Interstitial collagen in alcoholic human liver

Frej Stenbäck[1], Veli-Matti Wasenius[2] and Eero A. Sotaniemi[3]
[1]Department of Pathology, University of Oulu and [2]Radiotherapy and Oncology Clinic, University of Helsinki, and [3]Department of Internal Medicine, University of Oulu, Finland

Summary. The occurrence and intensity of staining for specific antibodies against the aminoterminal propeptide of type III procollagen (PIIIP), which is indicative of the synthesis and the degradation of that collagen type, was studied in sections from normal and alcoholic livers and compared with serum PIIIP levels, serum antipyrine clearance, fibronectin distribution and morphology as revealed by conventional stains and electronmicroscopy. Positive staining for PIIIP and fibronectin was observed in the perisinusoidal space of the normal liver and in portal tracts. In alcohol-induced fatty liver positive staining increased around the central veins, in alcoholic hepatitis increased staining reaction was seen to a limited extent in areas of cell injury. Extensive reticulin and PIIIP-positive areas were found in the periportal interstitium of the cirrhotic livers and in large fibrotic areas extending into the surrounding parenchyma in cases of active disease. The results show a distinct relationship between collagen type III metabolism, morphologically detectable hepatic injury and liver cell function tests, with tissue deposition occurring later in the disease process than biochemically detectable serum collagen levels and signs of altered liver cell function.

Key words: Liver - Alcohol - Cirrhosis - Collagen type III - Fibronectin - Reticulin

Introduction

The essential feature of alcohol induced liver cirrhosis is collagen accumulation (Pierce and Nakane, 1969; Popper and Udenfriend, 1970; Popper et al., 1981) however, staining characteristics specific for alcoholic liver disease have so far not been reported. Methods

employing special staining procedures for connective tissue such as elastica van Gieson, Mallory, Masson or Goldner staining have attempted to delineate specific changes but with limited success (Propper et al., 1981; Frei et al., 1984). Some authors (Ishii, 1967) postulate that collagen fibres are fundamentally identical with reticulin fibres, others that positive reticulin staining identifies precursors of collagen (Melcher, 1966) or a mixture of collagen type III, fibronectin and noncollagenous glycoproteins (Unsworth et al., 1982). Collagen increase has also been shown in biochemical (Rojkind et al., 1979; Niemelä et al., 1982; 1983) and immunohistochemical studies (Otto et al., 1977; Wick et al., 1978) with increased deposition of collagen types I and III in periportal and intralobular areas of the liver (Frei et al., 1984; Weigand et al., 1984).

Collagen type III is synthesized intracellularly in the smooth endoplasmic reticulum as a larger molecule, procollagen, which contains additional peptide extensions at both the aminoterminal and carboxyterminal ends. These peptides are cleaved off by specific proteases during the conversion of procollagen to collagen. The level of aminoterminal propeptide of type III procollagen (PIIIP) has been considered indicative of the active synthesis of type III collagen (Gay et al., 1975; Grimaud et al., 1980; Raedsch et al., 1983; Galambos et al., 1985). Other studies conclude that PIIIP levels need not only reflect increased depositions but also increased degradation of newly synthesized PIIIP or degradation of a tissue form still containing the aminoterminal propeptide (Niemelä et al., 1982; 1983).

In this study we have analyzed by immunohistochemical means the occurrence of type III collagen still containing its aminoterminal procollagen peptide in normal and diseased livers and compared the results with those obtained from determinations of PIIIP concentrations in the serum, serum antipyrine clearance, fibronectin analysis of tissue and sections as well as morphological studies using conventional histological stains; particularly reticulin and electron microscopy. The purpose was to

study the role an interstitial collagen, namely type III collagen plays in the progression and manifestation of alcoholic liver disease and its relationship to histologically identified structural changes.

Materials and methods

Patients

Twentyfour consecutive cases of alcoholism were investigated (mean age 52 yr, range 33-69 yr). The subjects met the following criteria: (a) all had consumed ethanol regularly for at least 10 yr; (b) in every case, the use of ethanol had caused disturbances in their social life, and (c) all had been hospitalized several times because of alcohol intoxication, delirium tremens, or both. None had any significant cardiac decompensation, and their kidney function, judged by creatinine clearance, was normal. Some subjects were treated with drugs such as digoxin, nitroglycerine, furosemide, and spasmolytics, but none were treated with compounds known to induce the hepatic microsomal enzyme system. As controls, 3 healthy subjects, 2 men and 1 woman (mean age 47 yr, range 35-64 yr) were also examined. Although they occasionally consumed ethanol, none was an alcoholic.

Study protocol

All the alcoholics were examined at the Clinical Research Unit (CRU). Blood samples for liver function tests were taken after an overnight fast. Three to 4 h later, a percutaneous liver biopsy specimen was taken with a Thru-Cut needle for diagnostic purposes. In most cases this was done 1 wk after admission. All samples were obtained with the patient's permission. This study was approved by the Ethical Committee of the Medical Faculty of Oulu University. From the same patients, though not all, blood samples were taken for determination of the serum antigens related to PIIIP.

Plasma antipyrine clearance was determined by a GLC method with phenacetin being an internal standard as previously described (Niemelä et al., 1982; 1983).

Liver histology

The liver biopsy specimens were fixed in neutral formalin, embedded in paraffin, sectioned serially, and stained with hematoxylin and eosin, periodic acid-Schiff after diastase digestion, van Gieson's stain, or Gomori's reticulin stain. The alcoholics were classified into five groups according to the liver histology; normal light microscopy, fatty liver, fatty liver with fibrosis, active cirrhosis some with alcoholic hepatitis and inactive cirrhosis. Patients classified as normal had either completely normal histological picture of the liver or very slightly altered liver parenchyma. Those with fatty liver had an accumulation of fat in the liver that exceeded 5% of the area. Patients with fatty liver and fibrosis, had an increase in connective tissue (> 5%). Patients with cirrhosis showed loss of hepatocytes, increased fibrosis,

presence of regenerative nodules, distortion of the lobular structure, and an abnormal vascular bed (Popper et al. 1981). Patients with inactive cirrhosis had normal serum albumin values and no signs of inflammation in the liver biopsy specimen at the time of investigation.

