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The Ultrastructure of Spiralled Collagen in Liver Fibrosis

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Abstract. An ultrastructural study was performed using oölong tea polyphenols for staining liver tissue from 5 patients with acute hepatitis, 20 patients with chronic hepatitis, and 5 patients with alcoholic liver disease. Spiralled collagen was seen in the region of the portal tract in all of the patients, but not in the periportal region, perisinusoidal region, or sinusoidal wall. The amount of spiralled collagen relative to the total amount of collagen fibrils was greater in patients with hepatitis than in those with alcoholic liver disease. The occurrence of spiralled collagen appears to be a degenerative phenomenon as indicated by observations of its three-dimensional structure.

Key words: Spiralled collagen - Hepatitis - Liver fibrosis - Ultrastructure

INTRODUCTION

Hepatic fibrosis is a consequence of severe liver damage, occurring in many chronic liver diseases as a forerunner of cirrhosis. In the fibrotic liver, the amounts of type I and type III collagen increase in the periportal, sinusoidal, and pericentral regions[1-3]. On the other hand, spiralled collagen has been observed in the skin of patients with Ehlers-Danlos syndrome (EDS-I and EDS-IV)[4, 5], dermal eruptions in pseudoxanthoma elasticum[6], elastofibromas[7], emphysematous lungs[8], amyloid kidneys[9] and rheumatoid arthritis[10]. PORTO et al.[11] have published an electron micrograph demonstrating the existence of spiralled collagen in the liver of chronic alcoholic patients. However, there is no information available on the occurrence of spiralled collagen in the liver of patients with acute and chronic hepatitis. For this report, we performed a systematic ultrastructural study of liver biopsies from 30 cases of liver disease, and also examined the three-dimensional structure of spiralled collagen.

MATERIALS AND METHODS

The patients reported on here had clinical signs consistent with liver disease, and the pathological data are summarized in Table 1. Liver specimens from 5 patients with acute hepatitis, 20 patients with chronic hepatitis, and 5 patients with alcoholic liver disease were used.

1. Immunolocalization study using anti-collagen type III antibody

Liver needle biopsy specimens were obtained and fixed in neutral formalin. The tissue samples were dehydrated and embedded in paraffin wax. The sections were cut, deparaffinized and dehydrated in a graded series of alcohol. The sections were then treated with 0.3% hydrogen peroxide for 30 min and rinsed with phosphate buffered saline (PBS). The sections were reacted for 45 min with the primary polyclonal antibody (Southern Biotechnology Associates, Birmingham, AL, USA) at a dilution of 1 : 1,000, then the sections were washed with PBS and incubated for 30 min with a 1 : 200 dilution of a biotinylated rabbit anti-goat IgG. The sections were washed with PBS and incubated with 0.05% 3,3-diaminobenzidine for 10-15 min. The sections were then washed with water and counterstained with Meyer's hematoxylin for 1 min, dehydrated in a graded series of alcohol and embedded in Mount-Quick (Daido Sangyo Co., Ltd., Tokyo, Japan).

2. Electron microscopy

Liver needle biopsy specimens were obtained, and these small pieces were fixed in 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide. The tissue samples were dehydrated in a graded series of alcohol and embedded in Epok 812. Ultrathin sections were cut on a Porter MT 5,000 ultramicrotome with a diamond knife, stained with 0.5% oölong tea polyphenol (OTE solution, Suntory Co.,

Tokyo, Japan) for staining connective tissues, and post-stained with uranyl acetate and lead citrate. The sections were examined at 75 kV under an Hitachi H-800 electron microscope.

For observation of their three-dimensional structures, ultrathin sections were cut 100 nm in thickness, stained with 0.5% oölong tea polyphenol, and post-stained with uranyl acetate and lead citrate. The sections were carbon-coated and examined at 200 kV under an Hitachi H-800 electron microscope. Stereopairs were obtained by taking pictures of the same field after tilting the specimen $-15°$ or $+15°$ from the original (0°) position.

