Ultrastructural localisation and size distribution of collagen fibrils in Glisson's sheath of rat liver: implications for mechanical environment and possible producing cells

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ABSTRACT

The ultrastructure and size distributions of collagen fibrils in Glisson's sheath were investigated in the rat liver to analyse the mechanical environment around the fibrils and their possible cells of origin. Glisson's sheath was found to contain 2 populations of collagen fibrils with different diameters and distinct localisations, namely fibroblast-associated and bile epithelium-associated. Fibroblast-associated collagen was composed of fibrils arranged in bundles and constituted the majority of the collagen in Glisson's sheath. Bile epithelium-associated collagen was represented by small dispersed groups of fibrils just beneath the basement membrane of the bile duct. The basement membrane of the bile duct was frequently reduplicated into a few or as many as 10 layers of laminae densae, with scattered collagen fibrils between these laminae. The diameters of the fibrils of both groups of collagen increased in relation to the calibre of the bile duct, whereas at any given place in Glisson's sheath bile epithelium-associated collagen fibrils had a smaller diameter compared with those of the fibroblast-associated fibrils. The increment in fibril diameter along the bile duct is considered to be correlated with the increase in mechanical stress acting on Glisson's sheath. The difference in diameter between the 2 populations as well as the incorporation of fibrils between the laminae densae of the basement membrane of the bile duct supports the view that the bile epithelium-associated collagen is produced by the epithelial cells of the bile duct, thus having a different origin from that of fibroblast-associated collagen. These findings provide the first evidence that the epithelial cells of the interlobular bile duct produce fibril-forming collagen. Furthermore, it is suggested that cholestasis stimulates the epithelial cells of interlobular bile duct to increased synthesis of fibril-forming collagen that is also produced by these cells under physiological conditions.

Key words: Interlobular bile ducts; morphometry; fibroblasts; basement membrane; fibrosis.

INTRODUCTION

The hepatic artery, portal vein and bile duct enter the liver at the hepatic hilum and divide into multiple branches that are surrounded by the distinctive connective tissue of Glisson's sheath or the portal tract along the periphery of hepatic lobules. Even in normal conditions the liver contains considerable quantities of collagen fibrils mainly in Glisson's sheath and in the liver capsule (Ohtani, 1988). These collagen fibrils are thought to help maintain the structural and functional integrity of the liver against frictional stresses caused by movement of the neighbouring abdominal wall and organs (Watanabe & Nishizono, 1994). An over-deposition of collagen in the liver constitutes hepatic fibrosis and is associated with diminished liver function as observed in hepatic cirrhosis (Kawasaki & Ogata, 1992).

The cellular origin of hepatic collagen fibrils has been studied under normal conditions and in fibrosis

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of the human liver (Clement et al. 1986; Gressner, 1991; Friedman, 1993) as well as in experimental models of fibrosis (Tuchweber et al. 1996; Desmouliere et al. 1997). Cultured hepatocytes and stellate cells have also been reported to synthesise various types of collagen found in vivo (Guzelian et al. 1981; Diegelmann et al. 1983; Kawase et al. 1986). Following the ligation of the rat common bile duct, the quantity of collagen fibrils in Glisson's sheath increases considerably and the epithelial cells proliferate markedly and transiently (Brooks et al. 1975). Available data in the literature point to the fact that the bile duct epithelial cells as well as fibroblasts in Glisson's sheath contribute to the synthesis of collagen, at least in fibrotic conditions such as after bile duct occlusion (Abdel Aziz et al. 1991).

It has been consistently reported that the diameter of collagen fibrils in the tissue shows considerable variation. Parry et al. (1978) measured the diameter of fibrils in various tissues and species, and found that their diameter correlated with the tensile strength of the tissue. Thereafter, detailed studies on the variations of diameter of fibrils have been performed in various tissues including the arterial wall (Buck, 1987), the dental alveoli (Ottani et al. 1998) and the tendon of the superficial digital flexor muscle (Patterson Kane et al. 1997*a*, *b*). However, the diameter of collagen fibrils has not been studied in the liver as far as we are aware. Recently Keene et al. (1995) reported that type XI collagen is restricted to thin fibrils, suggesting that a correlation exists between the diameter of the fibrils and the biochemical components of collagen.

In the present study, we investigated the ultrastructural distribution and diameter of collagen fibrils in Glisson's sheath in the rat liver. We report 2 distinct populations of fibrils in this sheath for the first time and this development, along with the available ultrastructural findings, raises the possibility that these 2 populations are produced by 2 different cell types in the sheath.

MATERIALS AND METHODS

In this study, 12 male Sprague–Dawley rats weighing 280 g (Charles River, Japan) were used. The liver was perfused with fixative containing 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, at 4 °C via a canula inserted into the abdominal aorta for $\sim$ 5 min. After fixation, the medial part of the left lobe was dissected out and immersed in 2.5% glutaraldehyde in the same buffer overnight.

The specimens were cut into small pieces of 250 μm thickness by a vibratory microtome (DTK-1000, Dosaka EM, Osaka, Japan) and processed further by the cold dehydration technique described elsewhere (Sakai & Kriz, 1987). Briefly, the specimens were immersed in 1.2% extracts of oolong tea (OTE, Suntory Co., Tokyo, Japan) in acetone buffer solution (0.05 M maleate buffer pH 6.0 containing 10% acetone), followed by 1% uranyl acetate in the same acetone buffer solution and then dehydrated with a graded series of acetone at 0 to -30 °C before embedding in Epon 812. Semithin sections were obtained with a diamond knife, stained with toluidine blue, and observed under a Nikon Optiphoto microscope. Ultrathin sections were cut with a diamond knife, doubly contrasted with uranyl acetate and lead citrate, and observed in a Hitachi H7100 electron microscope.

The diameter of bile ducts was measured on electron micrographs at a magnification of $\times$300–2000. The diameter of collagen fibrils was measured at a magnification of $\times$30000 on electron micrographs that included cross sectional profiles of bile ducts using a scale magnifying glass with a magnification of $\times$7. Measurements were obtained for 3 groups of cross sectional profiles of collagen fibrils of each population within a photographic area of 3 cm^2 in each section of Glisson's sheath. The magnification of electron micrographs was calibrated with a cross grating replica (Nisshin EM, Tokyo). The D period on the axial profile of collagen fibrils was measured to be 60 nm on the electron micrographs, suggesting about 10% of length shrinkage of the tissue.