Morphometry

Semiquantitative analysis of amount of reticulin positive fibres was carried out using a morphometric system (Ludwig and Elveback 1972; Stenbäck 1981). A grid lattice was super-imposed on a projected image at 250× magnification, covering almost the entire specimen. The number of points on fibres compared to total number of points was used as an expression of volume percentage of fibres.

Antibodies

Antisera to PIIIP were raised in rabbits and prepared as described previously (Niemelä et al. 1985). The antibodies were purified by immunoabsorption with the relevant antigen coupled to Sepharose 4B. Rabbit antisera to fibronectin were purchased from Dako A/S, Copenhagen, Denmark.

Immunoperoxidase staining

For the immunohistochemical studies the avidin-biotin modification (Hsu et al. 1981) of the peroxidase-antiperoxidase method was used. 5μm thick paraffin sections were deparaffinized and treated with 0.4% pepsin (Merck, Darmstadt, FRG). Endogenous peroxidase was blocked by incubating with H_2O_2. The sections were successively treated with rabbit antibodies, biotinylated anti-rabbit immunoglobulin (Vector Laboratories, Burlingame, Ca), dilution 1:500, avidin (Vector), dilution 1:1000, and biotinylated horseradish peroxidase complex (Vector). The peroxidase reaction was performed using 3-ethyl-9-methylcarbazole (Sigma Chemical Co., St. Louis, Mo.) as a substrate. Haematoxylin was used as counterstain.

Electron microscopy

Fresh tissue was fixed in 3% glutaraldehyde in phosphate buffer pH 7.4, postfixed in 1% osmium tetroxide in the same buffer, dehydrated, and embedded in Epon. 1μm sections were cut and stained with 1% toluidine blue for orientation by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Jeol JEM 100 B electron microscope.

Results

A regular distinct reaction with antibodies to PIIIP in the normal liver samples was seen in the perivascular space and the interstitial matrix of the portal region with

a continuous, slightly irregular PIIIP-positive strand in the central vein wall and around the bile ducts (Fig. 1). In these specimens quantitative alterations were minimal, as shown in Table 1, and the parenchymal compartment was almost 98%. The results are summarized in Table 2.

The 8 cases of fatty liver with the proportion of fat varying from 6% to 25% (Table 1) showed slight but variable differences from the normal pattern of antibodies to PIIIP (Table 1). A slight increase in PIIIP-positive staining was seen in the form of thickened discontinuous strands in the periportal connective tissue matrix, however, less distinct than that of fibronectin which showed positive rims around hepatic cells. PIIIP positive material formed a distinct widened positive area around the central vein in many places, though not everywhere (Fig. 2). The positive rim was continuous and thickened, though slightly varying in width.

Fatty liver and fibrosis was encountered in 7 patients with fat from 3% to 26% and fibrous tissue from 4.8% to 22% (Table 1). This was reflected in a slight increase in PIIIP positive periportal material and in perisinusoidal PIIIP staining activity (Fig. 3). PIIIP positive sprouts varying in length and width irradiated from the central vein areas into the sinusoids of the neighbouring hepatic cells. The increase in connective tissue when studied by conventional histological stains, i.e. for reticulin, was also located in the perivenular area, in the form of a narrow rim with extensions into the surrounding parenchyma (Fig. 6). Fibronectin activity was also distinct in perisinusoidal and periportal locations.

PIIIP staining increased in the perisinusoidal space in some areas in cases of active cirrhosis with hepatitis (Table 2). Thin discontinuous strands of PIIIP-positive material of varying thickness were seen in the perisinusoidal space, occasionally bordering on enlarged cystic spaces and sometimes occluding the space between the liver cell trabeculae, similarly to that of fibronectin. The staining intensity increased in the areas of focal necrosis with accumulations of leucocytes (Fig. 4). In active cirrhosis PIIIP staining was also noticeable on the border of the hepatic nodules and in the intervening connective tissue. Septae of varying length extended

from the portal areas to neighbouring ones and from the septa into the parenchyma, giving the septal border a sawtooth appearance. In some sections the border was sharp with abundant material in the septa, but less so pericellularly. Strands and cords of PIIIP positive material also extended from vessels into the perisinusoidal space forming pseudocapillary spaces (Fig. 5).

Inactive cirrhosis formed the largest group, 11 patients, in this series. Morphometrical analysis showed the amount of connective tissue to double in inactive cirrhosis as compared to active conditions (Table 1). The stainings for PIIIP varied in end-stage cirrhosis seen in wide continuous septa and around liver cells (Table 2). The fibrotic septa featured large areas with PIIIP-positive fibrils either surrounding individual liver cells (Fig. 6) or forming larger nodules (Fig. 7). Scanty perisinusoidal staining occurred occasionally at this stage. In inactive cirrhosis the location and amount of fibronectin corresponded to that of PIIIP, seen as fine diffuse material in the septa (Fig. 8). Comparison of collagen location findings obtained with conventional histological stains, e.g. van Gieson's or reticulin stain, showed an obvious coexistence of different types of collagen in the septae at this stage. The septae were complete, extending from one area to another, and regenerative features were also visible. Ultrastructural studies reflected collagen increase as depositions of electron-dense material in the perisinusoidal space and in the septae.

The percentages of reticulin positive connective tissue, fat and parenchyma by volume as indicated in Table 1 were also compared to serum levels of PIIIP (Fig. 9). A distinct correlation between the two was seen, however, in active liver cirrhosis serum levels were consistently higher and in inactive cirrhosis lower. The amount of connective tissue also affected liver function as measured by antipyrine halflife (Fig. 10). Values were, however, also higher here in active cirrhosis and lower in inactive cirrhosis when compared to average. Fat deposits also increased antipyrine clearance.