RESULTS

Type III collagen was seen in the portal and perisinusoidal regions of the fibrotic liver, and also along the sinusoidal wall (Fig.1). In cases of acute hepatitis, the portal tracts merely showed slight enlargement (Fig. 1A). In cases of chronic active hepatitis, numerous collagen fiber bundles were seen in the portal tract and the sinusoidal wall (Fig. 1B). Spiralled collagen (Fig. 2, arrows) was seen in portal fibrosis, but not in periportal tracts, the perisinusoidal region or sinusoidal wall (Fig. 2). Spiralled collagen (Fig. 3, arrows) was interspersed with normal collagen fibrils (with a mean diameter of 73.2 ± 4.8 nm) (Fig. 3). Spiralled collagen appeared as aggregated small filaments that were spiralled and retained the normal banding pattern (Fig. 4A). In cross-sections, the spiralled collagen had an irregularly-shaped flower-like appearance with a mean diameter of 174.7 ± 53.1 nm. The flower-like structure appeared to consist of aggregated small filaments (with a mean diameter of 33.6

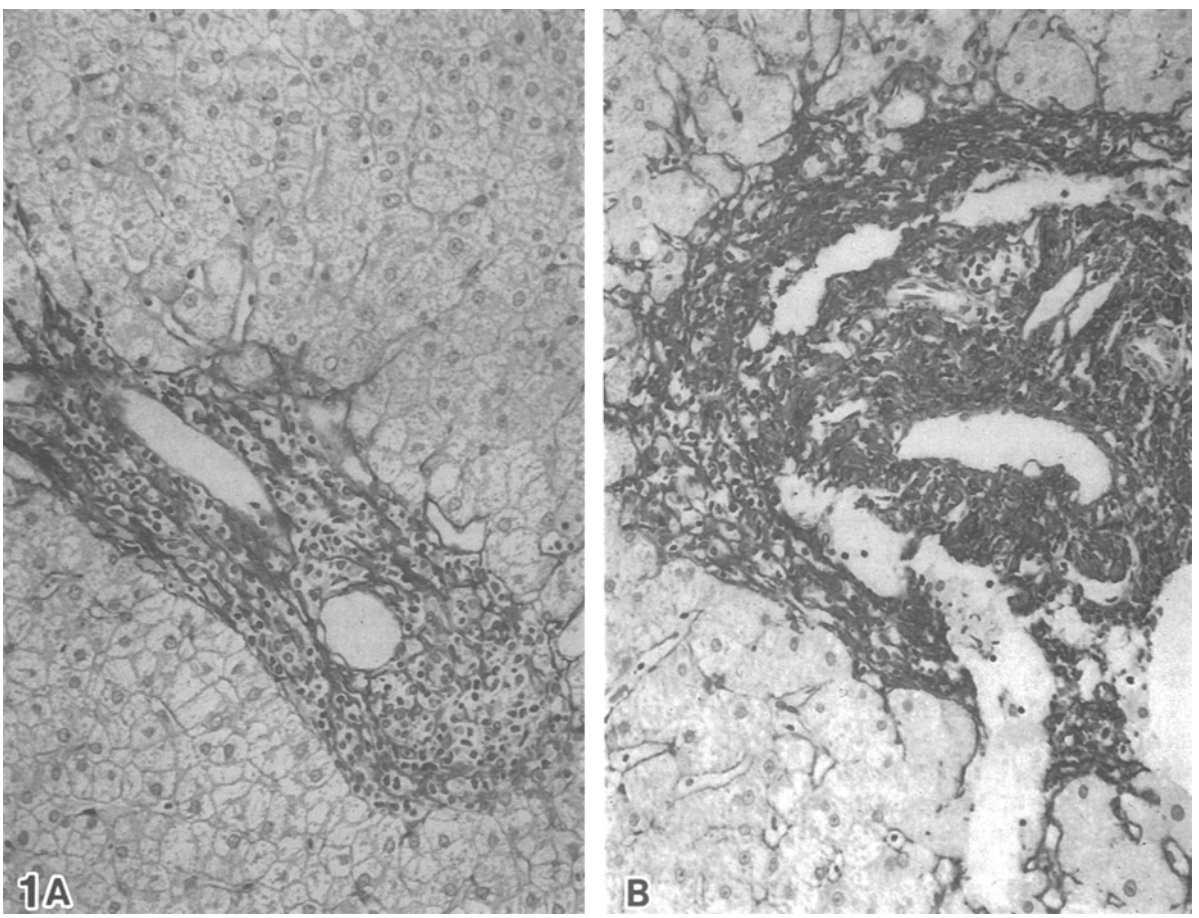
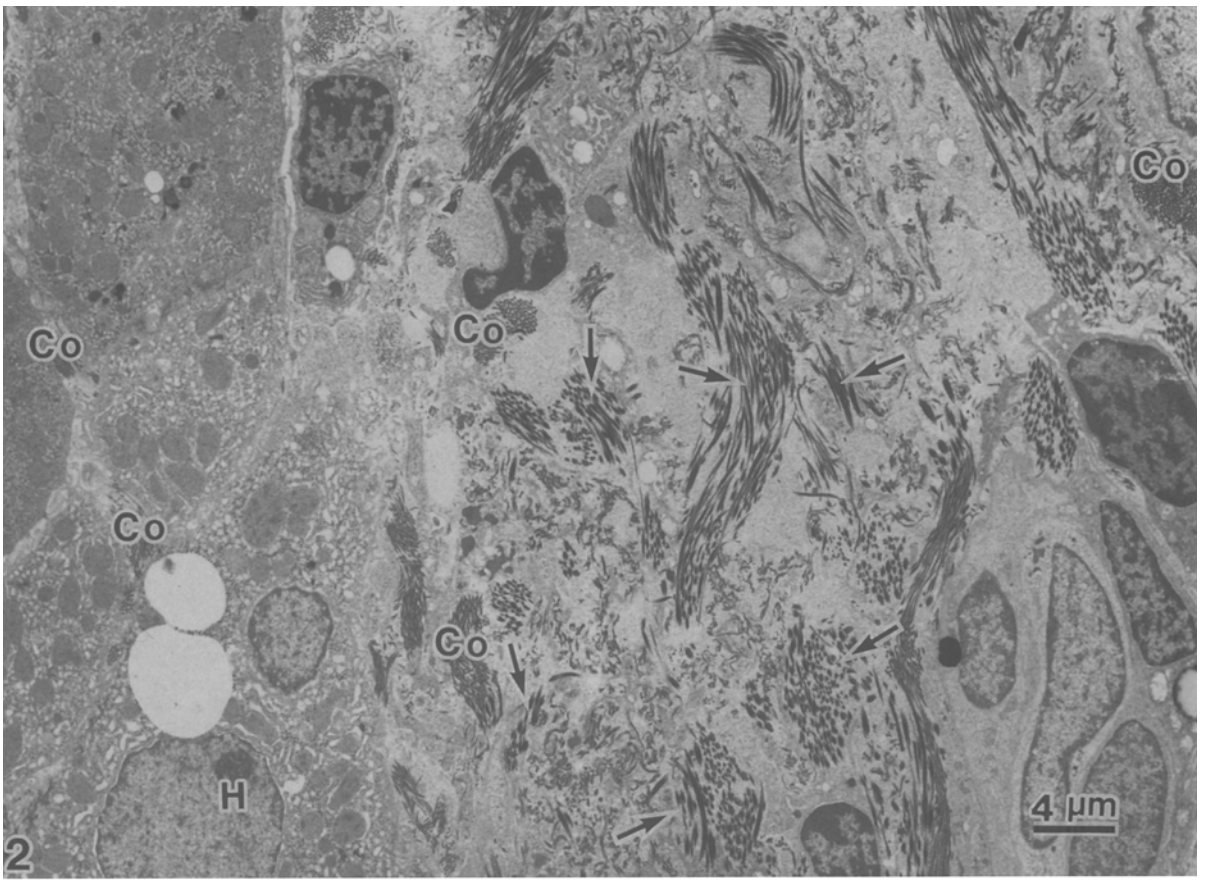


Fig. 1. Light micrographs showing immunolocalization of type III collagen. × 120. **A:** In this case of acute hepatitis (No. 3 in Table 1), a small amount of type III collagen is seen in the portal tract and sinusoidal wall. **B:** In this case of chronic active hepatitis (No. 21 in Table 1), a substantial amount of type III collagen is seen in the portal tract.