For immunocytochemistry, rats were perfused via the left ventricle with a periodate-lysine-paraformaldehyde fixative (McLean & Nakane, 1974) at room temperature, and small tissue pieces were removed. The tissues were immersed in the same fixative for 1 h. After fixation, the tissue samples were rinsed successively in a phosphate-buffered saline (PBS) solution containing 10% sucrose. Cryosections (thickness 2–4 μm) were cut using a Jung Frigocut 2800E (Leica, Nussloch, Germany), mounted on silanised glass slides (DAKO, Carpinteria, CA, USA) and rinsed 3 times in PBS (10 min each). The sections were processed for staining collagen types I and III and microfilaments. For staining collagens, the sections were incubated overnight at 4 °C with rabbit antirat collagen type I or rabbit antirat collagen type III (1:50 dilution, Chemicon, Temecula, CA, USA) and then incubated with fluorescein isothiocyanate

(FITC)-labelled swine antirabbit IgG (diluted 1:100, DAKO, Carpinteria, CA, USA) for 1 h at room temperature. In control experiments, incubation with the primary antibody was omitted. For staining of microfilaments, sections labelled with anticollagen antibodies and FITC-conjugated second antibodies were then incubated with rhodamine-phalloidin (diluted 1:200, Molecular Probes, Eugene, OR, USA) for 1 h at room temperature and washed for 30 min in PBS. All sections were examined using an Axioplan microscope (Zeiss, Oberkochen, Germany) and analysed using a CSU10 laser scanning confocal imaging system (Yokogawa, Tokyo, Japan).

RESULTS

Ultrastructure of Glisson's sheath

The interlobular bile ducts were lined by simple cuboidal or columnar epithelial cells (Figs 1, 2). The bile duct cells possessed scattered microvilli of irregular shape on their apical surface and numerous folds on their basolateral surface which occupied the intercellular spaces. The basement membrane, which separates the epithelial cells and the intercellular spaces from the interstitium, occasionally became multilayered with up to 10 layers of laminae densae and up to 5 μm in total thickness. The cytoplasm of duct epithelial cells contained well-developed rough endoplasmic reticulum and scattered mitochondria in addition to prominent Golgi apparatus located on the apical to lateral side of the nucleus.

The interlobular arteries and portal veins could easily be distinguished from each other on the basis of the smaller lumen of the former vessels and conspicuous structural differences in their walls (Figs 1, 2). The medial smooth muscle cells were arranged circularly in the arteries, whereas those in the portal veins were composed of an occasional inner longitudinal layer and a consistent outer circular layer. The elastic fibres in the arterial wall formed a conspicuous inner elastic lamina. In the portal veins, scattered elastic fibres were found mainly between the 2 layers of smooth muscle cells and contained a small mass of elastin together with an abundance of microfibrils. The endothelial cells were thicker in the arteries than in the portal veins.

The fibrous connective tissue was composed of fibroblasts and collagen fibrils, and contained a few lymphatics and nerve fibres (Figs 1, 2). The lymphatic vessels were represented by a thin layer of endothelial cells with occasional basal protrusions, which were connected to the surrounding structures by anchoring filaments.

Collagen fibrils and fibroblasts in Glisson's sheath

Most of the collagen fibrils in Glisson's sheath were arranged in bundles and were associated with fibroblasts (Figs 2, 3). The sheet-like processes of fibroblasts frequently formed compartments that were occupied by bundles of collagen and clear intercellular spaces. The bundles contained on average about 300 collagen fibrils. The fibroblast-associated collagen fibrils were connected to each other by fine filaments that were seen as a fuzzy decoration around the fibrils in their cross-sectional profiles. In longitudinal sections, the fibrils exhibited a regular banding pattern of 60 nm periodicity, typical for interstitial collagen composed of types I and III collagen.

The fibroblasts in Glisson's sheath had an irregular shape, sending out numerous sheet-like processes into the surrounding interstitium. The cell body contained moderate amounts of conspicuously dilated cisternae of rough-surfaced endoplasmic reticulum as well as a conspicuous Golgi apparatus. Small sized mitochondria and lysosomes were occasionally encountered.

In addition to fibroblast-associated collagen, Glisson's sheath contained another population of collagen fibrils, located just beneath the basement membrane of the bile duct epithelium (Figs 2, 3). Bile epithelium-associated collagen fibrils did not form discrete bundles as did those of fibroblast-associated collagen but were dispersed in small groups along the interstitial side of the basement membrane. They exhibited a similar banding pattern of ~ 60 nm periodicity as that of the fibroblast-associated collagen in longitudinal sections, but their diameter was conspicuously smaller. Interconnections between individual fibrils were occasionally observed where the fibrils came in close contact.

The basement membrane of bile duct epithelium had a conspicuous morphological peculiarity in addition to the above-mentioned association with collagen fibrils. It was frequently reduplicated into up to 10 layers of laminae densae (Fig. 4). The individual laminae in the laminated portions were interwoven with each other to enclose small clear domains in which collagen fibrils were occasionally encountered. In cross-sectional profiles of bile ducts, the laminated portions occupied about 20–30% of the total circumference of the basement membrane, and showed no particular distribution in the perimeter of the ducts.