Table 1. Number of patients with different types of alcoholic liver disease and percentage of connective tissue fat and parenchyma.

| Diagnosis	Number of patients	Connective tissue	Percentage of fat	Parenchyma
Normal liver	3	1.3 + 0.6	1 + 0.1	9.7 + 0.5
Fatty liver	8	1.1 + 0.2	15 + 9.6	83.4 + 9.6
Fatty liver and fibrosis	7	11.4 + 6.8	9.5 + 13.9	79.1 + 11.9
Active cirrhosis	9	15.7 + 18.9	2.2 + 1.8	81.7 + 19.1
Inactive cirrhosis	11	33.7 + 20.4	1.3 + 1	64.9 + 19.8

Table 2. Staining intensity and structure of the amino-terminal propeptide of type III procollagen in normal liver and in alcohol induced liver disease.

	intensity	structure
Normal liver		
portal	++	r
perivenular	+	r
sinusoidal	+	i
Fatty liver		
portal	++	r
perivenular	++	i
sinusoidal	+	i
Fatty liver and fibrosis		
portal	++	r
perivendular	++	i
sinusoidal	+	i
septal	+	i
Active cirrhosis		
portal	++	r-i
perivenular	++	i
sinusoidal	++	i
septal	++	i
Inactive cirrhosis		
portal	++ − +++	i
perivenular	++	i
sinusoidal	++ − +++	i
septal	++ − +++	i

