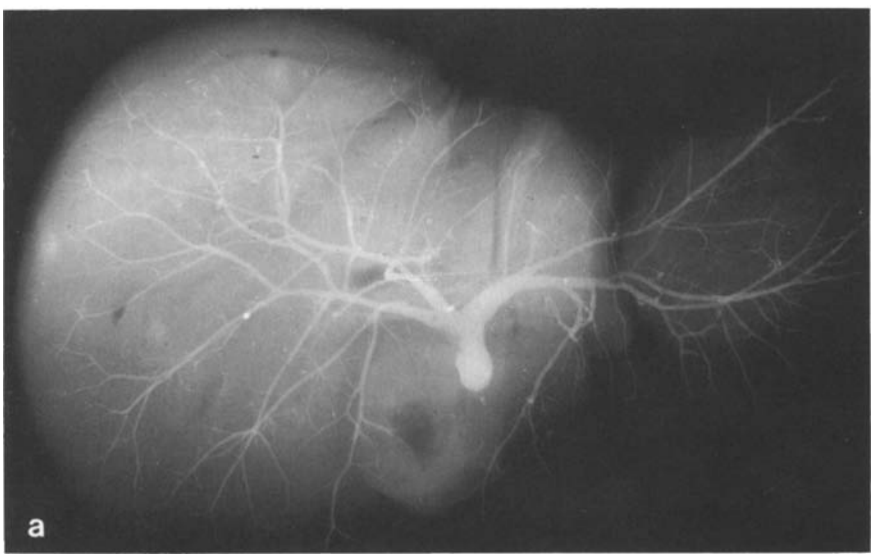


Fig. 1. Postmortem intrahepatic cholangiogram of a liver with hepatolithiasis viewed from postero-anterior direction. Intrahepatic calculi are seen as radiolucent materials within the dilated intrahepatic bile ducts of both lobes. CHD; common hepatic duct; reduced to seven tenths



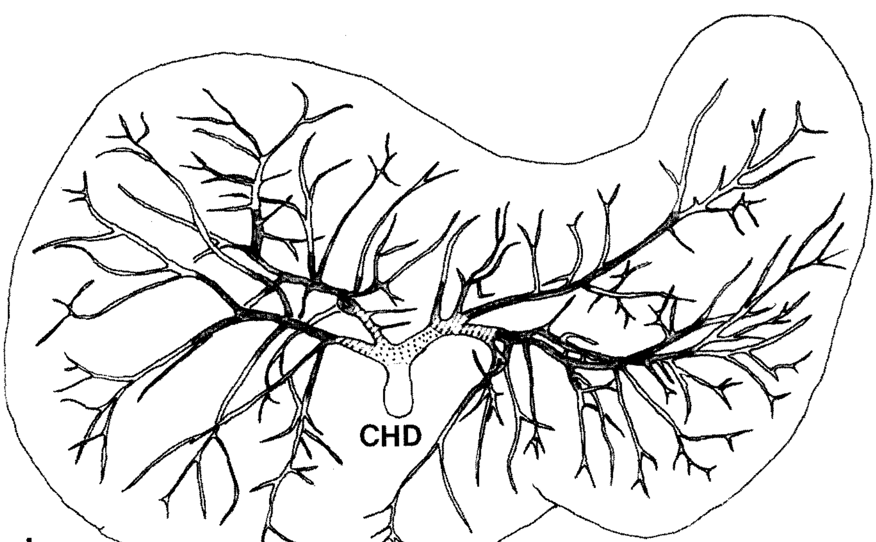


Fig. 2. A Postmortem intrahepatic cholangiogram of a normal liver of 43-year-old man who died of pulmonary embolism. The cholangiogram is postero-anterior direction; reduced to two thirds. **B** Trace of the **A**. Closed, hatched and dotted compartments were area, segmental and hepatic ducts, respectively; CHD, common hepatic duct

