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Microvasculature in the small portal tracts in idiopathic portal hypertension

A morphological comparison with other hepatic diseases

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Summary. The morphology of the microvasculature in the small portal tracts was examined in normal livers, idiopathic portal hypertension (IPH) and other hepatic diseases. The microvasculature examined was arbitrary divided into two groups: that near the limiting plate and that within portal tracts, particularly around bile ducts. Based on comparisons of histology, immunohistochemistry and vascular casts, it is suggested that the former corresponded to inlet venules and the latter to distributing portal veins and peribiliary capillary plexus. Both of these microvasculatures were positive for *Ulex europaeus* lectin I, and (infrequently and weakly) for factor VIII-related antigen. Morphometry disclosed that inlet venules were reduced in number in IPH compared with normal livers and that distributing portal veins, peribiliary capillary plexus and inlet venules were increased in extrahepatic portal obstruction, chronic active hepatitis and extrahepatic obstructive cholestasis. We believe that the change in the microvasculature reflects abnormal microcirculation in the small portal tracts, and that the reduction of inlet venules plays an important role in the development of portal hypertension in IPH.

Key words: Inlet venules – Peribiliary capillary plexus – Idiopathic portal hypertension – Morphometry – Vascular casts

Introduction

Pathological studies of the liver and portal venous system in idiopathic portal hypertension (IPH) have disclosed that there are narrowing, obliteration, subintimal thickening, thromboemboli and phlebosclerosis of the portal venous radicles, elastosis and sclerosis of the portal tract, thickening of hepatic veins, and atrophy of liver parenchyma (Mikkelsen et al. 1965; Nayak and Ramalingaswani 1969; Boyer et al. 1974; Aikat et al. 1979; Aida 1981; Kage 1981; Okuda et al. 1982; Nakanuma et al. 1984). In particular, the portal venous changes in the small portal tracts have been suggested as a major contributing factor to presinusoidal portal hypertension in IPH (Aida 1981; Kage 1981). However, a precise examination of the microcirculation in the small portal tracts in situ has not been made in IPH. In particular the respective pathologies of several elements including the peribiliary capillary plexus and the branches of portal veins in the small portal tracts have not been evaluated in IPH.

We describe here the morphology of the microcirculation of the small portal tract in IPH and compared the findings with those in normal livers, in extrahepatic portal venous obstruction (EHO), chronic active hepatitis (CAH) and extrahepatic obstructive cholestasis (EHC).

Materials and methods

Forty cases of IPH (surgical biopsy, 32 cases; autopsied livers, 8 cases; age range, 47–74 years; male to female = 2:38) were examined. Material from 4 autopsied cases of extrahepatic portal venous obstruction (age range, 46–75 years; male to female = 1:3) was available; 9 cases of CAH (surgical biopsy, 8 cases; autopsied liver, 1 case; age range, 36–66 years; male to female = 5:4) and 4 surgical biopsy cases of EHC (age range, 46–58 years; male to female = 3:1) were also studied. Thirty five normal livers (surgical biopsy, 2 cases; autopsied livers, 33 cases; age range, 31–67 years; male to female = 22:13) were used as controls. The diagnosis of idiopathic portal hypertension was according to the diagnostic manual of the Japanese Study Group for Portal Venous Blood Flow Abnormalities (1987). In this manual IPH consists of a variety of conditions

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including splenomegaly, anaemia and sustained portal hypertension in the absence of liver cirrhosis, blood diseases, parasites, and occlusion of the hepatic vein and extrahepatic portal vein. It is the equivalent of hepatoportal sclerosis or non-cirrhotic portal fibrosis in other countries terminology. One tissue block was obtained in each of biopsy cases and several tissue blocks were available in autopsy cases. They were fixed in 10% formalin and embedded in paraffin. Five μm sections were made from each tissue block and stained with haematoxylin and eosin (H&E) as well as elastica van Gieson (EVG).