Staining + = slight, ++ = moderate, +++ = distinct, r = regular, i = irregular
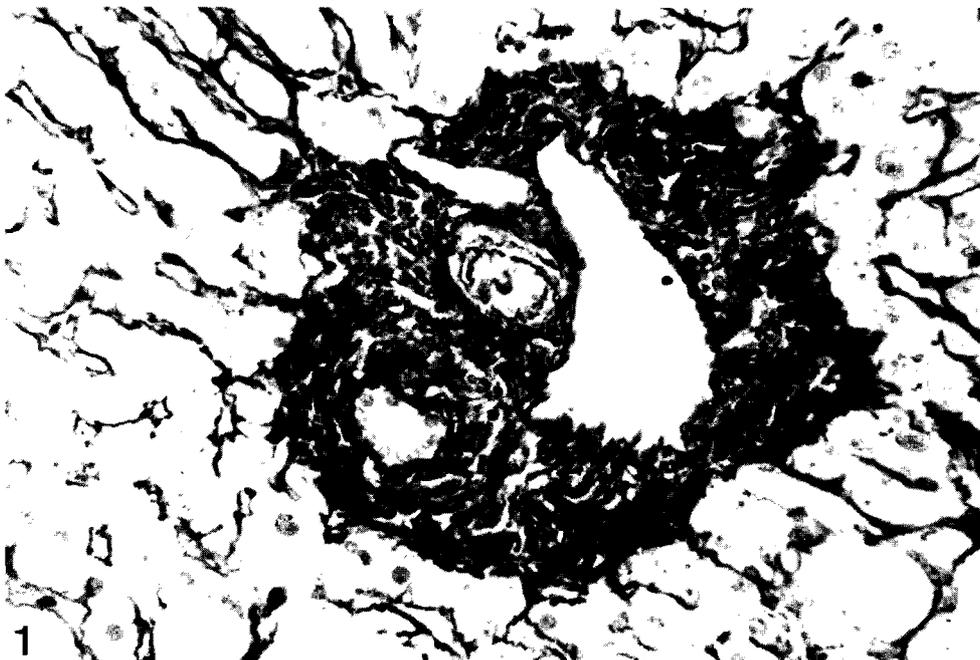
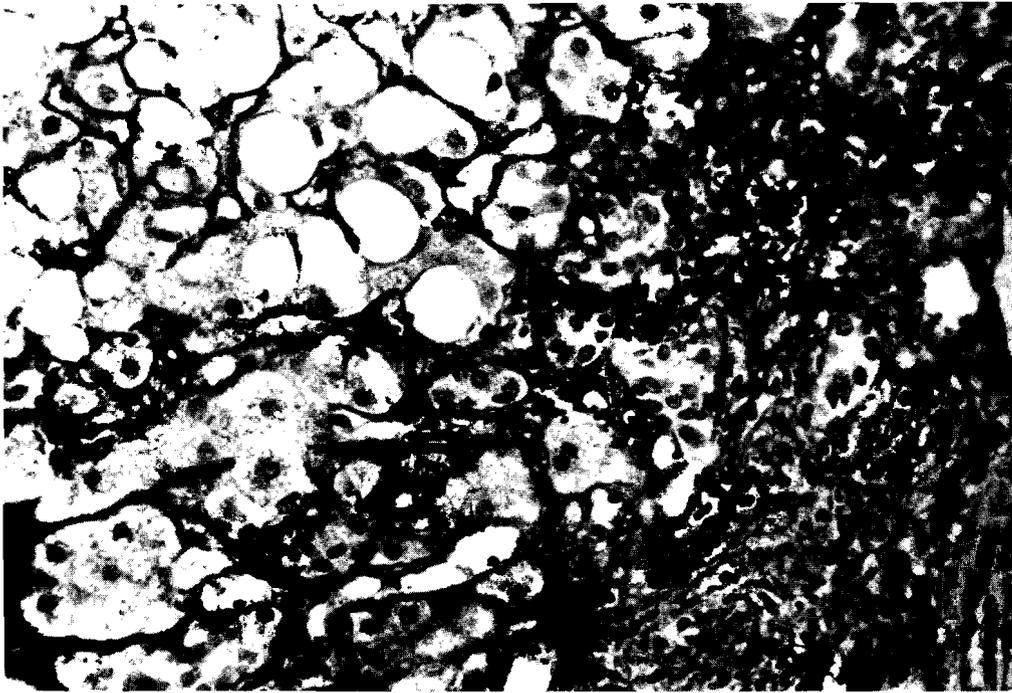**Fig. 1.** Distribution of PIIIP in portal tract of normal liver seen as distinct strands around vessels and ducts. PIIIP ×460
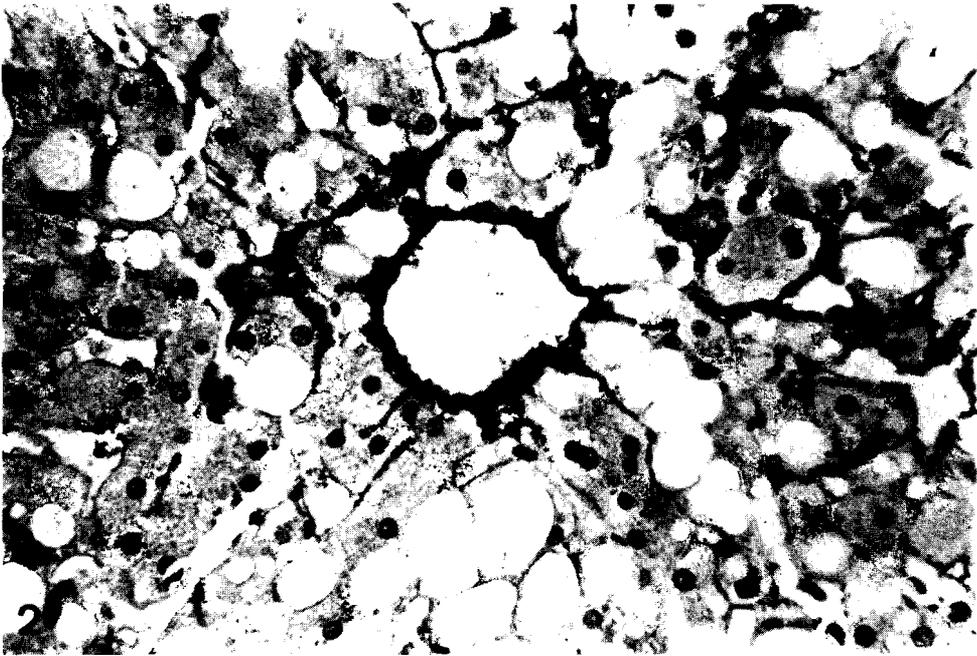
**Fig. 2.** Distinct PIIIP positive material around central vein in alcoholic liver. PIIIP ×380
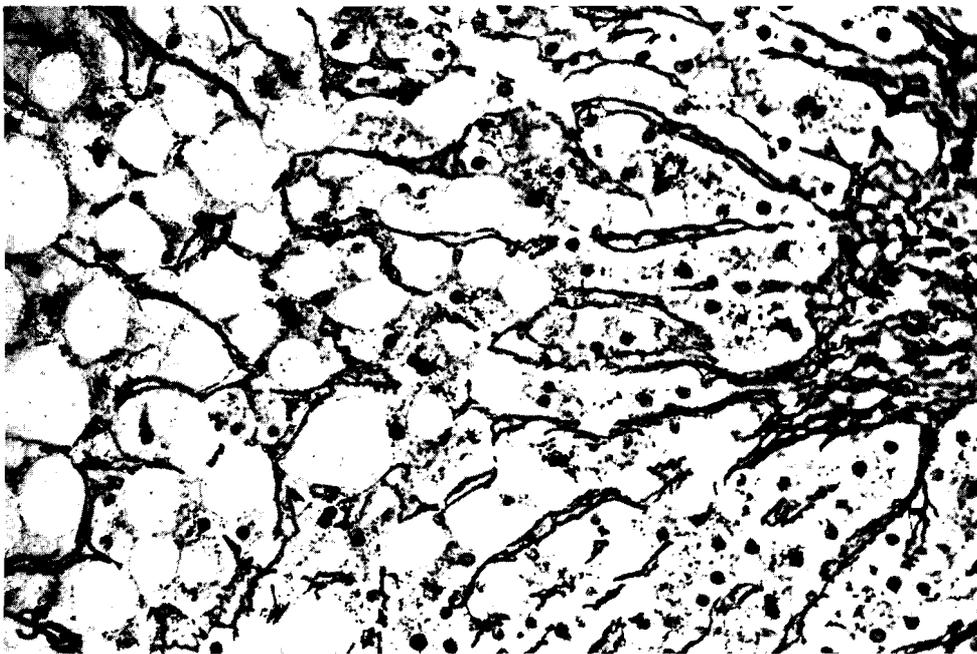
Fig. 3. Cords and strands of reticulin positive material extending into parenchyma in alcoholic fatty liver. Reticulin stain ×460