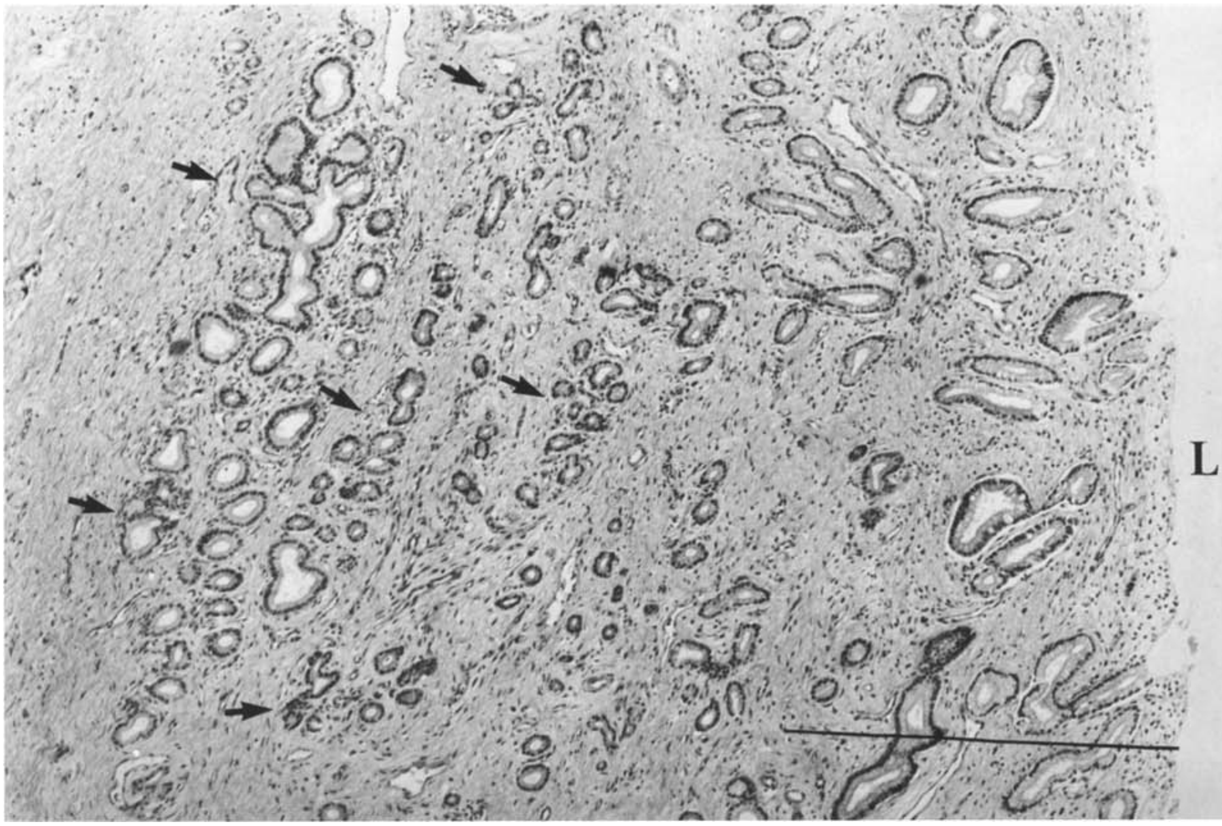


Fig. 3. Photomicrogram of the wall and periductal tissue of the stone-containing bile duct. There are many glandular structures within and around the bile duct. The glandular tissue take a form of tubular glands within the bile duct wall (intramural glands) and of tubuloalveolar glands in the periductal tissue (extramural glands) (*arrows*). The length of the bar in the photograph represents that of the thickened bile duct wall. *L*; lumen of the stone-containing bile duct; H&E (×40)

combined high iron diamine and Alcian blue at pH 2.5 (Spicer 1965), and Concanavalin A followed by horseradish peroxidase and diaminobenzidine (Katsuyama et al. 1978). In addition, a total of 2482 serial sections (5 μm in thickness) were made from 12 tissue blocks of 8 livers, and were stained with haematoxylin and eosin for evaluation of the three dimensional architecture of the glandular tissue.

Intrahepatic bile ducts were defined in this study as that part of the biliary tree proximal to the confluence of the hepatic ducts and were classified into hepatic (left and right), segmental (the first major branches of each hepatic duct: left lateral and medial, right anterior and posterior) and area ducts (the first major branches of each segmental duct) according to Healey and Schroy (1953) (Fig. 2). The area ducts are main ducts draining 8 hepatic areas (or segments), and range approximately from 0.2 to 0.1 cm in inner diameter when visualized by cholangiography (Fig. 2). The bile duct wall was defined as the dense collagenous tissue just beneath ductal epithelium. The periductal tissue was the loose connective tissue around the ductal wall. The bile duct wall was devoid of artery, vein or nerve bundle, all of which were present in the periductal tissue. Distinguishing the duct wall from the periductal tissue in intrahepatic bile ducts bearing stones was on occasion arbitrary.

Results

Intrahepatic calculi, when examined by cholangiograms or by macroscopic examination, were located within the hepatic, segmental and/or area ducts (Fig. 1). Bile ducts smaller than area ducts, i.e. septal and interlobular bile ducts and bile ductules, rarely contained stones but biliary sludge was occasionally seen within the smaller bile ducts. Of the 18 cases, 12 had stones in the left lobe alone, 5 in both lobes and 1 in right lobe alone. In 11 cases, the extrahepatic bile duct also contained gall stones. The stones were composed of brown pigment in all cases. The stone-containing ducts showed luminal dilatation as well as fibrosis of ductal walls and periductal tissue.

On microscopic examination, there were a number of glandular elements within and around the stone-containing bile duct walls (Fig. 3). The glandular structures were divided into intra- and extramural glands on the basis of their location and also their morphology, though their distinction was arbitrary on occasion. The intramural glands showed clear abundant cytoplasm and basally situated nuclei, resembling pseudopyloric gland metaplasia in chronic cholecystitis (Figs. 3, 4A). Goblet cells were also occasionally intermingled in these glandular epithelia (Fig. 3). Serial