For the characterization of the vascular endothelium, all of the formalin-fixed and paraffin-embedded 5 μm sections were stained for factor VIII-related antigen (FVIII-R-Ag) and with *Ulex europaeus* agglutinin I (UEA-I) using the avidin-biotin-peroxidase complex (ABC) method according to Hsu et al. (1981). Anti-human FVIII-R-Ag antibodies and biotinylated UEA-I were purchased from Dakopatts (Denmark) and Vector Laboratories (Burlingame, California), respectively. Protease digestion was done prior to FVIII-R-Ag stain by immersion of sections for 30 min in 0.1% trypsin (trypsin type I, Sigma Chemical Co., St. Louis) in Tris-HCl buffered saline (TBS). The specificity of the stains was confirmed by substituting TBS and non-immune rabbit serum for the primary anti FVIII-R-Ag antisera and UEA-I, and also by absorption of anti FVIII-R-Ag antibodies by factor VIII (Diagnostica Stago, Asnieres, France) and of biotinylated UEA-I by L(−)-fucose (Wako Pure Chemicals, Osaka).

In the present study, the microvasculature in the small portal tract was defined as small vessels, free of smooth muscles. Since these were recognized most clearly on UEA-I-stained sections (*vide infra*), they were arbitrary divided in these sections into two groups: vessels abutting on the limiting plate and those within the portal tract remote from the limiting plate. The former vessels were very likely to correspond to inlet venules and the latter vessels to distributing portal veins and peribiliary capillary plexus (*vide infra*).

In all small portal tracts less than 300000 μm^2 in area, the circumference and area of the portal tract were measured on UEA-I-stained sections, using a computed imaging analytical apparatus (Nexus 6400, Kashiwagi Laboratory, Tokyo), a personal computer (PC-9801, NEC, Tokyo) and a video camera (BK5001, Hitachi Electronics, Tokyo). The number of vessels abutting on the limiting plate per unit length of the limiting plate and the number of small vessels within portal tracts per unit area of the portal tract were calculated. Statistically, Student's *t* test was employed with a significant level less than 5%.

Hepatic arterial casts and portal venous casts were made from two normal adult autopsied livers, respectively. At autopsy, the portal venous trunk of one liver and hepatic arteries of another liver were cannulated by polyethylene tubes. After perfusing the portal vein or hepatic arteries with Ringer's solution, synthetic resins Mercox (Dainippon Ink Co. Ltd., Tokyo) diluted in 1:1 by methylmethacrylate monomer free of hydroquinone (Nakarai Chemicals, Kyoto) were injected into the portal vein at the pressure of 10–20 mmHg and into the hepatic arteries at the pressure of 100–150 mmHg. After immersing the livers in a water bath (60° C) for 24 hr to polymerize the resins, the livers were macerated in 30% NaOH solution in a water bath (60° C) until tissues became completely corroded. The vascular casts thus obtained were washed in running water, dried in air and observed under a stereomicroscope. Then, the well-prepared peripheral portions of the casts (1 × 1 cm) were cut off by a razor, mounted on metal stubs and sputter-coated with palladium-platinum by an ion coater (IB-2, Eiko Engineering Co. Ltd., Ibaragi, Japan). The coated casts were observed under the scanning electron microscope (Hitachi, S-511) with an accelerating voltage of 25 kV.

In order to evaluate the three dimensional architecture of the microvasculature in the small portal tracts, 200 serial sections (6 μm in thickness) were obtained from a normal liver as well as from an IPH liver, respectively, and all were stained with UEA-I.

Results

The microvasculature in the small portal tracts was difficult to discern on H&E or EVG stains in normal livers. While the endothelium of portal venous and hepatic arterial radicles were positive for both FVIII-R-Ag and UEA-I in all cases (Fig. 1) the endothelium of the microvasculature was focally and weakly positive for FVIII-R-Ag in about 30% of cases and almost negative for FVIII-R-Ag in the remaining 70% of cases (Fig. 1 A). The endothelium was positive for UEA-I in all cases (Fig. 1 B). These UEA-I-positive vessels were more or less dense around bile ducts and also at the limiting plate (Fig. 1 B). Sinusoidal lining cells were negative for FVIII-R-Ag or UEA-I