Fig. 2. An electron micrograph showing the region of portal fibrosis in a case of acute hepatitis (No. 3 in Table 1). Spiralled collagen (arrows) is seen in the portal tract, but not in the periportal region. Co: collagen fibrils, H: hepatocyte.

Fig. 3. In this case of chronic active hepatitis (No. 21 in Table 1), spiralled collagen (arrows) is interspersed with normal collagen.


Co
Co
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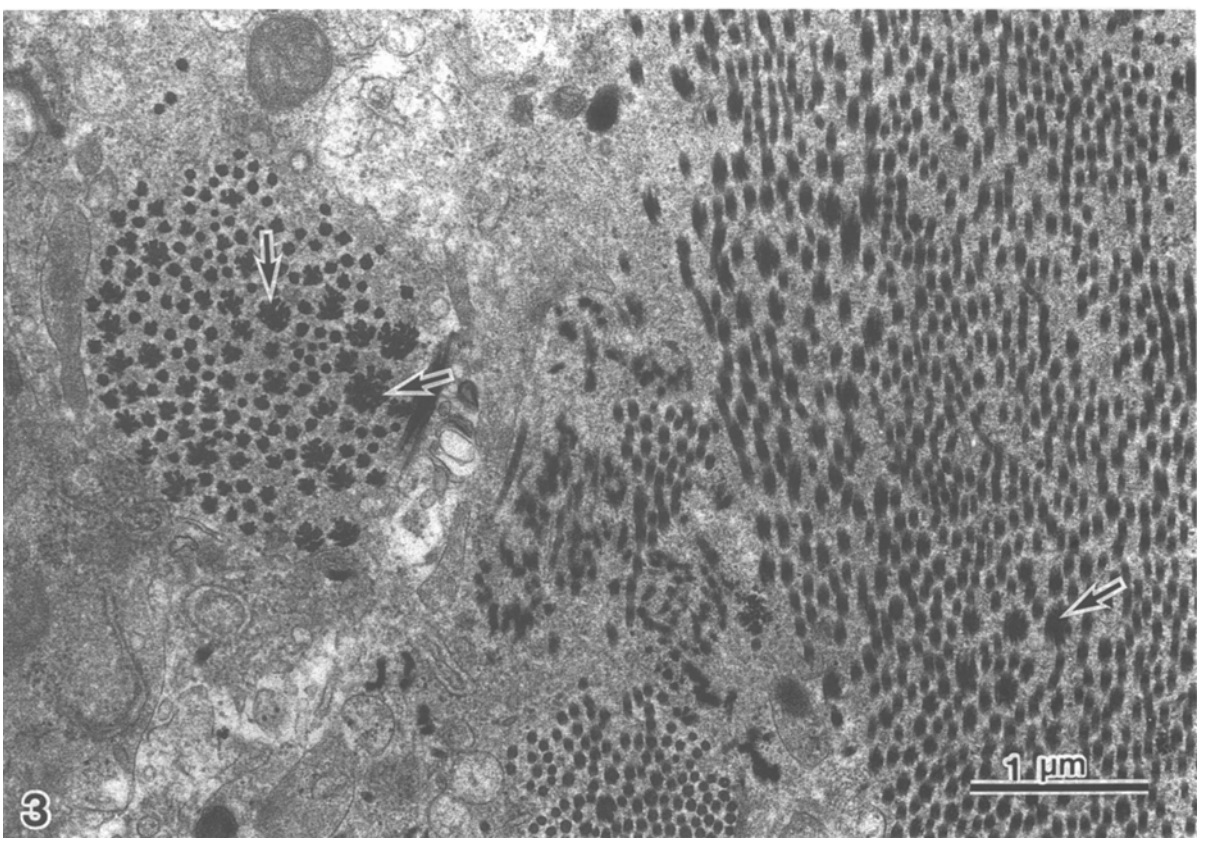
H
4 µm
2


1 µm
3

±8.2 nm) which were not homogeneous in size (Fig. 4B). In two cases of chronic active hepatitis, spiralled collagen was seen in regions of perivenular fibrosis. In cases of alcoholic hepatic fibrosis, spiralled collagen was seen in the portal tract. The central vein was not seen in cases of alcoholic liver disease, because the biopsy specimens were too small. The amount of spiralled collagen relative to the total amount of collagen fibrils was greater in cases of hepatitis than in cases of alcoholic liver disease (Table 1).