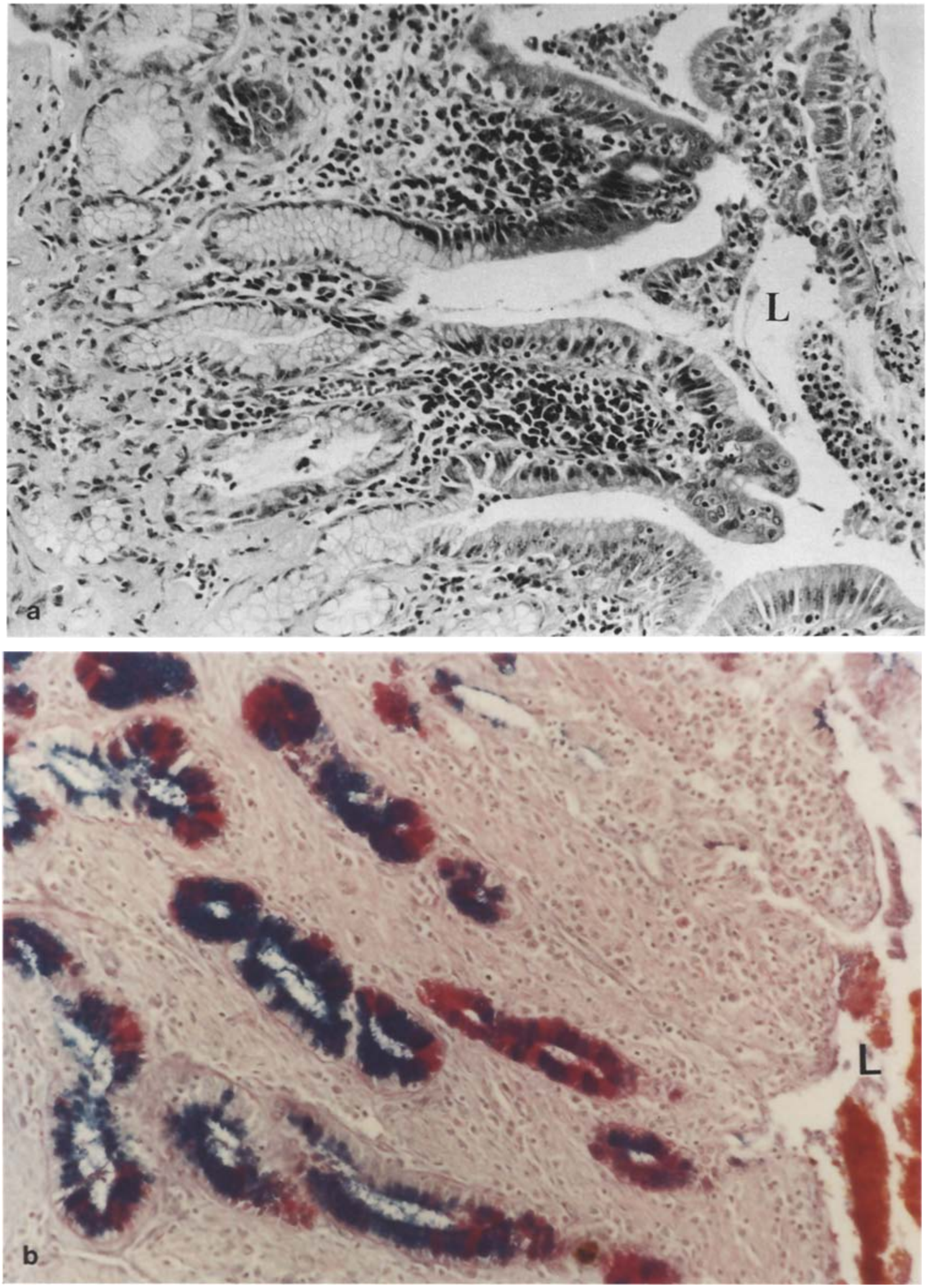


Fig. 4a, b

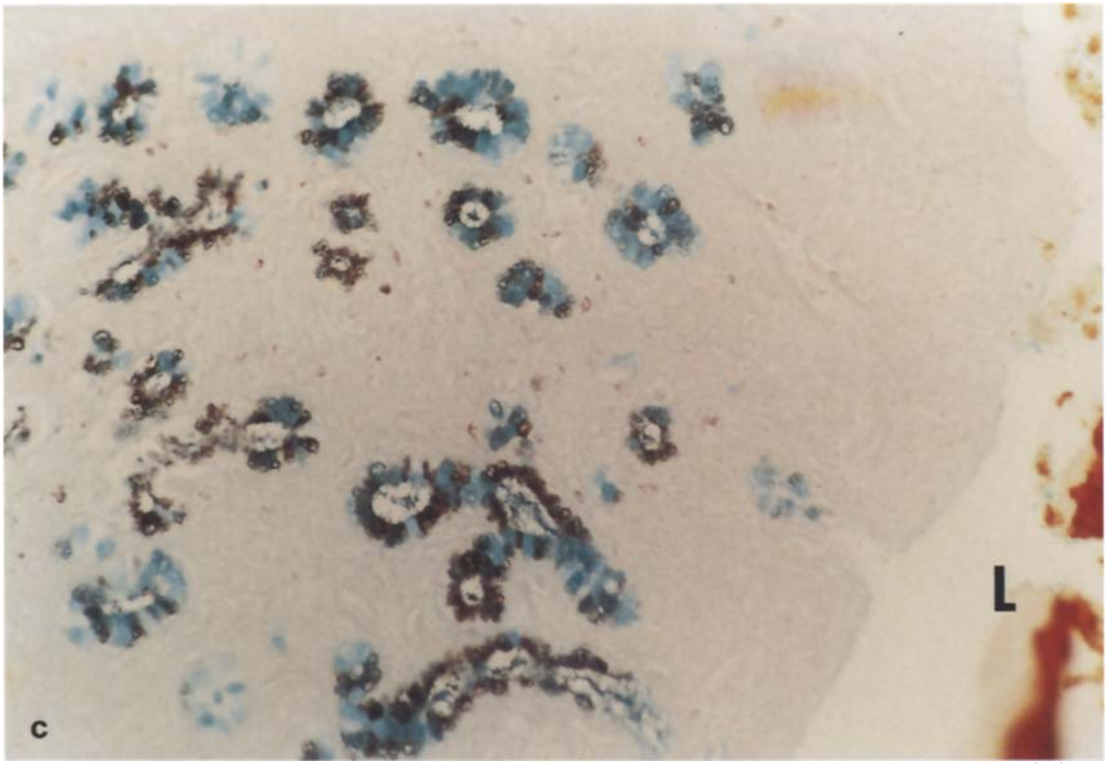


Fig. 4. a High power view of the intramural tubular glands in hepatolithiasis. The glandular epithelia are continuous with surface epithelia lining the bile duct. *L*, bile duct lumen. H&E, (× 100). **b** The intramural tubular glands in hepatolithiasis. The tubular glands are stained both red and blue in their entirety, indicating of the presence of both neutral and acidic mucus. The neutral and acidic mucus