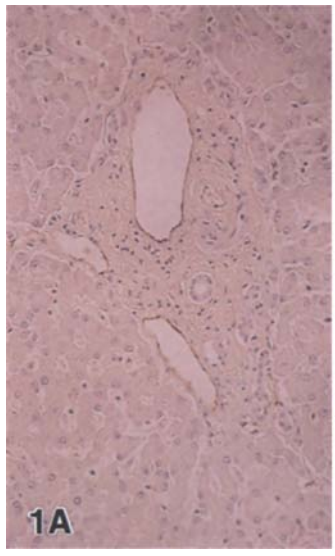
Fig. 1. Serial sections of the normal liver. **A** A hepatic artery and portal venous radicles are positive for factor VIII-related antigen. Factor VIII-related antigen (ABC method) and haematoxylin, ×40. **B** In addition to artery and portal veins, several small vessels in the vicinity of the limiting plate (*arrows*) and within the portal tract (*arrowhead*), particularly around bile ducts, are positive for UEA-I. UEA-I (ABC method) and haematoxylin, ×40

Fig. 2. Scanning electron microscopic view of small portal venous casts. The portal radicle (*large white arrows*) regularly give off distributing portal veins (*small white arrows*) from which inlet venules (*arrowheads*) are branched. Inlet venules, in turn, give rise to sinusoid (*S*). *Bar*, 500 μm

Fig. 3. Higher magnification of Fig. 2. *Arrows*: distributing portal vein. *Arrowheads*: inlet venules. *S*: sinusoid. *Bar*, 200 μm

Fig. 4. Scanning electron microscopic view of small hepatic arterial casts. Plexus casts (peribiliary capillary plexus, *arrows*) are seen around the hepatic artery (*HA*). A *large arrow* indicates the direction of blood flow. *S*, sinusoid. *Bar*, 100 μm