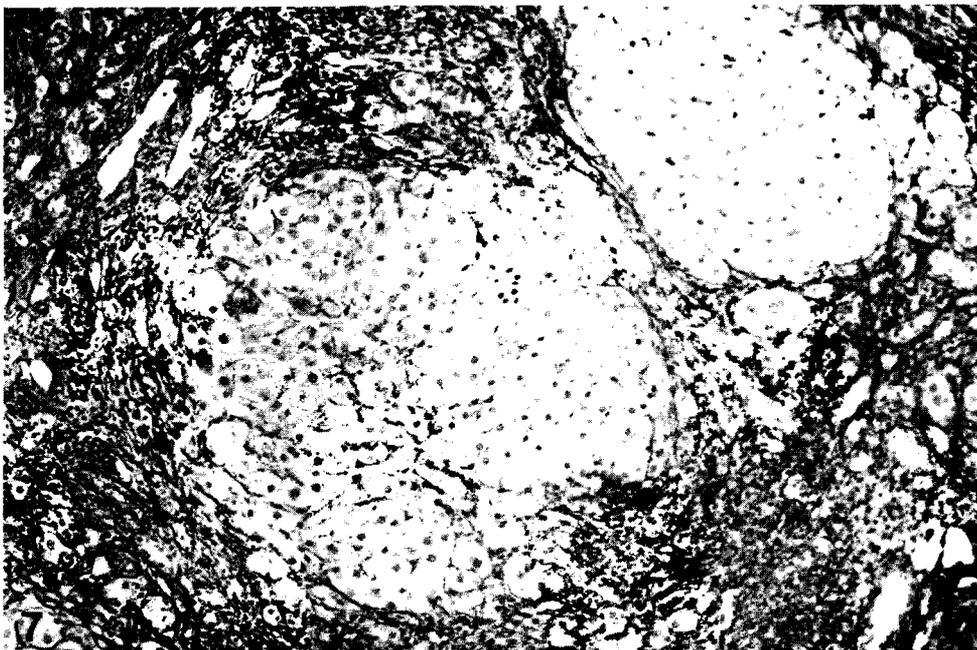
Fig. 4. Extensive involvement of the liver in patient with active cirrhosis with PIIIP positive material extending into surrounding parenchyma. PIIIP ×280
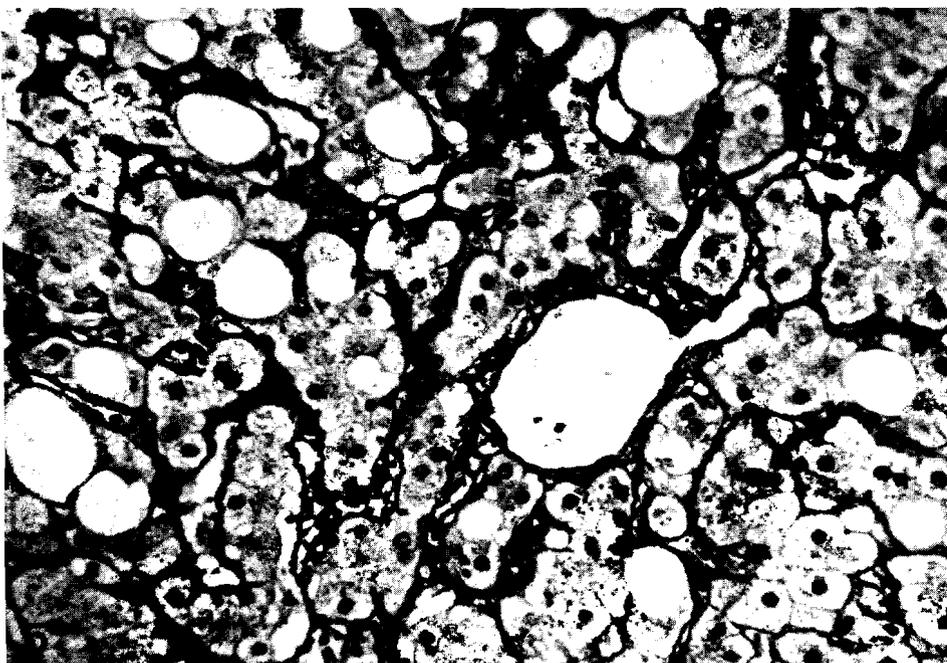
**Fig. 5.** Abundant PIIIP positive material in septa in liver of patient with active cirrhosis. PIIIP ×360
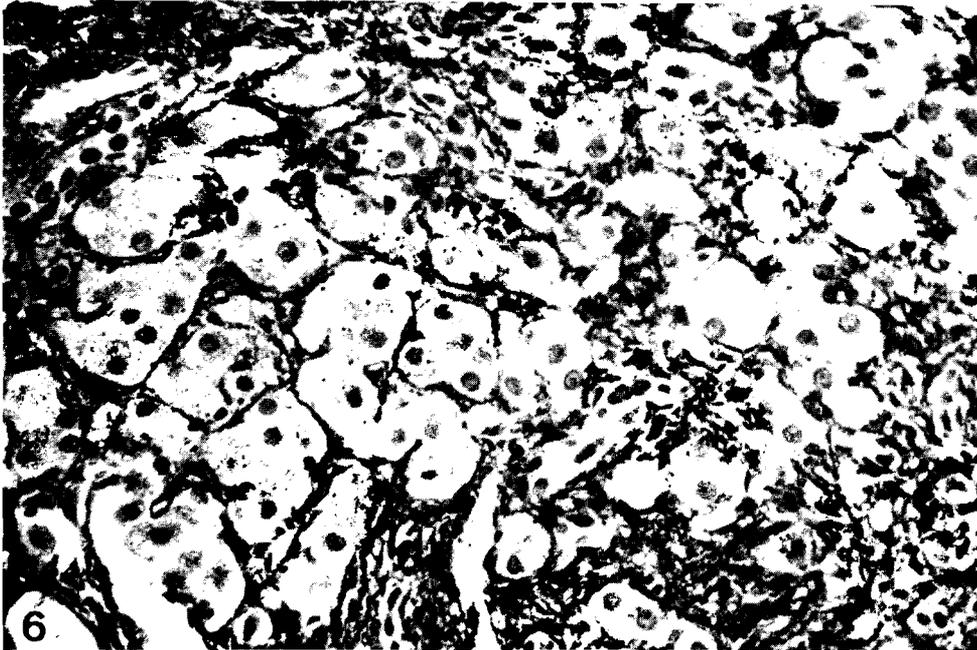
Fig. 6. Alcoholic cirrhosis with PIIIP positive materials between individual liver cells. PIIIP ×360
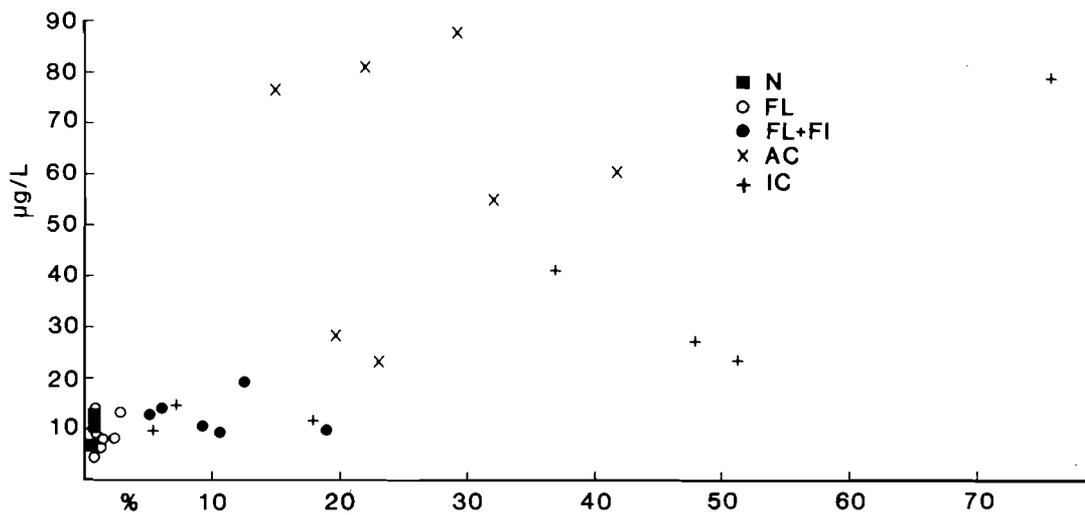