are equal in amount. *L*, bile duct lumen. Alcian blue (pH 2.5) and periodic acid-Schiff, (× 100). **c** The intramural tubular glands in hepatolithiasis. The glandular tubules are stained both blue and brown in their entirety, indicating of the presence of carboxylated and sulfated mucus. The carboxylated and sulfated mucus are equal in amount. *L*, bile duct lumen. High iron diamine and Alcian blue (pH 2.5), (× 100)

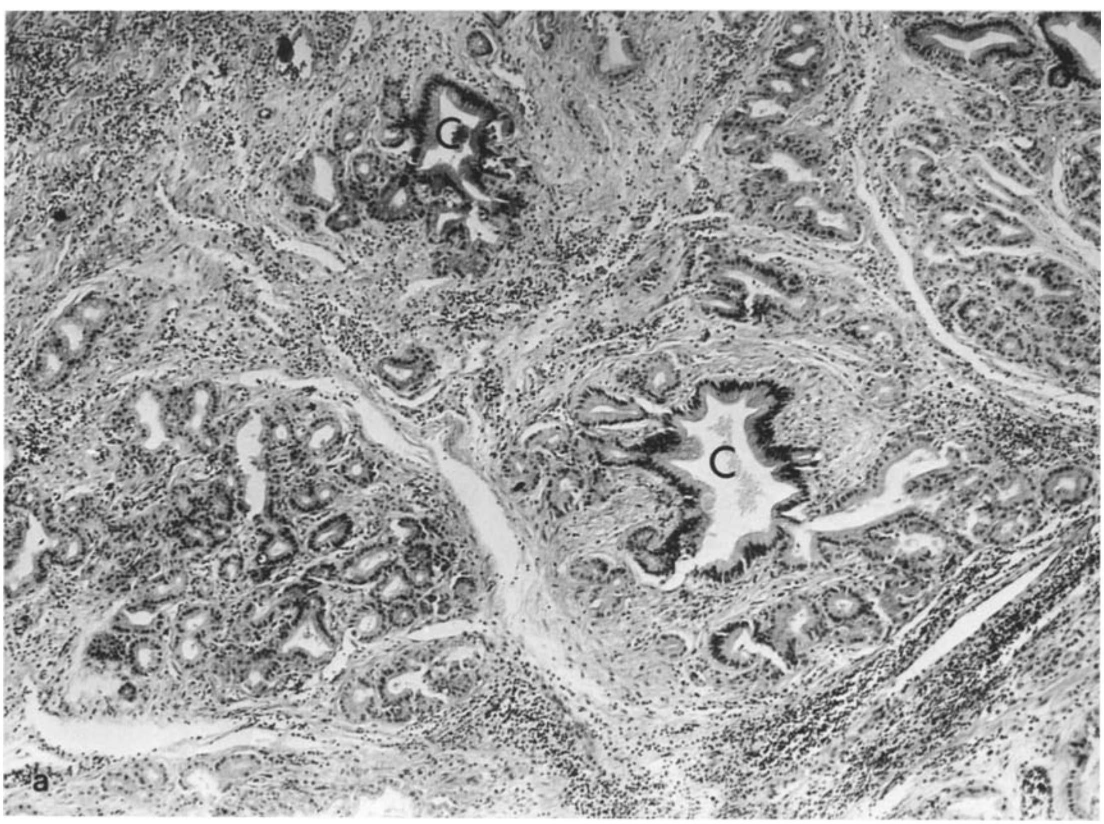
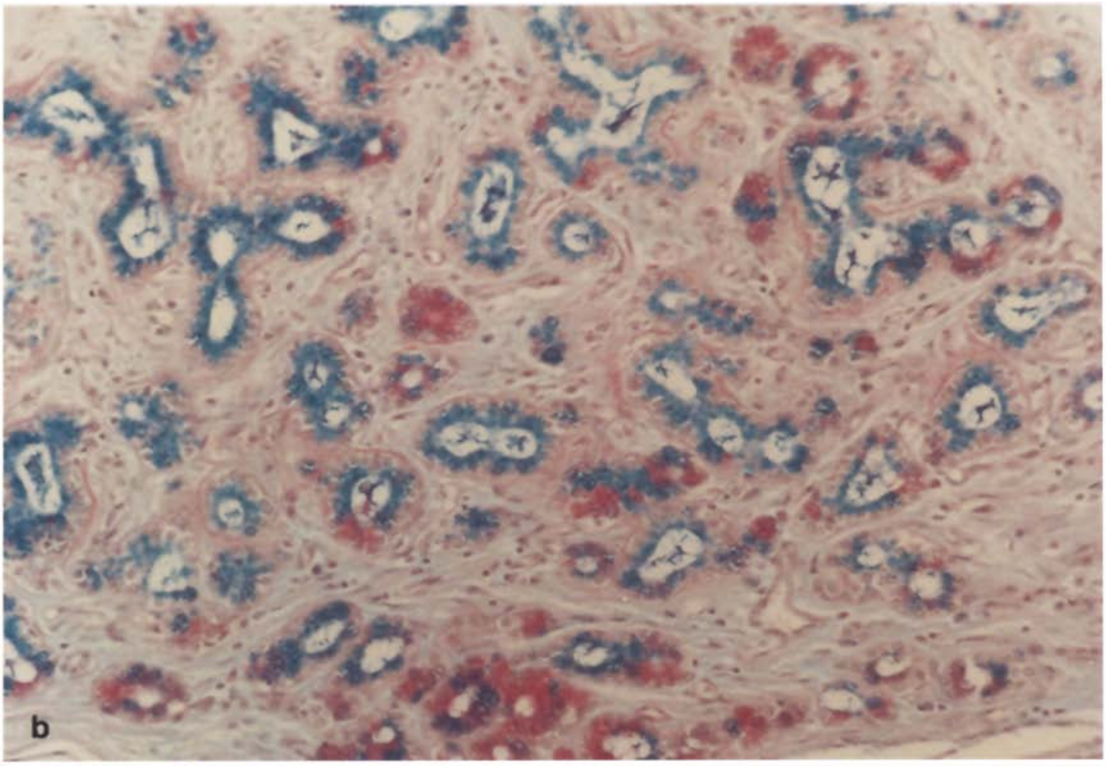