Fig. 5. Scanning electron microscopic view of small hepatic arterial casts. Network of peribiliary capillary plexus (*PCP*) are seen around the hepatic artery on the left upper part. The plexus are communicating with sinusoid (*S*) via radicular portal vein (*arrow*). *HA*, hepatic artery. *Bar*, 200 μm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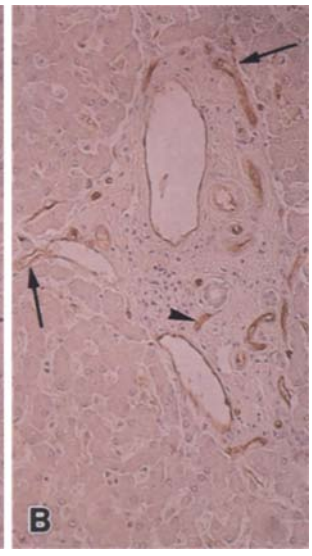
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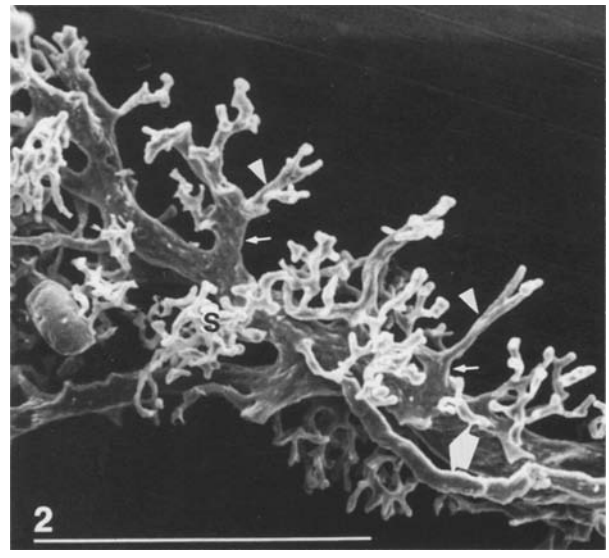
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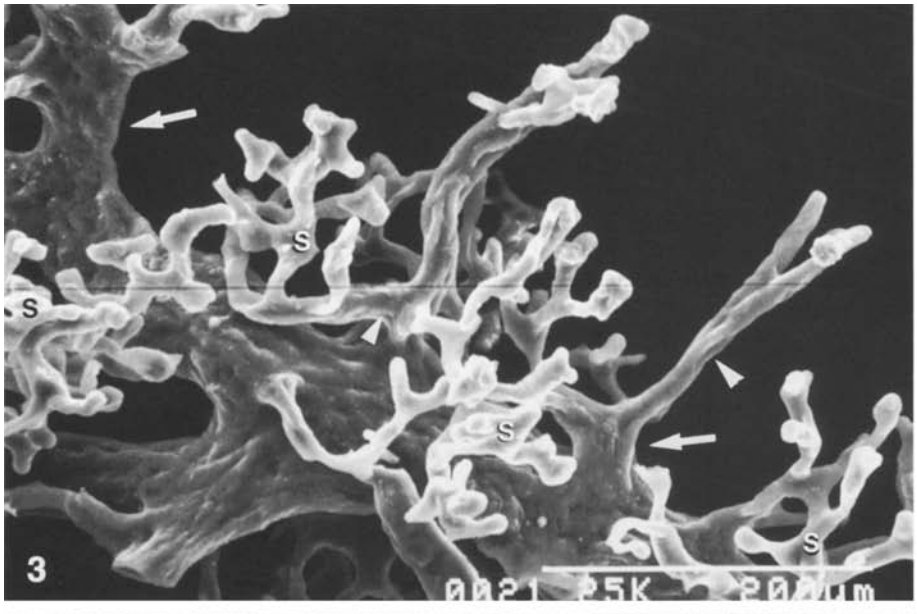
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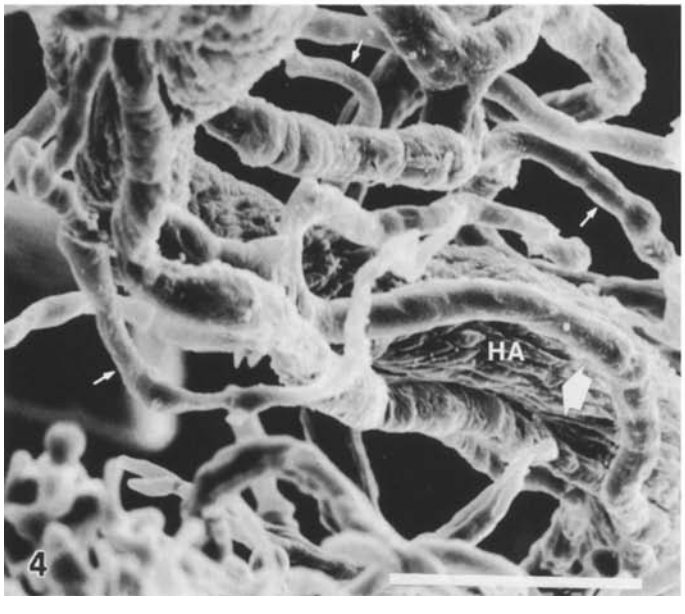
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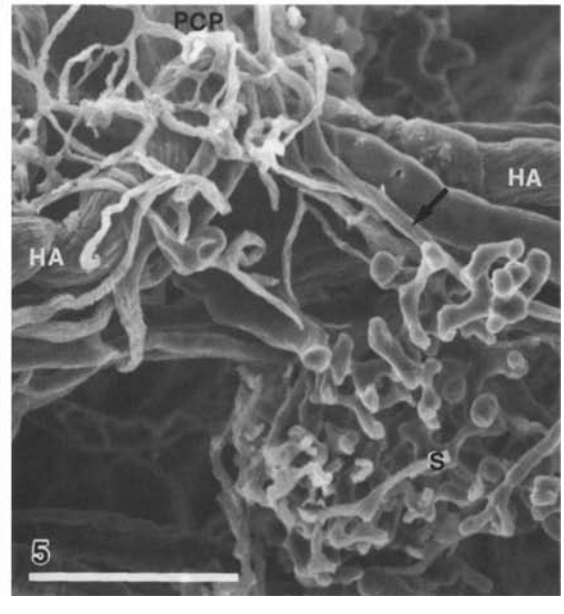
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Table 1. Expression of factor FIII-related antigen and *Ulex europaeus* agglutinin I in endothelium of the microvasculature in the small portal tract and sinusoids