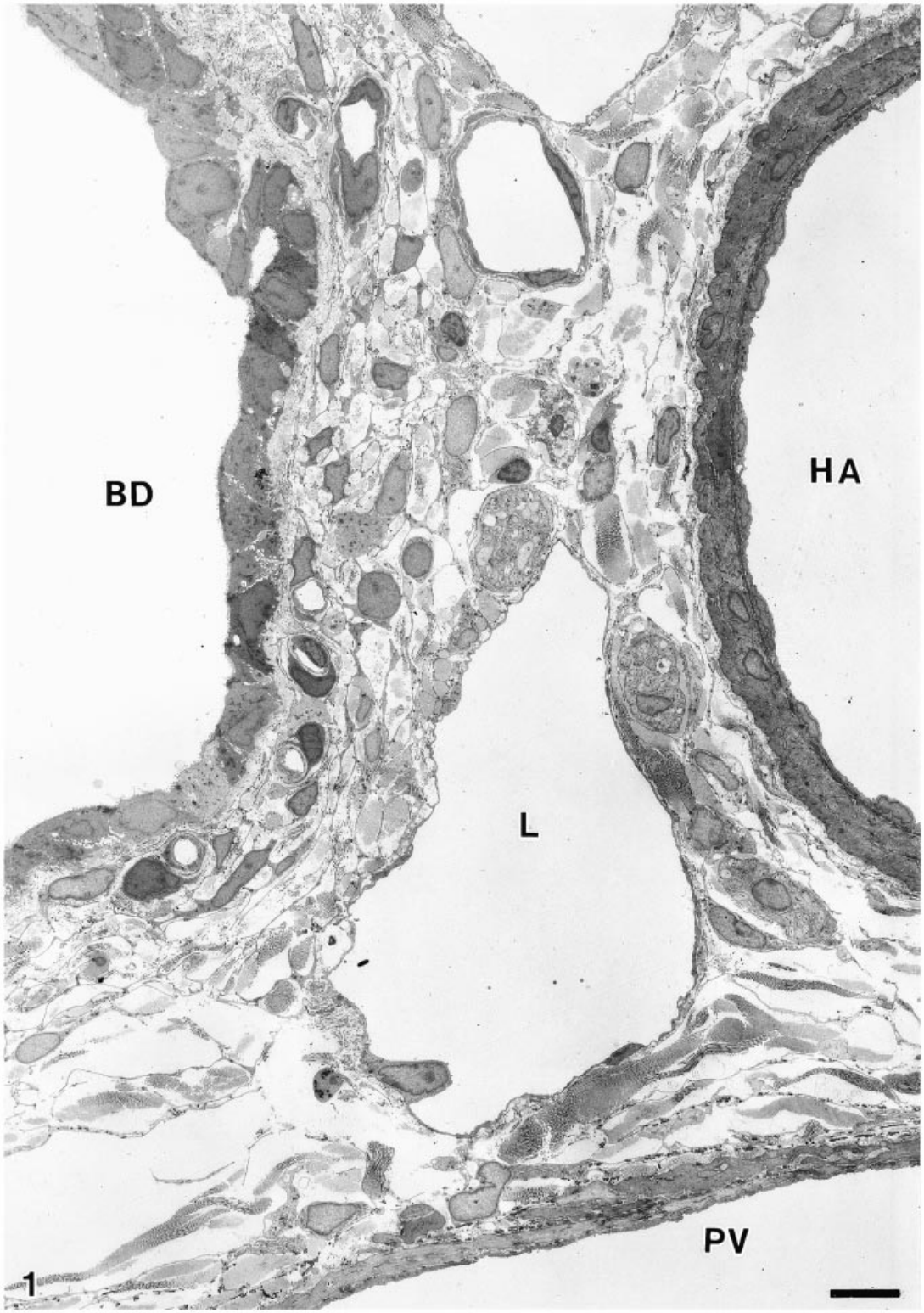

Fig. 1. Low power electron micrograph of Glisson's sheath in rat liver. Three conspicuous ductal structures are recognisable: the hepatic arteriole (HA), interlobular bile duct (BD), and interlobular portal vein (PV). Lymphatic ducts (L) are conspicuously thin walled and have a much smaller calibre than the 3 ductal structures. The blood capillaries in the Glisson's sheath represent the peribiliary plexus surrounding the bile duct. Bar, 10 μm.

Morphometry of collagen fibrils in Glisson's sheath

The diameters of collagen fibrils of the populations were measured in 12 sections of Glisson's sheath containing cross or slightly oblique sectional profiles of bile ducts to evaluate differences of their 2 collagen populations. We found 2 empirical rules which govern the size distribution of the fibrils (Figs 5, 6). First, in

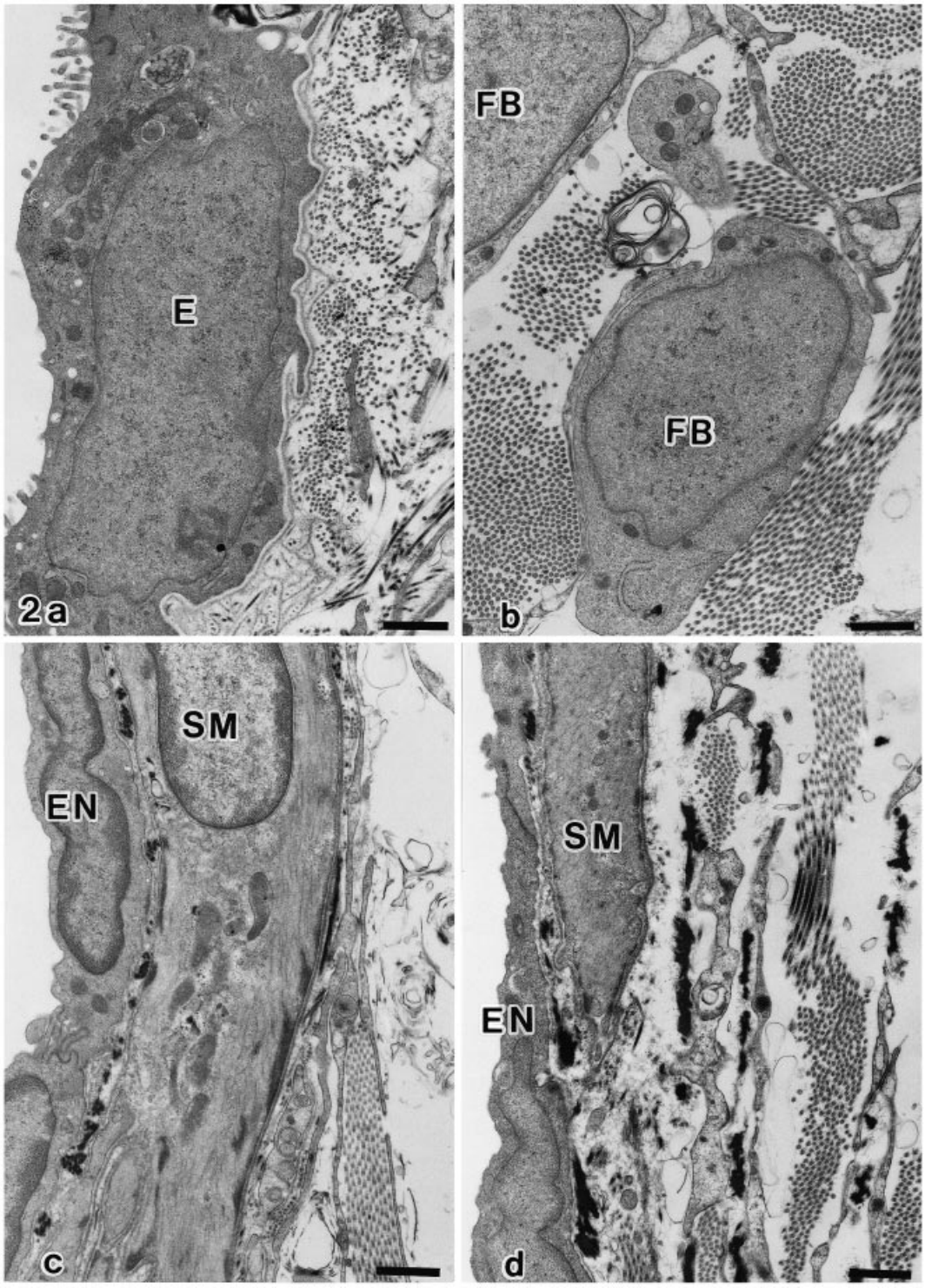

Fig. 2. High power electron micrographs of different structures of Glisson's sheath in the rat liver. (*a*) Bile duct cells showing scattered microvilli of irregular shape, on their apical surface and numerous folds on their basolateral surface which occupy the intercellular space bordered by epithelial cells (E) and basement membrane. (*b*) Fibrous connective tissue composed of fibroblasts (FB) and bundles of collagen fibrils surrounded by sheet-like processes of fibroblasts. (*c*) Endothelium (EN) of a hepatic arteriole showing the usual pinocytotic vesicles and tight junction between cells. The medial smooth muscle cells (SM) are arranged circularly. Elastic fibres make up a nearly complete inner elastic lamina. (*d*) Interlobular vein containing an inner longitudinal layer and an outer circular layer of smooth muscle cells. Elastic fibres are composed of small masses of elastin. Note that the hepatic artery and portal vein are not associated with collagen on their interstitial surfaces as occurs in the bile duct. Bars (*a–d*), 1 μm.