Fig. 7. Hepatic nodules in cirrhotic liver surrounded by abundant PIIIP positive material in thick strands, scanty staining in perisinusoidal locations. PIIIP ×220

Interstitial collagen in alcoholic liver
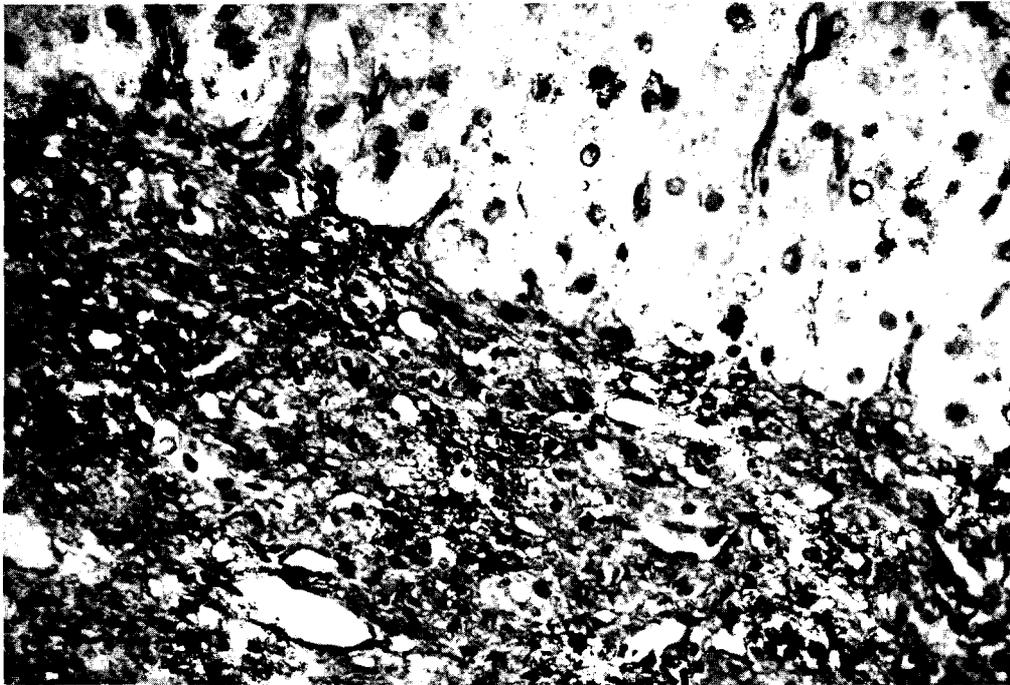
Fig. 8. Fibronectin positive material finely distributed in septa of cirrhotic liver. Fibronectin ×360

Fig. 9. Serum PIIIP levels (ug/L) and morphometrically detected deposits of connective tissue. N=normal liver, FA=fatty liver, FA+FT=Fatty liver and fibrosis, AC=Active cirrhosis and IC=inactive cirrhosis.
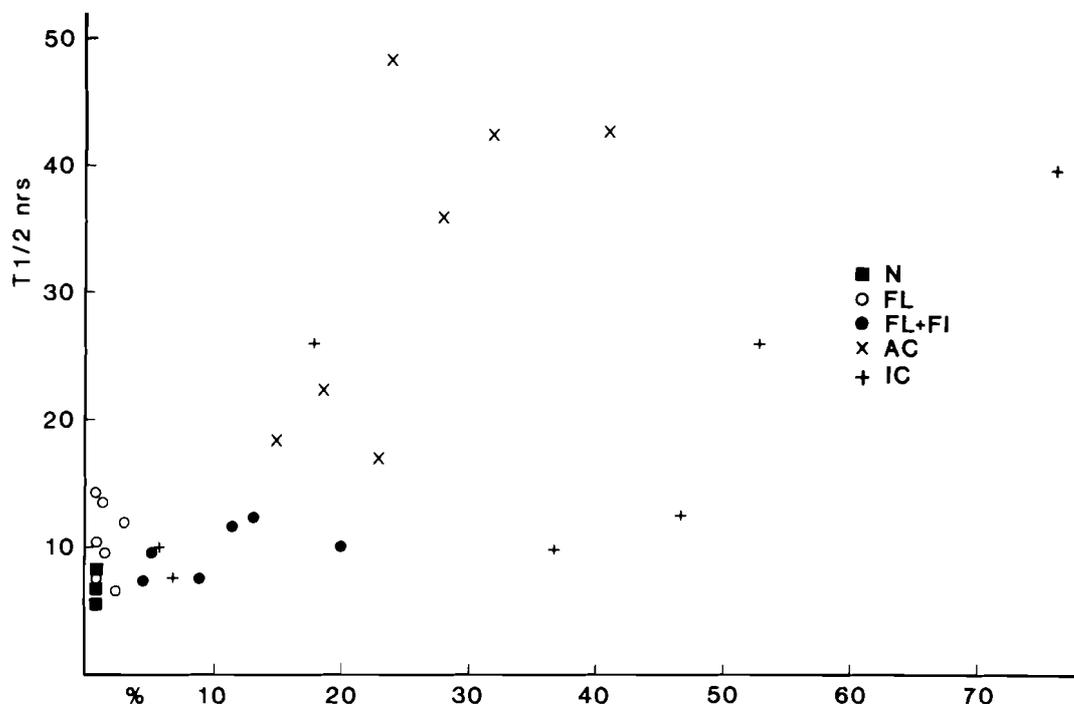
Fig. 10. Antipyrin clearance and morphologically detected deposits of connective tissue. N = normal liver, FA = fatty liver, FA + FT = Fatty liver and fibrosis, AC = Active cirrhosis and IC = inactive cirrhosis.

Discussion

The intensity of PIIIP-positive staining was found here to be closely related to histologically detectable alcoholic liver disease. Deposits of PIIIP-positve material increased in patients with early alcohol-induced liver injury. In many areas in fatty livers PIIIP was identified in a pericentral location, similar to the perivenular hepatic fibrosis reported previously (Popper and Udenfriend 1970; Popper et al., 1981). Histological signs of inflammation were associated with increased PIIIP deposits but only to a limited extent. An increase in PIIIP positive staining was observed in perisinusoidal locations in alcoholic livers prior to the development of cirrhosis, similar to findings in alcohol-fed baboons (Sato et al., 1986). These changes may precede or correlate with the capillarization of liver sinusoids found in the active and late stages of cirrhosis (Phillips and Steiner, 1966; Schaffner and Popper, 1963). Fibrosis as determined by conventional histological stains contained large PIIIP positive deposits in this study. No PIIIP staining was observed in the hepatocytes which coincides with most reports (Gay et al. 1975; Grimaud et al., 1980; Bianchi et al., 1984) though intracytoplasmic collagen III has been described in cirrhotic liver (Sakakibara et al., 1985).