Looking at properly adjusted pairs of such pictures (Fig. 5A, B) with a stereoscopic binocular lens permits a three-dimensional visualization of spiralled collagen. In longitudinal section, spiralled collagen appeared to consist of aggregated small fibrils which were not homogeneous in size. Sometimes, the normal collagen fibrils branched off, and changed into small fibrils (Fig. 5, 6).

DISCUSSION

Tissue specimens from patients with chronic hepatitis and alcoholic liver disease showed marked portal

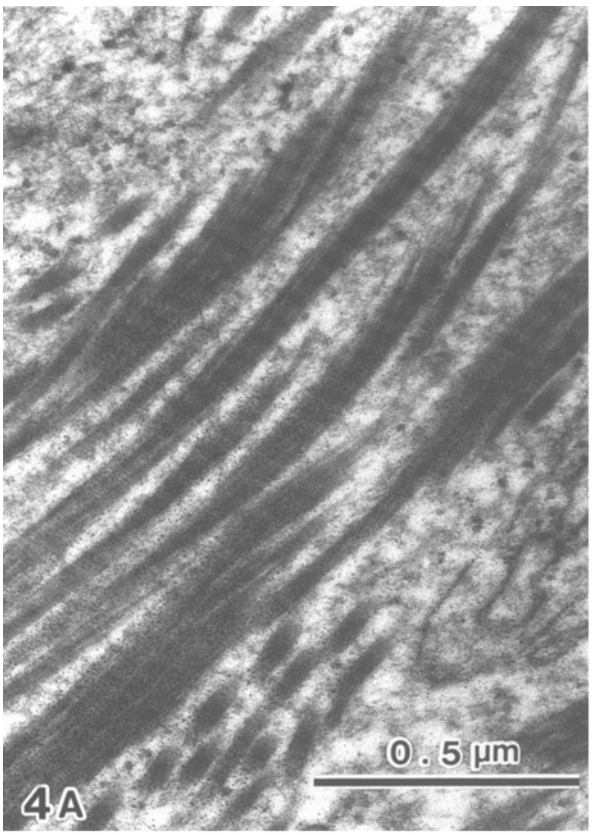
Table 1. Cases of liver diseases

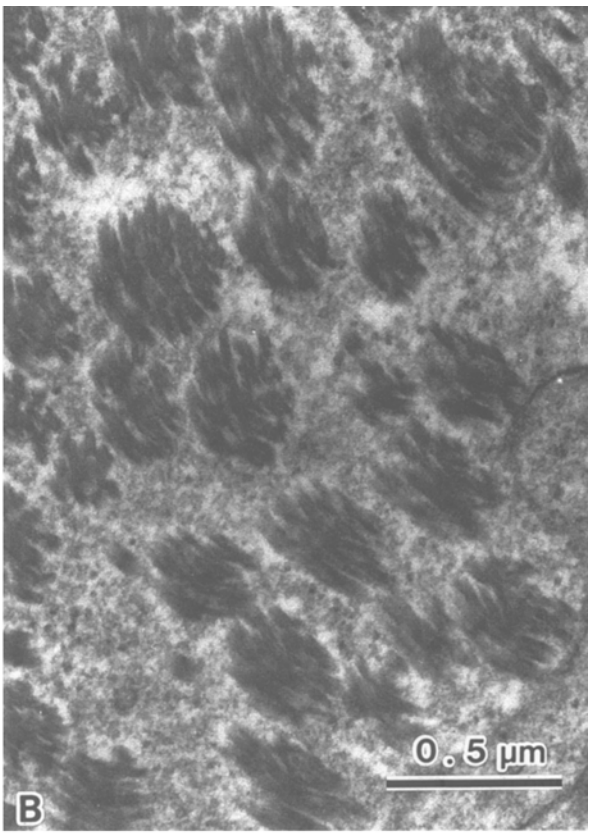
No.	Age	Sex	Diagnosis	The amount of spiralled collagen
1	30	M	Acute hepatitis (type A)	+
2	33	F	Acute hepatitis (type A)	+
3	35	M	Acute hepatitis (type A)	+++
4	37	M	Acute hepatitis protracted (type A)	+++
5	46	F	Acute hepatitis (type B)	+++
6	19	M	Chronic active hepatitis (HBV carrier)	+++
7	26	M	Chronic inactive hepatitis (type C)	+
8	27	M	Chronic inactive hepatitis (type C)	+++
9	29	M	Chronic active hepatitis (type C)	+++
10	40	F	Chronic active hepatitis with mild activity (type C)	+++
11	47	M	Chronic active hepatitis (type C)	++
12	52	M	Chronic active hepatitis (type C)	++
13	52	M	Chronic active hepatitis, lupoid hepatitis compatible	+++
14	52	F	Chronic active hepatitis (type C)	+
15	52	F	Chronic active hepatitis (type B)	++
16	54	F	Chronic active hepatitis (type C)	+