Fig. 5 a



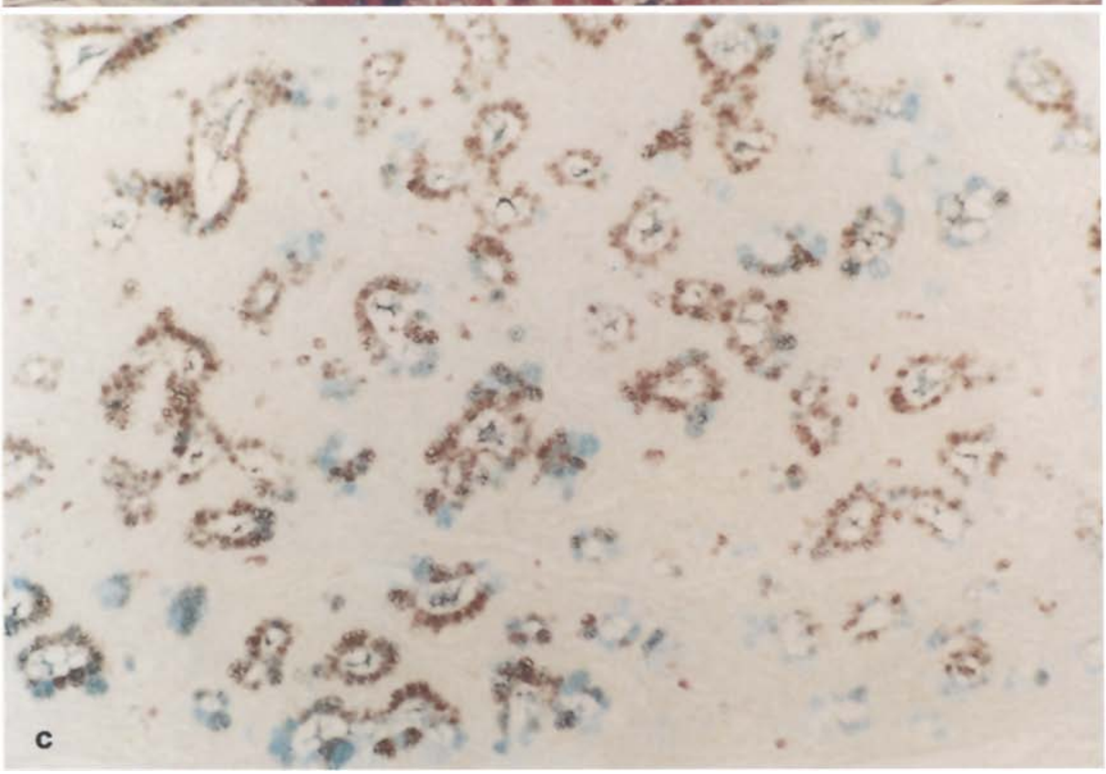


Fig. 5. a Photomicrogram of the extramural tubuloalveolar glands in hepatolithiasis. The constituent cells consist of low-columnar serous and high columnar clear mucous cells. The glandular acini are surrounded with fibrous band, forming lobules. Conduits of these glands are seen. *C*, conduit of extramural glands (H&E, ×40). **b** The extramural tubuloalveolar glands in hepatolithiasis. The glandular mucous acini are stained both red and blue, indicating of the presence of both neutral and acidic mucus. Acidic mucus is more abundant than neutral mucus. Serous acini were not stained or stained only faintly along the luminal border. Alcian blue (pH 2.5) and periodic acid-Schiff, (×100). **c** The extramural tubuloalveolar glands in hepatolithiasis. The glandular mucous acini are stained both blue and brown, indicating of the presence of both carboxylated and sulfated mucus. Sulfated mucus is more abundant than carboxylated mucus. Serous acini were not stained or stained faintly only along the luminal border. High iron diamine and alcian blue (pH 2.5), (×100)