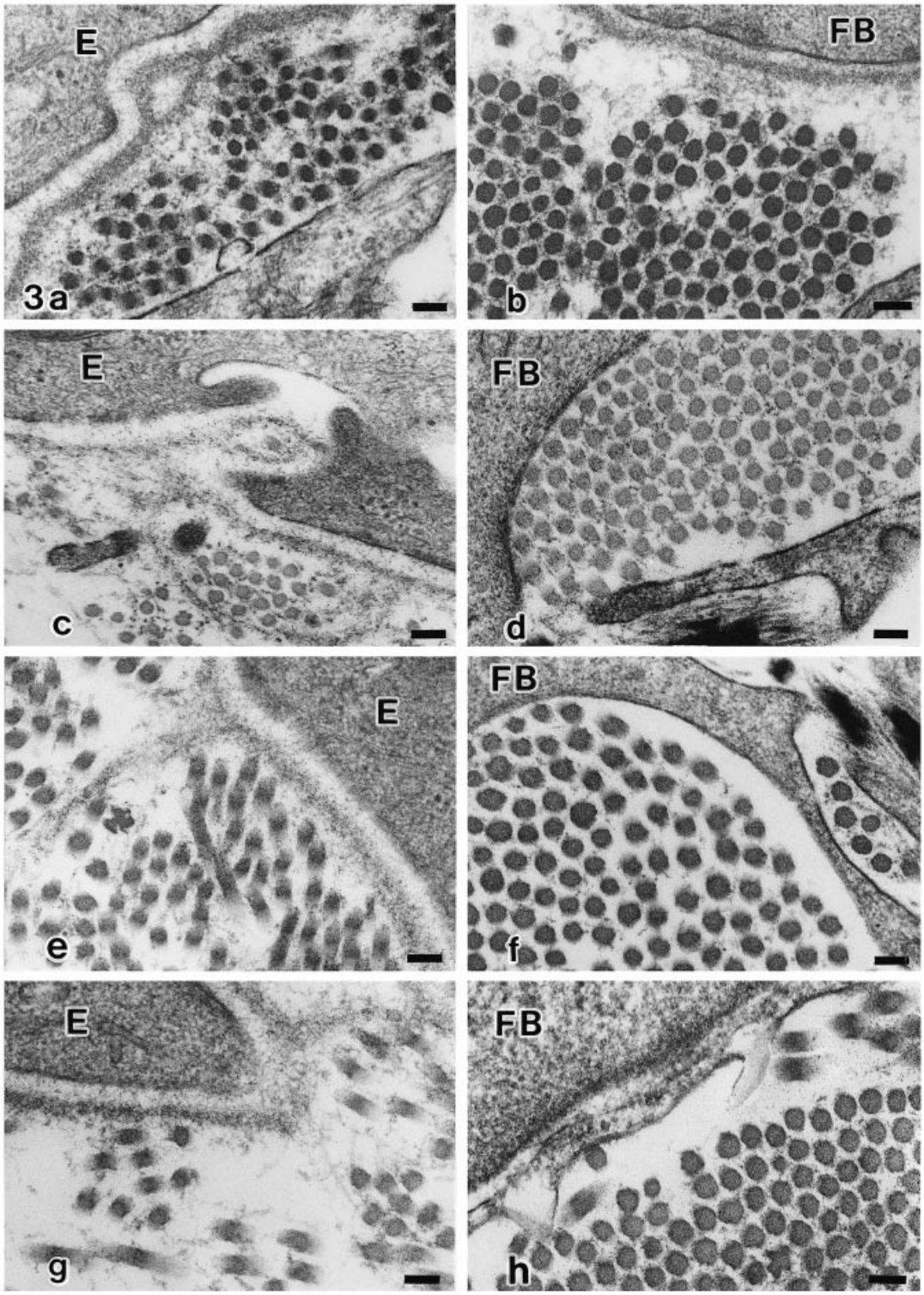

Fig. 3. High power electron micrographs of 2 populations of collagen fibrils from various sections of Glisson's sheath in the rat liver. The left column (*a*, *c*, *e*, *g*) shows bile epithelium-associated collagen fibrils and the right column (*b*, *d*, *f*, *h*) fibroblast-associated fibrils. The calibre of the bile ducts was 12.8 μm in the first row (*a*, *b*), 25.9 μm in the second row (*c*, *d*), 64.8 μm (*e*, *f*) in the third row, and 74.1 μm in the fourth row (*g*, *h*). Note that the diameter of the fibrils is smaller in the bile epithelium-associated collagen (left column) than in the fibroblast-associated (right column), and in proportion to the calibre of bile duct (from above to below). Bars (*a–h*), 0.1 μm.

any given section of Glisson's sheath the diameter of unit fibrils was significantly smaller in the bile duct-associated collagen than in the fibroblast-associated collagen. Second, in either population, the diameter of the fibrils increased as the size of the bile duct in the section became larger, namely, the closer was the