17	56	F	Chronic active hepatitis with severe fibrosis, precirrhosis (type C)	+
18	58	M	Chronic active hepatitis (type C), precirrhosis with fatty liver	++
19	58	F	Chronic active hepatitis (type C)	+++
20	59	M	Chronic active hepatitis with moderate activity (type C), irregular fatty morphosis	++
21	59	F	Chronic active hepatitis with mild activity (type C)	+++
22	63	F	Chronic active hepatitis precirrhosis (type C)	+++
23	64	F	Chronic active hepatitis (type C)	++
24	67	M	Chronic active hepatitis with moderate activity (type C)	+++
25	69	F	Chronic active hepatitis with superimposed acute necroinflammation (type C)	+++
26	58	M	Moderate liver fibrosis, alcoholic liver disease, compatible	++
27	62	M	Alcoholic liver cirrhosis with severe inflammation	+
28	63	M	Alcoholic fatty liver	+
29	64	M	Alcoholic liver cirrhosis	+
30	69	F	Alcoholic liver cirrhosis	++

+ : few, ++ : several, +++ : many (more than 10% of the total amount of collagen fibrils).

Fig. 4. Electron micrographs of spiralled collagen in a case of chronic active hepatitis (No. 21 in Table 1). **A:** Spiralled collagen has a spiralled appearance and displays the normal banding pattern in a longitudinal section. **B:** In a cross-section, spiralled collagen has an irregularly-shaped flower-like appearance.

Fig. 5. Electron micrographs showing stereopairs of spiralled collagen in the same field (No. 9 in Table 1). **A:** The original (0°) position. **B:** A tilted angle of +15°.