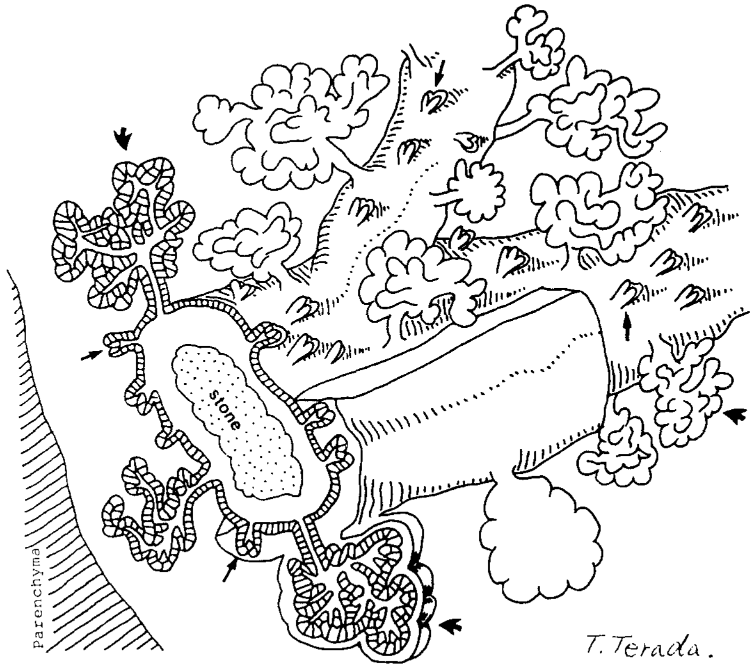


Fig. 6. Schematic stereogram of the bile ducts in hepatolithiasis. The bile duct is irregularly dilated with the lumen anchoring gall stones. The bile duct wall shows fibrous thickenning. Intramural glands (*straight arrows*) are tubular glands located within the ductal wall, whereas extramural glands (*curved arrows*) are tubuloalveolar glands situated in the periductal tissue. The extramural glands communicate with the bile duct lumen via conduits

section observations revealed that the intramural glands were tubular with a few branches (Fig. 4A) and these glands were confined within the bile duct walls. They communicated directly with ductal lumina and there were continuous transitions between glandular and surface epithelia lining bile ducts (Fig. 4A), though a majority of those lining ducts were denuded postsurgically or postmortem. In contrast, the extramural glands were characteristically ensheathed by a fibrous band, thus forming glandular lobules (Figs. 3, 5A). These glands consisted of mucous and serous acini which were intermingled in various proportions in each glandular lobule. Serial section observations further disclosed that the extramural glands were branched tubuloalveolar in type (Fig. 5A) and never communicated with the liver parenchyma. Rather, the glands were connected with biliary lumina via their own conduits which were lined by columnar epithelia and had their own thin fibrous walls (Fig. 5A). The schematic stereogram of bile ducts in hepatolithiasis, composed by reconstruction of serial sections, is shown in Fig. 6.

The main mucus histochemical results are shown in Table 1. In brief, the cytoplasm of intramural glands was positive for periodic acid-Schiff, alcian blue at pH 2.5 and high iron diamine (Fig. 4B, C), suggesting that the glands contained neutral, carboxylated and sulfated mucus glycoproteins. Some of the glandular acini were stainable only with periodic acid-Schiff, implying that such glandular elements were pyloric gland metaplasia. Almost all glands were positive for mucus, though the amount was small in some glandular epithelial cells. In contrast, mucus histochemistry clearly showed that the extramural glands were seromucous, consisting of both mucous and serous cells. The serous cells were more abundant than the mucous. The mucous cells contained both neutral, carboxylated and sulfated mucus glycoproteins (Fig. 5B, C) as was found intramural glands. Although mucous cells stainable with only one single stain were present in small amounts; the majority of the mucous cells of both glands were stained positively on serial sections with double stains as well as single stains, suggesting that neutral, carboxylated and sulfated mucins were present in a single mucous cell of both glands. The mucus of intramural and extramural glands was negative for Concanavalin A affinity. The mucus, with histochemical characters identical to intra- and extramural glands, was incorporated within the brown pigment stones and within the biliary sludge (Fig. 7A, B).

Discussion

Intrahepatic bile ducts bearing calculi showed numerous glandular elements within as well as around the ductal walls. Their morphology and histochemical characters have been examined in this study. These glands were divided into intramural and extramural according to their location. Glandular elements are known to be present normally around the intrahepatic large bile ducts (Terada et al. 1987), though their normal number is

Table 1. Reaction patterns of bile duct epithelia and intraductal contents to several staining methods for mucosubstances in the liver with hepatolithiasis

Materials examined	Staining reactions to:							
	AB2.5/PAS	HID/AB2.5	AB2.5	AB1.0	HID	Col.ir.	d-PAS	ConA
Mucous cells of intramural glands	Bl, + + +, (W) M, + + +, (W)	Br, + + +, (W) Bl, + + +, (W)	Bl, + + +, (W)	Bl, + + +, (W)	Br, + + +, (W)	Bl, + + +, (W)	M, + + +, (W)	Br, ±, (L)
Mucous cells of extramural glands	Bl, + + +, (S) M, + +, (S)	Br, + + +, (S) Bl, + +, (S)	Bl, + + +, (S)	Bl, + +, (S)	Br, + +, (S)	Bl, + + +, (S)	M, + +, (S)	Br, ±, (L)
Serous cells of extramural glands	Bl, ± (L) M, ±, (L)	Br, ±, (L) Bl, ±, (L)	Bl, ±, (L)	Bl, ±, (L)	Br, ±, (L)	Bl, ±, (L)	M, ±, (L)	Br, ±, (L)
Epithelia lining bile ducts	Bl, +, (L) M, +, (L)	Br, +, (L) Bl, +, (L)	Bl, +, (L)	Bl, +, (L)	Br, +, (L)	Bl, +, (L)	M, +, (L)	Br, ±, (L)
Pigment stones & biliary sludge	Bl, + + M, + +	Br, + Bl, + +	Bl, +	Bl, +	Br, +	Bl, +	M, +	Br, ±