Materials		Vessels abutting on the limiting plate		Small vessels within portal tract		Sinusoidal lining cells	
		FVIII	UEA-I	FVIII	UEA-I	FVIII	UEA-I
NL	($n=35$)	9 (26%)	35 (100%)	12 (34%)	35 (100%)	9 (0%)	0 (0%)
IPH	($n=40$)	18 (45%)	40 (100%)	16 (40%)	40 (100%)	22 (55%)	34 (85%)
EHO	($n=4$)	1 (25%)	4 (100%)	1 (25%)	4 (100%)	1 (25%)	4 (100%)
CAH	($n=9$)	7 (78%)	9 (100%)	7 (78%)	9 (100%)	9 (100%)	9 (100%)
EHC	($n=4$)	3 (75%)	4 (100%)	3 (75%)	4 (100%)	4 (100%)	4 (100%)

Figures are positive cases. Parentheses are percentage of positive cases. FVIII, factor VIII-related antigen. UEA-I, *Ulex europaeus* agglutinin I. NL, normal liver. IPH, Idiopathic portal hypertension. EHO, extrahepatic portal obstruction. CAH, chronic active hepatitis. EHC, extrahepatic obstructive cholestasis

(Fig. 1). These histochemical characteristics are shown in Table 1. Scanning electron microscopic observations of vascular casts disclosed that the portal venous casts regularly gave off distributing portal veins (Elias 1946), which in turn branched into many inlet venules communicating with the sinusoid (Figs. 2 and 3). Hepatic arterial casts, however, disclosed that the arterial radicle gave off vascular plexus around the bile ducts (peribiliary capillary plexus) (Murakami et al. 1974) (Fig. 4) and also that the peribiliary plexus were infrequently continuous with the sinusoid via radicular portal veins (Ohtani 1979) (Fig. 5). Serial section observations showed that portal veins regularly gave off distributing portal veins and inlet venules (Fig. 6). Distributing portal veins were situated within portal tracts, while inlet venules were seen near the limiting plate. In contrast, hepatic arteries gave off anastomosing capillary channels within the portal tracts, particularly around bile ducts. These capillary channels were rarely located near the limiting plate.

In IPH, the portal venous radicles showed variable degrees of stenosis, disappearance and sclerosis (Figs. 7, 8 and 9), and portal tracts themselves also showed elastosis and sclerosis in variable degrees (Figs. 7 and 8). The microvasculature in the portal tracts was focally and weakly positive for FVIII-R-Ag in about 40% of cases and almost negative for FVIII-R-Ag in the remaining cases. The microvasculature was positive for UEA-I in all cases. UEA-I-stained sections disclosed that the microvasculature tended to be sparse in the sclerotic portal tracts (Figs. 7 and 8) and be normal or slightly increased in non-sclerotic portal tracts (Fig. 9). Aberrant vascular channels (Fukuda et al. 1985), which were scattered adjacent to non-sclerotic portal tracts, were positive for FVIII-R-Ag and UEA-I (Fig. 9). Serial section observations revealed that portal venous radicles rarely gave off distributing portal veins and inlet venules and that small vessels within the portal tracts were mainly connected with hepatic arterial branches. Some of sinusoidal lining cells, particularly those in areas with severe parenchymal atrophy, were positive for FVIII-R-Ag and UEA-I (Fig. 7) in a considerable number of cases (Table 1).