0.5 µm
4A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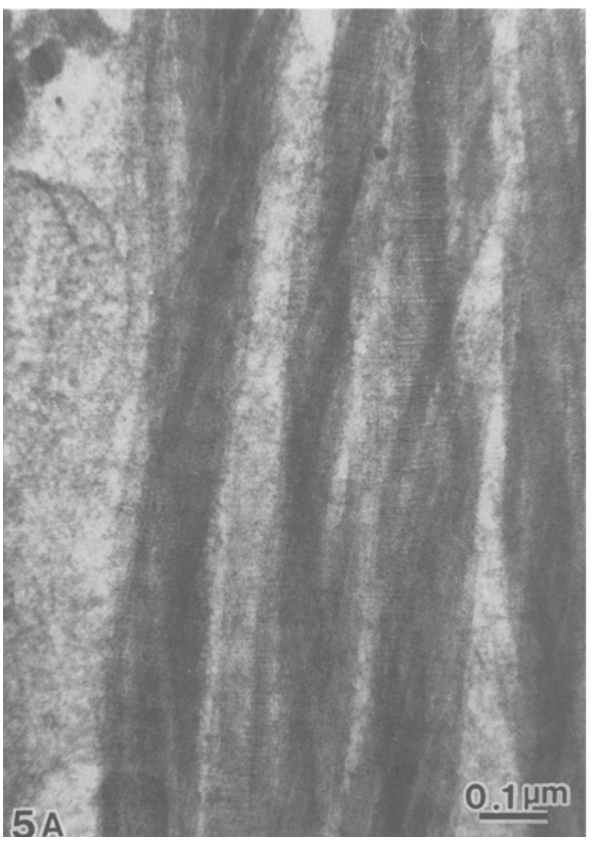
B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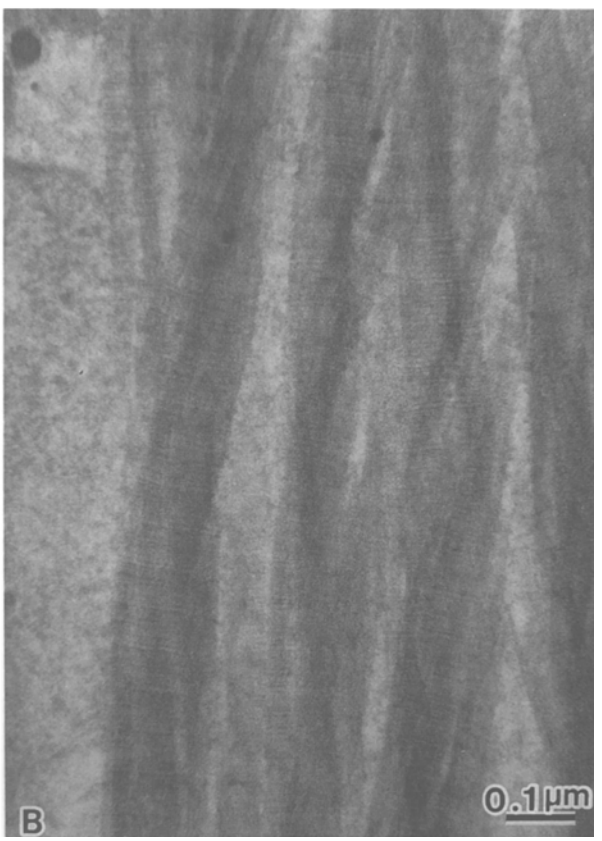
5A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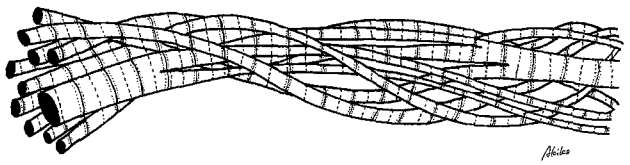

Fig. 6. A drawing of spiralled collagen based on information derived from observations made with a stereoscopic binocular lens.

fibrosis, and those from patients with acute hepatitis showed slight fibrosis in the portal tract. We detected spiralled collagen not only in cases of liver cirrhosis, but also in the slightly fibrotic liver (Table 1). It is unclear why the spiralled collagen found consistently in this study has escaped notice in previous studies of liver fibrosis using electron microscopy[12-14]. It is possible that in routine electron microscopic preparations collagen fibrils are not evident. Collagen fibrils are not stained by uranium and lead stains, so they are not readily visible by electron microscopy. Moreover, the aim of most research in this area has been limited to fibrosis of the Disse space[12-14]. In this study, spiralled collagen was not seen in the Disse space of the sinusoidal wall.

The occurrence of spiralled collagen is thought to be a degenerative phenomenon similar to that occurring in the lungs of rats exposed to nitrogen dioxide[15], rheumatoid connective tissues[10], collagen treated with urea[15], or liver tissue treated with different types of proteolytic enzymes[16]. On the other hand, VOGEL et al.[5] have concluded that the occurrence of spiralled collagen in Ehlers-Danlos syndrome is due to a genetic defect in collagen synthesis and polymerization. In this present study, we concluded that the occurrence of spiralled collagen in liver fibrosis is a degenerative phenomenon as indicated by observations of its three-dimensional structure (Fig. 6).

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