Abbreviations and codes are as follows: AB2.5/PAS, combined Alcian blue at pH 2.5 and PAS; HID/AB2.5, combined high iron diamine and Alcian blue at pH 2.5; AB2.5, Alcian blue at pH 2.5; AB1.0, Alcian blue at pH 1.0; HID, high iron diamine; Col.ir., colloidal iron; d-PAS, periodic acid-Schiff after diastase digestion; ConA, Concanavalin A followed by horseradish peroxydase and diaminobenzidine; Bl, blue; Br, brown; M, magenta; + + +, strongly positive; + +, moderately positive; +, weakly positive; ±, little or none; (W), whole cytoplasm; (S), supranuclear cytoplasm, (L), luminal border

small. The extramural tubuloalveolar glands in hepatolithiasis are very similar in morphology and location to the intrahepatic peribiliary glands in normal livers; the glands in normal livers and hepatolithiasis were branched tubuloalveolar glands composed of seromucous acini and located in peribiliary tissue outside ductal walls (Terada et al. 1987). The finding that stones were found in the biliary tree, hepatic, segment and/or area ducts, where peribiliary glands are present in physiological condition (Terada et al. 1987), supports the above-mentioned suggestion, and further implies that calculus formation is related to the presence of the peribiliary glands. From these morphological and mucus histochemical observations, the extramural glands in hepatolithiasis seem likely to result from a proliferation (hyperplasia) of the preexisting peribiliary glands. Similar phenomena have been observed in the bronchial glandular system which proliferates under inflammatory conditions such as chronic bronchitis (Reid 1963) as well as in choledocholithiasis where peribiliary glands around the common bile duct are more abundant when compared with controls (Schein et al. 1979).

Tubular mucous glands were also seen to be abundant within the affected bile duct walls in hepatolithiasis, but such glandular elements were very few within the intrahepatic bile ducts walls in normal livers (Terada et al. 1987). Intramural glands in hepatolithiasis were confined within the bile duct walls and their glandular epithelium was continuous with the epithelium lining bile ducts, when present. Therefore, it is likely that the intramural glands might have resulted from invagination and subsequent proliferation of the covering epithelium lining bile ducts. A similar phenomenon has also been described in the human gall bladder which lacks such glands in normal situations and show metaplastic glands in the presence of inflammation and calculi (Latio 1980). Biliary epithelia of the extrahepatic bile ducts and gall bladder have been shown to proliferate to give rise to glandular formations in their walls under experimental conditions (Hou 1961; Lee 1981). Therefore, biliary epithelia seem to have the potential of producing glandular structures in the presence of irritation or inflammation. A well-known example of this is "adenomatous hyperplasia" of intrahepatic bile ducts seen in bile ducts infested with *Clonorchis Sinensis* (Hou 1955), which seems very similar to the intramural glands in hepatolithiasis. Thus, it seems reasonable that the intramural tubular glands in hepatolithiasis arise from covering the epithelium lining bile ducts, followed by their proliferation and metaplasia.