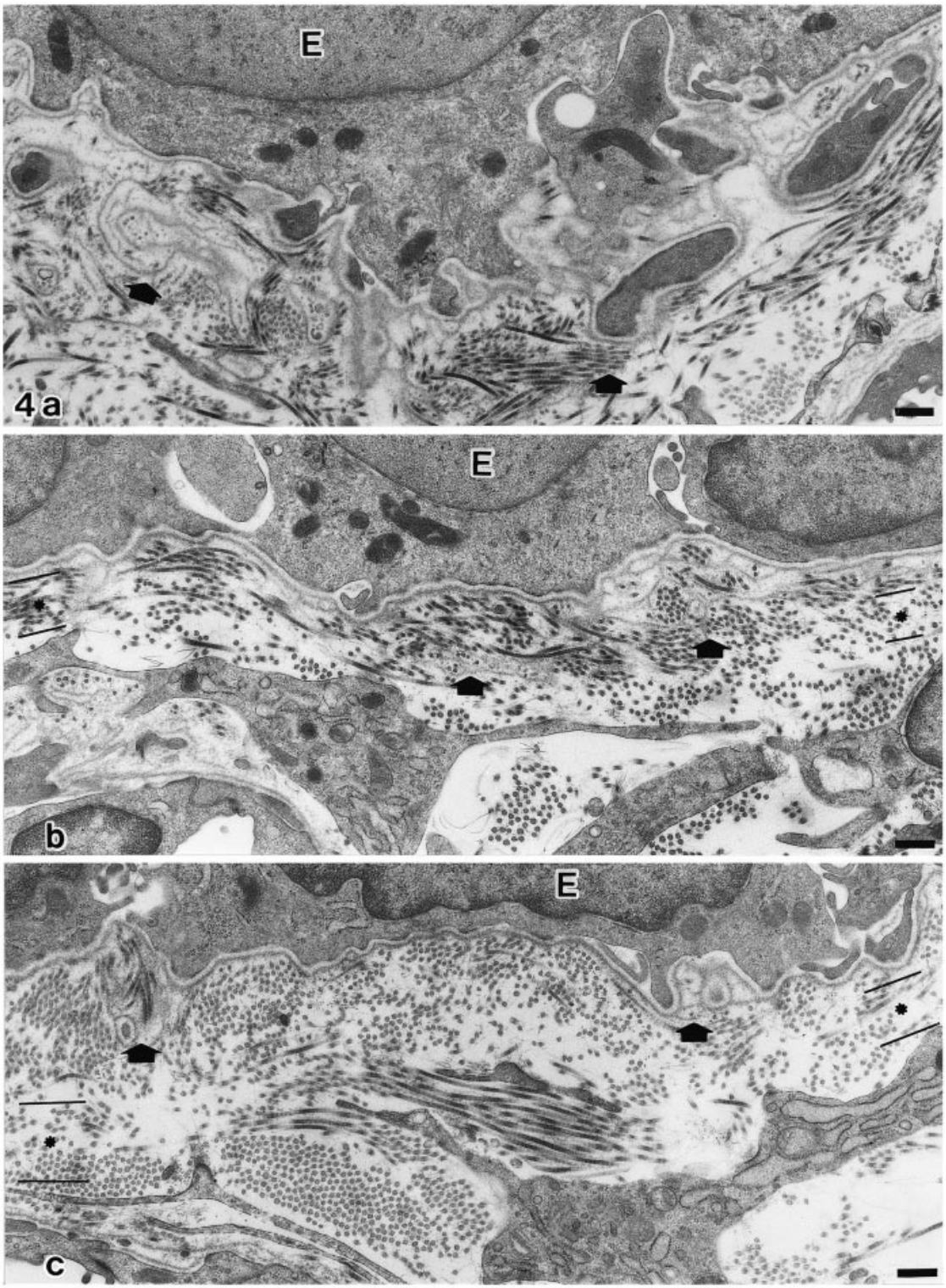

Fig. 4. Electron micrographs showing the basement membrane of bile duct and their associated collagen fibrils in Glisson's sheath of rat liver. The basement membrane is frequently reduplicated being composed of several layers of laminae densae and containing occasional collagen fibrils between the laminae (arrows). The bile epithelium-associated collagen is spatially separated from the interstitial fibroblasts and their associated collagen fibrils by a loose interstitial space containing relatively few collagen fibrils (asterisks). Bars, 0.5 μm.

section to the hilus of the liver. Thus the diameters of bile epithelium-associated collagen exhibit discrete differences in any given part of Glisson's sheath, although the ranges of size distribution of the 2 populations overlapped considerably as a whole, fibroblast-associated collagen ranging between 37 and 93 nm and bile duct-associated collagen between 30 and 80 nm.