The central change in alcohol induced liver diseases is collagen accumulation, however specific changes of prognostic significance are less known (Popper and Udenfriend 1970; Popper et al., 1981). About 70% of

liver collagen is of types I and III, which are present in approximately equal amounts in normal liver (Sakakibara et al., 1985; Grimaud et al., 1980). Increased depositions of both collagen types occurs in cirrhosis in varying proportions depending on the stage of the disease, with type III predominant in early fibrosis and type I in late cirrhosis (Rojkind and Martínez-Palomo, 1976; Grimaud et al., 1980; Murata et al., 1984). The increase in PIIIP in this study was distinct also in later stages of disease. Connective tissue proliferation (Table 1) and PIIIP staining activity in this study were most distinct in inactive cirrhosis.

Hepatic fibrosis takes place mainly in three anatomically different forms; proliferation of fibroblasts in the portal tracts, the perihepatocellular area and the periductular area (Popper et al., 1981). Periductular staining for PIIIP was seen in all types of livers in this study and was related to the severity of the disease, while pericellular collagen accumulation appeared to be more closely associated with clinical signs of disease in the patient when compared to serum levels of PIIIP (Niemelä et al., 1982; 1983). This process of polymerisation and possible pathological cross-linking of collagen fibrils may be initiated and enhanced by substances from necrotic hepatocytes, whereupon the balance of interaction between the epithelial and mesenchymal cells may be disturbed (Popper and Uderfriend, 1970; Popper et al., 1981). However, in this study comparatively small amounts of PIIIP deposits were found in areas of necrosis and inflammation. Liver

fibrosis occurs either through condensation and increase of collagen fibres after parenchymal collapse or through the formation of new collagen fibres (Popper and Udenfriend, 1970). The occurrence of the latter process in cirrhosis is supported by the increase in PIIIP propeptide, which is indicative of active collagen metabolism. PIIIP positive material was also seen in the connective tissue of the portal triads and septae in the form of fibrillar structures in the intercellular matrix, and increasing with severity of disease (Niemelä et al., 1982; 1983). The causes of increased PIIIP deposits may be excessive formation, particularly in active disease, and insufficient degradation in end-stage cirrhosis.

Codistribution of collagen type III with fibronectin has been shown for many tissues in the body (D'Ardenne et al., 1983). In normal and cirrhotic human liver alterations in distribution and amount of fibronectin and collagen III are also similar (Kojima et al., 1981; Yamamoto et al., 1984; Clement et al., 1986). Fibronectin production increased early in experimental liver injury (Ballardini et al., 1985; Martínez-Hernández, 1985) thus forming a matrix for subsequent collagen deposits (Martínez-Hernández, 1985). This was also seen in this study indicating the significance of fibronectin in human disease as well.

Serum assays of collagen III precursors have been used for monitoring the development of fibrosis in liver diseases in alcoholics (Rojkind, 1984; Savolainen et al., 1984). The antigens detected by this radioimmunoassay, however, exist in serum in at least three different forms, differing in antigenic properties and molecular sizes (Niemelä et al., 1982; 1983). These studies showed a correlation between serum measurements and immunohistochemical results. In active liver cirrhosis serum values were somewhat higher indicating the occurrence of active synthesis prior to deposition in the liver. Also, in end-stage disease where serum levels were decreasing tissue deposits were extensive, possible due to a slow turnover at this stage or metabolic alterations of PIIIP to a less soluble form. Morphometrical analysis of collagen in liver disease (Ludwig and Elveback, 1972; Volmers and Lüders 1981; Weigand et al., 1984) have shown correlation to clinical findings and collagen serum levels, seen also in this study (Fig. 9).

Assays of liver cell function such as antipyrine halflife (Niemelä et al., 1982; 1983; Sotaniemi et al., 1977, 1986) also related to PIIIP distribution and morphometrically assayed collagen content (Fig. 10) indicating the significance of PIIIP amount and distribution on hepatic function. The finding that liver cell function is more severely altered in active cirrhosis than in inactive lesions points to the significance of the disease process itself, not only total amount of collagen (Sotaniemi et al., 1986).

References

Ballardini G., Faccani A., Fallani M., Berti S., Vassi V., Castaldini C., Biagini G., Garbisa S. and Bianchi F.B. (1985). Sequential behaviour of extracellular matrix glycoproteins in an experimental model of hepatic fibrosis. Virchows. Arch. (Cell Pathol.) 49, 317-324.

Bianchi F.B., Biaginic G., Ballardini G., Cenacchi G., Facchini A., Pisi E., Laschi R., Liotta L. and Garbisa S. (1984). Basement membrane production by hepatocytes in chronic liver disease. Hepatol. 4, 1167-1172.

Clement B., Grimaud J.A., Campion J.P., Deugnier Y. and Guillouzo A. (1986). Cell types involved in collagen and fibronectin production in normal and fibrotic human liver. Hepatol. 6, 225-234.

D'Ardenne A., Burns J., Sykes B.C. and Kirkpatrick P. (1983). Comparative distribution of fibronectin and type III collagen in normal tissues. J. Pathol. 141, 55-69.

Frei A., Zimmerman A. and Weigand K. (1984). The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. Hepatol. 4, 830-834.

Galambos M.R., Collins D.C. and Galambos J.T. (1985). A radioimmunoassay procedure for type III procollagen., its use in the detection of hepatic fibrosis. Hepatol. 5, 38-42.

Gay S., Fietzek P.P., Remberger K., Eder M. and Kühn K. (1975). Liver cirrhosis: immunofluorescence and biochemical studies demonstrate two type of collagen. Klinische Wochenschrift 53, 205-208.

Grimaud J.A., Druguet M. and Peyrol S. (1980). Collagen immunotyping in human liver: light and electron microscope study. J. Histochem. Cytochem. 28, 1145-1156.

Hsu S., Raine L. and Fanger H. (1981). A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am. J. Clin. Pathol. 75, 734-738.

Ishii T. (1967). Das Silberbild der Retikulinfasern des Lymphknotens (mit besonderer Berücksichtigung de Übergangs in die kollagenen Fasern). Verch. Anat. Ges. 62, 353-359.

Kojima N., Isemura M., Yosizawa Z., Ono T., Shinada S., Soga K. and Aoyagi Y. (1981). Distribution of fibronectin in fibrotic human livers at various states. Tohoku J. exp. Med. 135, 403-412.