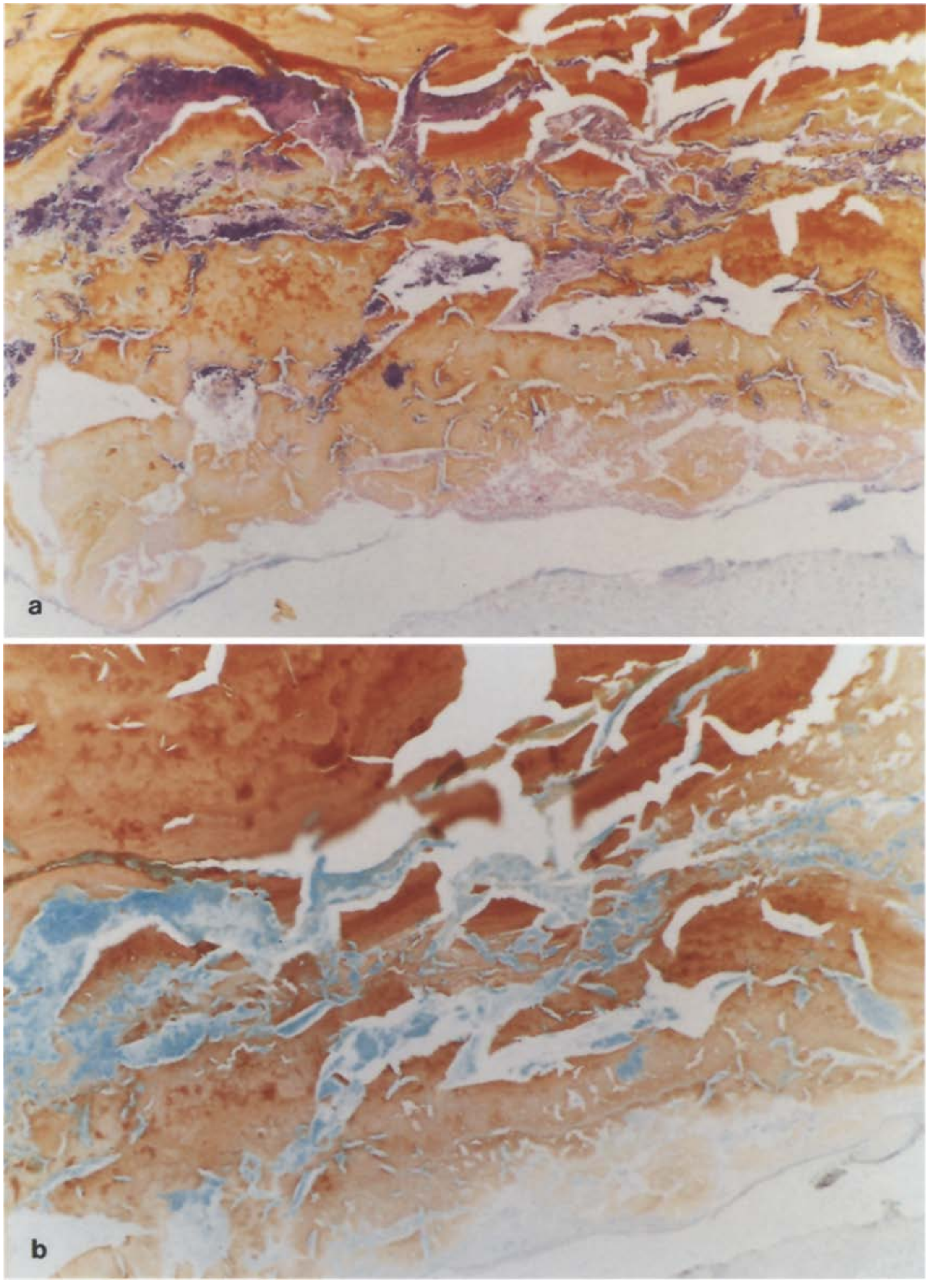


Fig. 7. a Brown pigment stones and biliary sludges in the bile duct. Both neutral and acidic mucus are seen incorporated within the stones. Alcian blue (pH 2.5) and periodic acid-Schiff (×100). **b** Brown pigment stones and biliary sludges in the bile duct sulfated mucus is found in the stones. Alcian blue (pH 1.0), (×100)

Finally, much mucus was shown in mucous cells of both glands as well as within the brown pigment stones and biliary sludge. A causative relationship between hypersecreted mucus and stone formation has been suggest from many points of view, irrespective of the chemical composition of the gall stones. Mucus has been shown to exist within gall stones by histochemistry (Wormack et al. 1974), chemistry (King 1959), and spectrophotometry (Sutor et al. 1974). In addition, bile with gall stones has been shown to contain more mucus glycoproteins than bile without stones (Bouchier et al. 1965; Lee et al. 1979). Thus, excessive mucus secretion from the intra- and extramural glands may lead to diminished solubility of both unconjugated bilirubin and calcium bilirubinate and trap calcium salts of bilirubin, phosphate and carbonate, initiating a nidus for gall stones.

References

Bouchier IAD, Cooperband SR, Elkodosi BM (1965) Mucous substance and viscosity of normal and pathologic human bile. Gastroenterology 49:343–353

Healey JE, Schroy PC (1953) Anatomy of the biliary ducts within the human liver. Arch Surg 66:599–616

Hou PC (1955) The pathology of clonorchis sinensis infestation of the liver. J Pathol 70:53–64

Hou CT (1961) Repair of the extrahepatic bile ducts after mechanical and chemical injury. J Pathol 82:83–94

Katsuyama T, Spicer SS (1978) Histochemical differentiation of complex carbohydrates with variants of Concanavation A-horseradish peroxidase method. J Histochem Cytochem 26:233–250

King JS, Boyce WH (1959) Analysis of renal calculous matrix compared with some other matrix materials and with uromucoid. Arch Biochem Biophys 82:455–461

Latio M (1980) Morphology and histochemistry of non-tumorous gall bladder epithelium. A series of 103 cases. Pathol Res Pract 167:335–345

Lee SP, Lim TH, Scott AJ (1979) Carbohydrate moietis of glycoproteins in human hepatic and gall-bladder bile, gall bladder mucosa and gall stones. Clin Sci 56:533–538

Lee SP (1981) Hypersecretion of mucus glycoprotein by the gall bladder epithelium in experimental cholelithiasis. J Pathol 134:199–207

Mowry RW (1963) The special value of methods that color both acidic and vicinal hydroxy groups in the histochemical study of mucins: with revised directions for the colloidal iron stain, the use of alcian blue 8GX and their combinations with the periodic acid-Schiff reaction. Ann NY Acad Sci 106:402–423

Nakanuma Y, Ohta G, Nagakawa T, Matsubara F (1981) Histological studies on the livers with intrahepatic gallstons. Jpn J Gastroenterol 78:874–882

Ohta G, Nakanuma Y, Terada T (1984) Pathology of hepatolithiasis: cholangitis and cholangiocarcinoma. In: Intrahepatic calculi, Alan R. Liss, Inc., New York, pp 91–113

Reid L (1963) An experimental study of hypersecretion of mucus in the bronchial tree. Br J Exp Pathol 44:437–445

Schein CJ, Mahadevia P (1979) Surgical significance of the histopathology of the common bile duct. Am J Surg 137:763–767

Spicer SS (1965) Diamine method for differentiating mucosubstances histochemically. J Histochem Cytochem 13:211–234

Sutor DJ, Wooley SE (1974) The organic matrix of gallstones. Gut 15:487–491

Terada T, Nakanuma Y, Ohta G (1987) Glandular elements around the intrahepatic bile ducts in man; their morphology and distribution in normal livers. Liver 7:1–8

Wormack NA, Zeppa R, Irvin GL (1963) The anatomy of gallstones. Ann Surg 157:670–686

Yamamoto K (1982) Intrahepatic periductal glands and their significance in primary intrahepatic lithiasis. Jpn J Surg 12:163–170

Accepted January 28, 1988