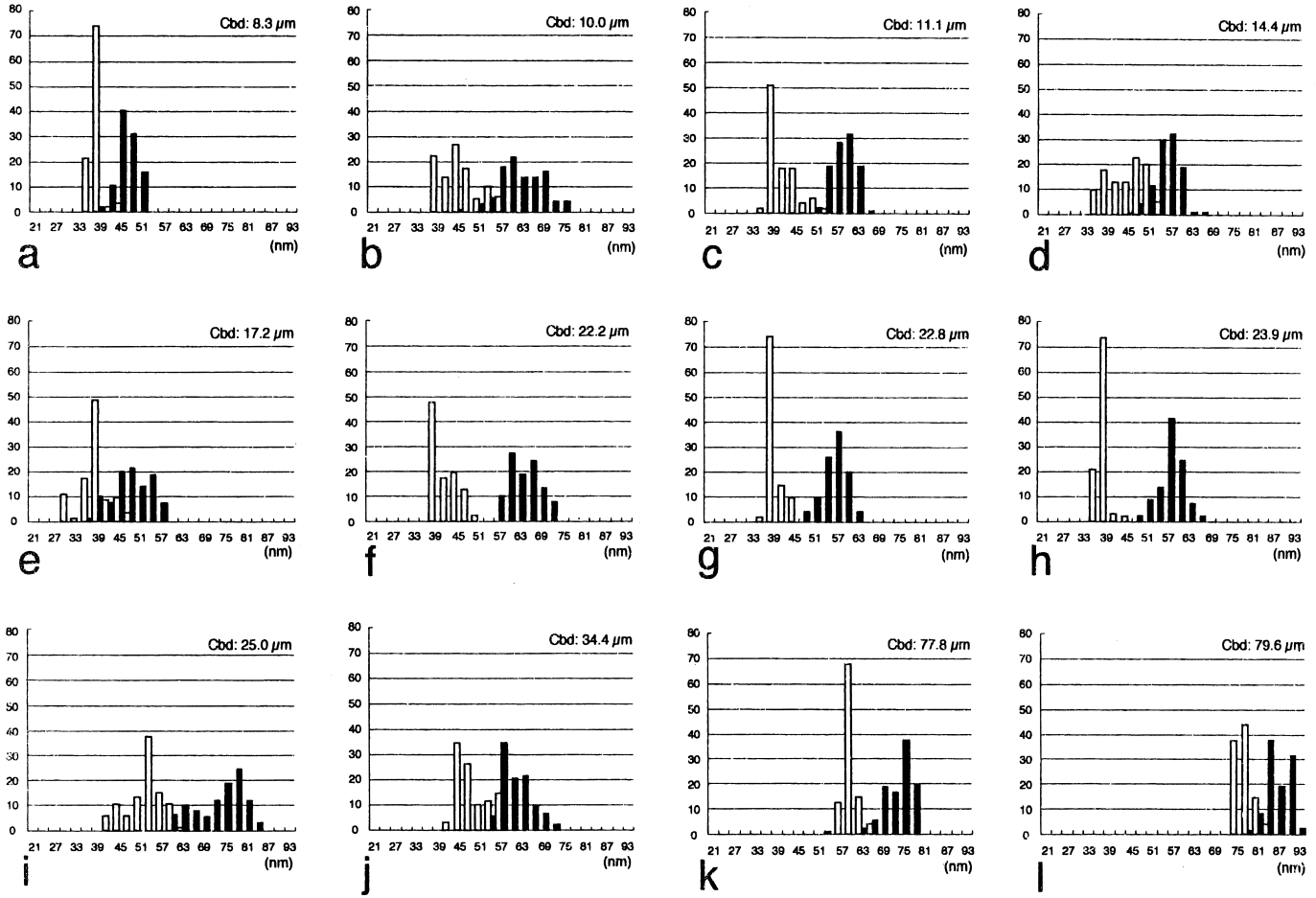

Fig. 5. Histograms showing diameter distribution of 2 populations of collagen fibrils in various sections of Glisson's sheath of the rat liver. The white and dark columns represent the diameter distribution of fibrils from the bile epithelium-associated and fibroblast-associated collagen, respectively. The histograms are arranged in ascending order of calibre of the bile duct (Cbd) shown in the upper right corner. Note that the diameter of the fibrils is smaller in the bile epithelium-associated collagen (white columns) than in the fibroblast-associated collagen (dark columns), and in proportion to the caliber of bile duct (from *a* to *l*).

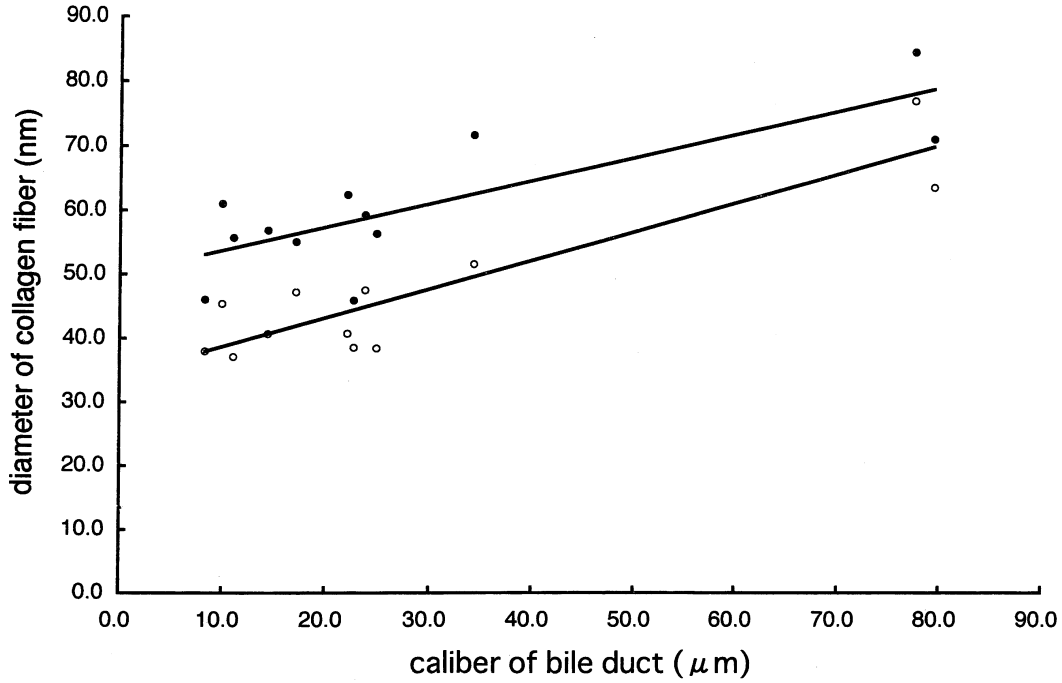

Fig. 6. Relationship between the calibre of bile duct and the diameter of collagen fibrils of 2 populations in various sections of Glisson's sheath in the rat liver. The diameter of the fibrils is larger in the fibroblast-associated collagen (filled symbols) than in the bile epithelium-associated collagen (open symbols). In both populations the diameter of collagen is related to the calibre of bile duct. The regression line for the bile epithelium-associated collagen is represented by the equation $Y = 0.357X + 50.1$ ($r = 0.640$) and that for the fibroblast-associated collagen, $Y = 0.445X + 34.3$ ($r = 0.820$).

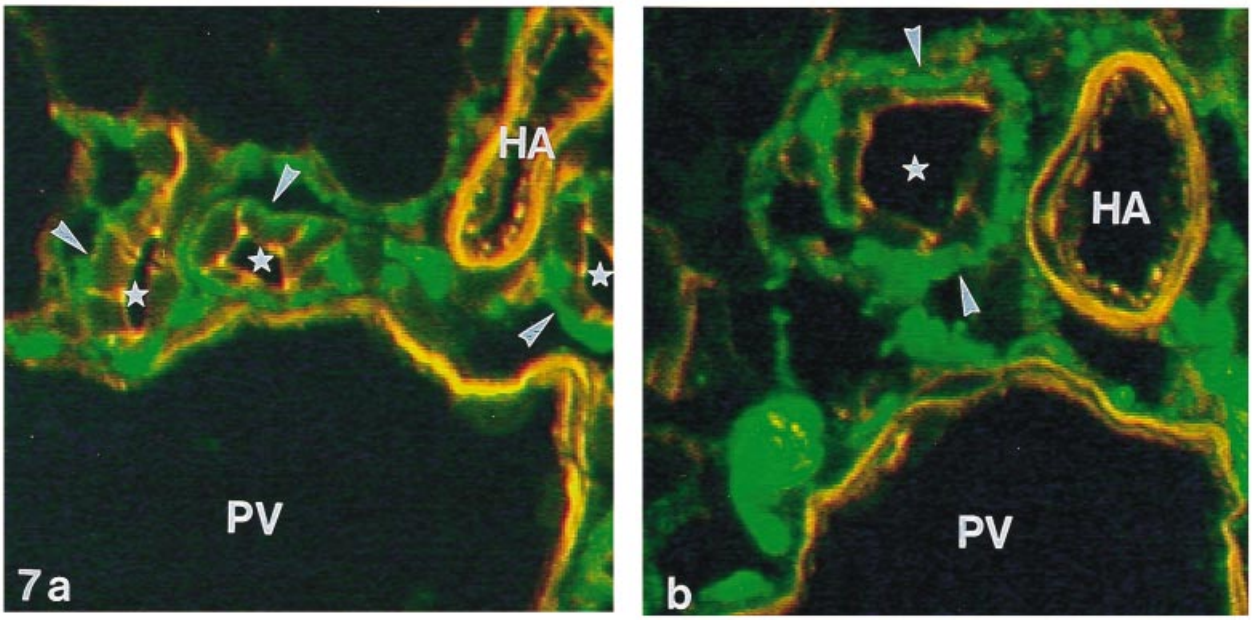

Fig. 7. Double labelling of rat liver using antibodies against rat collagen (type I or type III) and rhodamine-phalloidin. The similar labelling pattern between collagen type I (*a*) and type III (*b*) is observed in Glisson's sheath of the normal rat liver. Note the intense signals for fibrous collagens (arrowheads) beneath the epithelial cells of interlobular bile duct (asterisks). PV, portal vein; HA, hepatic artery. ×1500.