Ludwig J. and Elveback L.R. (1972). Parenchyma weight changes in hepatic cirrhosis. A morphometric study and discussion of the method. Lab. Invest. 26, 338.

Martínez-Hernández A. (1985). The hepatic extracellular matrix. II. Electron immunohistochemical studies in rats with CC1₄-induced cirrhosis. Lab. Invest. 53, 166.

Melcher A.H. (1966). Gingival reticulin: Identification and role in histogenesis of collagen fibres. J. Dent. Res. 45, 426-439.

Murata K., Kudo M., Onuma F. and Motoyama T. (1984). Changes of collagen types at various stages of human liver cirrhosis. Hepato-gastroenterol. 31, 158-161.

Niemelä O., Risteli L., Sotaniemi E.A. and Risteli J. (1982). Heterogeneity of the antigens related to the aminoterminal propeptide of type III procollagen in human serum. Clin. Chim. Acta 124, 39-44.

Niemelä O., Risteli L. and Sotaniemi E.A. (1983). Aminoterminal propeptide of type III procollagen in serum in alcoholic liver disease. Gastroenterol. 85, 254-325.

Niemelä O., Risteli L., Parkkinen J. and Risteli J. (1985). Purification and characterization of the aminoterminal

propeptide of human type III procollagen. Biochem. J. in press.

Ott U., Hahn E., Moshuids E., Bode J.C. and Martini G.A. (1977). Immunohistologischer Nachweis von Typ I-und Typ III-Kollagen in Leberbiopsien: Frühe und späte Verändrungen bei alkoholischer Lebererkrakung. Verhand. Deutsch Gesells. innere Med. 83, 537-540.

Phillips M.J. and Steiner J.W. (1966). Electron microscopy of cirrhotic nodule. Tubularization of the parenchyma by biliary hepatocytes. Lab. Invest. 15, 802-817.

Pierce G.B. and Nakane P.K. (1969). Basement membrane, synthesis and deposition in response to cellular injury. Lab. Invest. 21, 27-41.

Popper H. and Udenfriend S. (1970). Hepatic fibrosis. Correlation of biochemical and morphological investigations. Am.J. Med. 49, 707-721.

Popper H., Tung S.N. and Gerber M.A. (1981). Pathology of alcoholic liver disease. Seminars in Liver Disease. 1, 203-216.

Raedsch R., Stiehl A., Sieg A., Walker S. and Kommerell B. (1983). Biliary excretion of procollagen type III peptide in healthy humans and in patients with alcoholic cirrhosis of the liver. Gastroenterol. 85, 1265-1270.

Risteli J., Bächinger H.P., Engel J., Furthmayr H. and Timpl R. (1980) 7-S collagen, Characterization of an unusual basement membrane structure. Eur. J. Biochem. 108, 239-250.

Risteli L. and Timpl R. (1981). Isolation and characterization of pepsin fragments of laminin from human placental and renal basement membranes. Biochem. 193, 749-755.

Rojkind M. and Martínez-Palomo A. (1976). Increase in type I, II and type III collagens in human alcoholic liver cirrhosis. Pro. Nat. Acad. Sci. 73, 539-543.

Rojkind M., Giambrone M.A. and Biempica L. (1979). Collagen types in normal and cirrhotic liver. Gastroenterol. 76, 710-719.

Rojkind M. (1984). The blue glass and the predictive value of serum amino-terminal propeptide of type III procollagen as a marker of liver fibrosis. Hepatol. 4, 977-978.

Sato S. and Leo M.A. (1986). Ultrastructural localization of type III procollagen in baboon liver. Am. J. Pathol. 122, 212-217.

Savolainen E.R., Goldberg B., Leo M.A., Vélez M. and Lieber C.S. (1984). Diagnostic value of serum procollagen peptide measurements in alcoholic liver disease. Clin. Experim. Res. 8, 384.

Sakakibara K., Igarashi S. and Hatahara T. (1985). Localization of type III procollagen aminopeptide antigenicity in hepatocytes from cirrhotic human liver. Virschows Arch. (Pathol. Anat.) 408, 219-228.

Schaffner F. and Popper H. (1963). Capillarization of hepatic sinusoids in man. Gastroenterology 44, 239-242.

Sotaniemi E.A., Ahlqvist J., Pelkonen R.O., Pirttiaho H. and Luoma P.V. (1977). Histological changes in the liver and indices of drug metabolism in alcoholics. Europ. J. clin. Pharmacol. 11, 295-303.

Soataniemi E.A., Niemelä O., Risteli L., Stenbäck F., Pelkonen R.O., Lahtela J.T. and Risteli J. (1986). Fibrotic process and drugs metabolism in alcoholic liver disease. Clin. Pharm. Ther 40, 46-55.

Stenbäck F. (1983). Applicability of morphometrical methods in clinical cytology. Acta Stereol. 2, 319-328.

Unsworth D.J., Scott D.L., Almond T.J., Beard H.K., Holborow E.J. and Walton K.W. (1982). Studies on reticulin. I, Serological and immunohistological investigation of the occurrence of collagen type III, fibronectin and the non-collagenous glycoprotein of pras and glynn in reticulin. Br. J. Exp. Path 63, 154.

Volmer J. and Lüders C.J. (1982). Morphometric investigations of the portal tracts of the liver, the differentiation of variable progressionin chronic persistent hepatitis. Virchows Arch. (Pathol. Anat.) 392, 321-337.

Voss B., Rautenberg J. and Allam S. (1980). Distributions of collagen type I and type III and of two collagenous components of basement membranes in the human liver. Pathol. Res. Pract. 170, 50-60.

Weigand K., Zaugg P.Y., Frei A. and Zimmermann A. (1984). Long-term follow-up of serum N-terminal propeptide of collagen type III levels in patients with chronic liver disease. Hepatol. 4, 835-838.

Wick G., Brunner H., Penner E. and Timpl R. (1978). The diagnostic application of specific antiprocollagen sera. II. Analysis of liver biopsies. Int. Arch. Allerg. Appl. Immunol. 56, 316-324.

Yamamoto M., Sumiyoshi H., Nakagami K. and Tahara E. (1984). Distribution of collagen types I, III and V in fibrotic and neoplastic human liver. Acta Pathol. Jpn., 34, 77-86.