Table 2. Number of vessels abutting on the limiting plate per 200 μm of the limiting plate

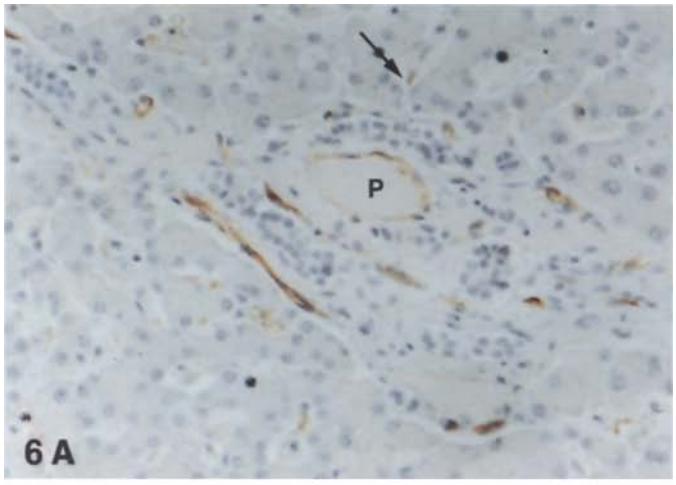
Materials		No. of vessels ($m \pm SD$)
NL	($n=35$)	1.09 ± 0.42[a]
IPH	($n=40$)	0.72 ± 0.59[a]
EHO	($n=4$)	1.86 ± 0.56[a]
CAH	($n=9$)	1.88 ± 0.49[a]
EHC	($n=4$)	1.97 ± 0.23[a]

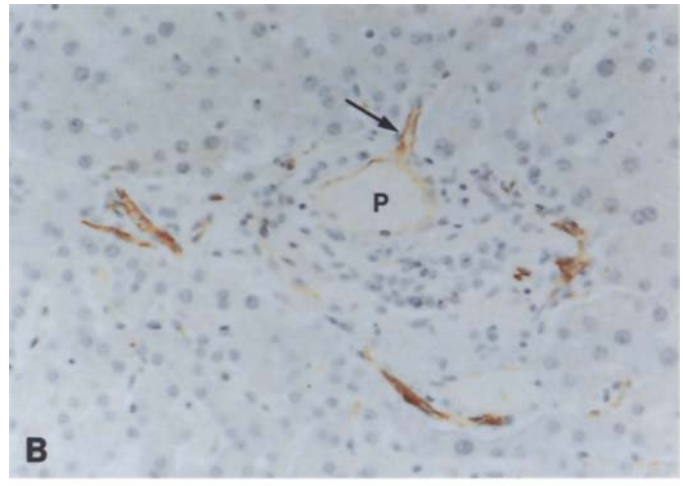
NL, normal liver. IPH, Idiopathic portal hypertension. EHO, extrahepatic portal obstruction. CAH, chronic active hepatitis. EHC, extrahepatic obstructive cholestasis

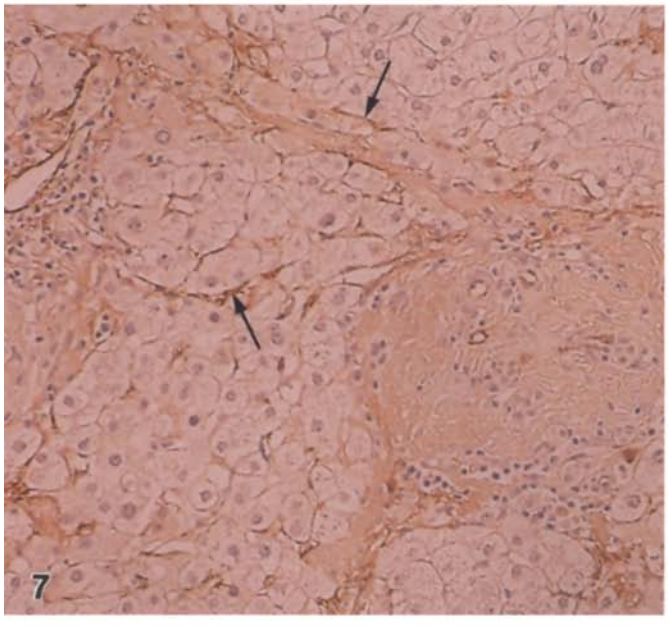
[a] $p < 0.005$ (NL vs IPH, EHO, CAH and EHC)