Immunolocalisation of collagen type I and type III in rat liver Glisson's sheath

To identify the type of fibrous collagens observed by electron microscopy, we localised the type-specific antibodies to rat collagen types I and III in Glisson's sheath. To characterise the artery, vein and bile duct, we stained microfilaments with rhodamine-phalloidin (Fig. 7). Intense labelling by rhodamine-phalloidin was observed in the cytoplasm of vascular smooth

muscle cells. The interlobular arteries were distinguished from the interlobular veins by their wider lumina and thicker smooth muscle layers. Microfilaments of the bile duct epithelial cells exhibited an apical and intercellular distribution showing an asymmetric pattern. Intense rhodamine-phalloidin labelling was observed beneath the apical plasma membrane of epithelial cells similar to that of microfilaments in other exocrine cells (Kurihara & Uchida, 1987). Immunofluorescence for collagen types I or III showed that both were distributed similarly in the rat liver Glisson's sheath. A periductal expression of types I and type III fibrous collagen was observed in normal rat liver. Bile duct epithelial cells were continuously surrounded by a moderately thick layer of both collagen types. On the other hand, spot-like labelling with anti-collagen type I or type III was observed around the interlobular veins and arteries. Labelling intensity along the artery was less than that along the vein.

DISCUSSION

Distribution of collagen fibrils in the liver

Under normal conditions the liver contains considerable quantities of collagen fibrils mainly in Glisson's sheath and in the liver capsule, and fibrils are also found in isolation or in bundles in the space of Disse to form sheaths for housing the hepatic sinusoids (Ohtani, 1988). The quantity of collagen fibrils in the normal liver is modest (12.4 mg and 1.3 mg of collagen fibrils/g of wet tissue weight in the human and rat liver, respectively; Ohtani, 1988), whereas it increases conspicuously in various types of hepatic fibrosis after injury to hepatic tissue. There are 2 main models of hepatic fibrosis, namely parenchymal fibrosis after damage to hepatocytes and periportal fibrosis in Glisson's sheath following ligation of the bile duct.

Early studies on the site of production of collagen in the liver showed that hepatocytes, stellate cells, and endothelial cells are engaged in production of extracellular matrix components in normal human liver, and that in fibrosis hepatocytes are transformed to synthesise types III and IV collagen (Clement et al. 1986). On the other hand, studies on the localisation of mRNA of procollagen types I, III and IV using in situ hybridisation revealed that in the liver types I, III and IV procollagen expression takes place predominantly in nonparenchymal cells in normal and CCl_4-induced fibrotic rat liver (Milani et al. 1989).

Cellular sources of matrix proteins in an experimental model of cholestatic fibrosis were investigated by Abdel-Aziz et al. (1991) who demonstrated that several cell types, including bile duct cells and stellate cells with the possible involvement of hepatocytes, participate in the formation of extracellular matrix components which constitute periportal fibrosis in Glisson's sheath. Recently Desmouliere et al. (1997) studied the process of fibrotic change after ligation of the bile duct in detail, and reported that extracellular matrix deposition occurs very early in Glisson's sheath surrounding proliferating ductules, and precede myofibroblastic differentiation.

In the present study we investigated the ultrastructural distribution of collagen fibrils in Glisson's sheath in normal rat liver and demonstrated that there are 2 distinct populations of fibrils, one associated with interlobular bile ducts and the other associated with fibroblasts. Morphometric analysis revealed that the diameters of the 2 populations were significantly different in any given section of Glisson's sheath, although their diameter varied in relation to the calibre of the bile duct. These 2 populations differ distinctly in the diameter and in the size of bundles, and were frequently separated from each other by fluid filled spaces.

Interestingly, the collagen fibrils associated with the interlobular bile duct had a close relation to the basement membrane of the bile duct. The basement membrane is sometimes divided into several laminae densae, and the laminated portion of the basement membrane frequently includes solitary fibrils in the space between the laminae densae. It has been repeatedly reported that the epithelial cells of the interlobular bile duct produce type IV collagen constituting the basement membrane (Martinez Hernandez, 1984). Thus it is reasonable to assume that the collagen of fibrils associated with the bile duct together with that constituting the basement membrane are synthesised and secreted by the epithelial cells of the interlobular bile duct. We showed with immunohistochemistry that both the bile duct-associated and fibroblast-associated collagen contains types I and III collagen.

The production of fibril forming collagen by epithelial cells has also been reported for other types of epithelium. Corneal epithelium synthesises striated types I and III collagen (Trelstad et al. 1974) and secretes them across the basal lamina. In the egg-shell membrane of the hen collagens similar to types I and V are synthesised by the epithelial cells of part of the oviduct (Wong et al. 1984). However, Perche et al. (1990) demonstrated with detailed experiments including in situ hybridisation that the type I collagen

Table. *Diameter of collagen fibrils in various tissues and animals reported in the literature*

Collagen diameter	Source	Species	Literature
Cartilage			
9–19 nm	Proximal tibial growth plate	Chicken	Howlett CR (1979)
20 nm	Pericellular ring of auricular cartilage	Rat	Kostovic Knezevic et al. (1981)
15–40 nm	Cartilage	Sea lamprey	Wright & Youson (1983)
50–200 nm	Articular cartilage of femoral condyles	Baboons	Pidd & Gardner (1987)
30–50 nm	F1 layers of loosely fibrous zone of articular cartilage of mandibular condyle	Rat	Mizuno et al. (1990)
40–100 nm	F2 layers of densely fibrous zone of articular cartilage of mandibular condyle	Rat	Mizuno et al. (1990)
97.6 ± 18.9 nm	Peripheral zones of temporomandibular joint discs	Bovine	Kuc & Scott (1994)
72.4 ± 14.3 nm	Centre of temporomandibular joint discs	Bovine	Kuc & Scott (1994)
Subepithelial connective tissue			
67–91 nm	Skin of the right shoulder	Human	Hino et al. (1982)
40 nm	Vocal fold mucosa	Human	Sato (1998)
50.8–70.1 nm	Free buccal gingiva	Rhesus monkey	Ottani et al. (1998)
50.3–71.8 nm	Attached buccal gingiva	Rhesus monkey	Ottani et al. (1998)
38.5–42.1 nm	Hard palate rugae	Rhesus monkey	Ottani et al. (1998)
39.0–43.5 nm	Hard palate interrugal	Rhesus monkey	Ottani et al. (1998)
45.3–53.5 nm	Free portion of hard palate mucosa	Rhesus monkey	Ottani et al. (1998)
Eye			
75–90 nm	Cornea (anterior 1/4 of stroma)	Human	Waring & Rodrigues (1980)
24–180 nm	Vitreous body	Rabbit	Pac et al. (1989)
40.6 ± 5.0 nm	Cornea	Rabbit	Yamabayashi et al. (1991)
217.3 ± 50 nm	Sclera	Rabbit	Yamabayashi et al. (1991)
101 ± 5 nm	Tenon's capsule, infantile esotropic group	Human	Shauly et al. (1992)
86 ± 5 nm	Tenon's capsule, nonstrabismic group	Human	Shauly et al. (1992)
24–43 nm	Cornea	Various	Meek & Leonard (1993)
118.3–1268.0 nm	Sclera	Human	Meller et al. (1997)
48.0–113.0 nm	Cornea	Human	Meller et al. (1997)
30–60 nm	Optic nerve lamina cribrosa	Monkey	Abe et al. (1995)
Peripheral nerve			
46.1 nm	Proximal sciatic nerve	Rat	Muona et al. (1989)
45.5 nm	Distal sciatic nerve	Rat	Muona et al. (1989)
14–25 nm	Perineurium	Spiny lobster	Baerwald et al. (1991)
Skeletal muscle			
30–50 nm	Trachea, visceral muscles	Guinea pigs	Gabella (1991)
70–100 nm	Stapedius muscle	Squirrel monkey	Yoshihara et al. (1993)
30–70 nm	Semitendinosus muscle	Bovine	Nishimura et al. (1994)
Tendon			
318 ± 12 nm	Tail tendon	Rat	Gotoh & Sugi (1985)
20–290 nm	Flexor and extensor tendon	Human	Neurath & Stofft (1992)
124–170 nm	Calcanean tendon tissues	Rat	Baranauskas et al. (1998)
20–360 nm	Superficial digital flexor tendon	Horse	Kobayashi et al. (1999)

between epithelial cells of the quail oviduct originates from mesenchymal cells and not from the epithelial cells.

The production of fibril-forming collagen, such as types I and III, by epithelial cells of the interlobular bile duct, has been reported in an experimental model of cholestatic fibrosis (Abdel Aziz et al. 1991). Our present findings provide the first evidence that the epithelial cells of the interlobular bile duct produce fibril-forming collagen as well as basement membrane-forming collagen under physiological conditions. It is suggested that cholestasis stimulates the epithelial cells of interlobular bile ducts to increase synthesis of fibril-forming collagen, which is also produced by these cells under physiological conditions.

Variations in the diameter of collagen fibrils

Variations in diameter of collagen fibrils in the connective tissues were reported for the first time by Parry et al. (1978) who studied fibril ultrastructure in rat tail tendon. These authors suggested that the diameter of the fibrils is correlated with the age of the individuals as well as the mechanical stress acting on the tissue. Flint et al. (1984) studied the diameter of collagen fibrils from various parts of the skin of

various species, and suggested a correlation between the distribution of the fibrils, biochemical conditions and the functional load acting on the tissue. Thereafter variable diameter of collagen fibrils has been reported in various tissues including cartilage (Howlett, 1979; Kostovic Knezevic et al. 1981; Wright & Youson, 1983; Mizuno et al. 1990; Kuc & Scott, 1994), subepithelial connective tissue (Hino et al. 1982; Ottani et al. 1998; Sato, 1998), eye (Waring & Rodrigues, 1980; Pac et al. 1989; Yamabayashi et al. 1991; Shauly et al. 1992; Meek & Leonard, 1993; Meller et al. 1997), peripheral nerve (Muona et al. 1989; Baerwald et al. 1991; Abe et al. 1995), skeletal muscle (Gabella, 1991; Yoshihara et al. 1993; Nishimura et al. 1994) and tendon (Gotoh & Sugi, 1985; Neurath & Stofft, 1992; Baranauskas et al. 1998; Kobayashi et al. 1999), as shown in the Table. These data show that collagen fibrils generally have a large diameter in tendons and sclera where they are obviously exposed to strong mechanical stress.

The relationship between the diameter of collagen fibrils and the age of individuals is complex. Parry et al. (1978) stated that the diameter of collagen fibrils increased with age in rat tail tendon. On the other hand, Flint et al. (1984) surveyed a wide range of samples and showed that diameter increased during the growth period and decreased in late maturity. Recently Patterson Kane et al. (1997*a*) measured the diameters of fibrils in the tendon of the superficial digital flexor muscle in the horse and reported that diameter was constant between the ages of 2 and 10 y. These results suggest that age-dependent alteration in diameter is almost negligible in adulthood.

A relationship between the diameter of collagen fibrils and mechanical stress acting on the tissue has been suggested by Parry et al. (1978) who pointed out that the diameter of fibrils and tensile strength of the tissue are correlated in several types of tissues. Matthew & Moore (1991) compared the diameter distribution of fibrils in healing tissue after complete and partial transection and suggested that the different diameters can be explained by different levels of mechanical stress.

The biochemical factors that determine the diameter of fibrils are manifold. Currently, 2 distinct collagens are considered to have a major role in determining the diameter of collagen fibrils, namely collagen V and pN collagen III (Adachi et al. 1997). Evidence has been provided that both collagen type V and pN collagen III copolymerise with type I collagen, thereby reducing the diameter of collagen fibrils (Romanic et al. 1991; Adachi & Hayashi, 1986). Flint et al. (1984) reported a correlation between the diameter of collagen fibrils and the type and amount of glycosaminoglycan (GAG). The leucine-rich repeat class of proteoglycans, such as decorin, fibromodulin and lumican bind to fibrillar collagens in vitro, leading to delayed fibril formation and the formation of thinner fibrils (Vogel et al. 1984; Vogel & Trotter, 1987; Hedbom & Heinegard, 1989; Rada et al. 1993). Recently Svensson et al. (1999) showed that mice lacking a functional fibromodulin gene exhibit irregular shaped, thinner collagen fibrils in the Achilles tendon compared with wild type animals.

The variations in diameter of fibrils in Glisson's sheath shown in the present study is related to 2 different components. The first, the correlation of fibril diameter with the calibre of the bile duct, is most probably the result of difference in mechanical stress acting on the tissue. The liver shifts rhythmically with respiration, and its hilum is connected to the duodenohepatic ligament containing the bile duct, hepatic artery, and portal vein. Thus Glisson's sheath, which surrounds a larger bile duct and is therefore located closer to the hepatic hilum, is thought to receive greater mechanical stress. The second component, the difference between bile duct-associated and fibroblast-associated collagen, is thought to reflect a difference between the cells producing them. We found that both populations contain both types I and III collagen. The biochemical background of the 2 different components covering the variable diameter of collagen fibrils in Glisson's sheath requires further analysis.

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