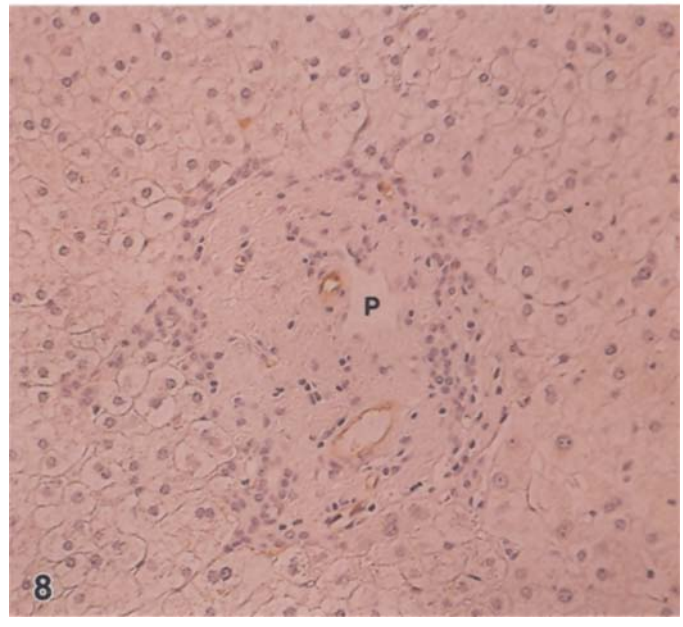
In EHO, the immunohistochemical characteristics of the endothelium of the microvasculature were almost the same as in IPH (Fig. 10 and Table 1), although vessels in portal tracts were more abundant than in normal livers and IPH (Fig. 10). In CAH and EHC, the microvasculature was also abundant and was positive for FVIII-R-Ag in about 80% of cases and for UEA-I in all cases (Fig. 11 and Table 1). Sinusoidal lining cells, particularly those in periportal areas, were rather strongly positive for FVIII-R-Ag and UEA-I in all cases (Fig. 11 and Table 1).

The morphometric data are shown in Tables 2 and 3. In IPH, the number of vessels abutting on the limiting plate per unit length of the limiting plate was reduced compared with that in normal









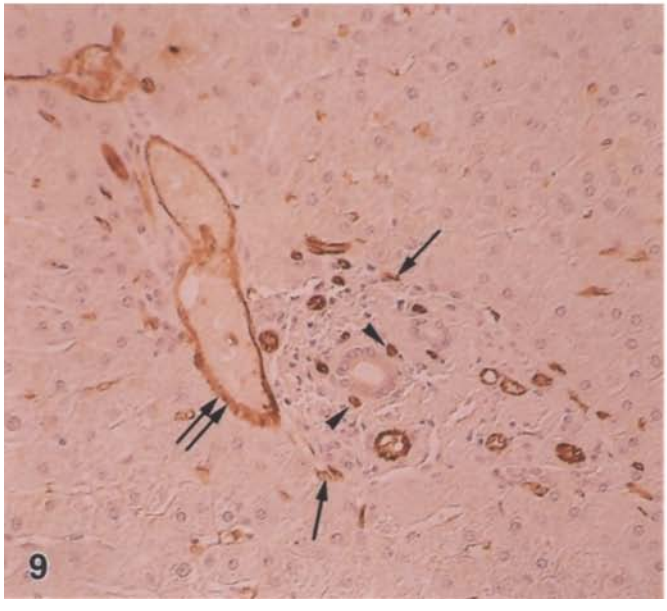


Fig. 6. Serial sections in the normal liver. The vessels abutting the limiting plate (*arrows*) communicate with portal venous radicles (*P*). The distance of A and B is 18 μm. UEA-I (ABC method) and haematoxylin, × 60

Fig. 7. Small portal tracts and atrophic parenchyma in idiopathic portal hypertension. Few portal radicles and microvasculatures are present in the sclerotic portal tracts. Reaction products of UEA-I are present in some part of the sinusoidal lining cells (*arrows*). UEA-I (ABC method) and haematoxylin, × 100

Fig. 8. A sclerotic portal tract in idiopathic portal hypertension. The portal venous radicle (*P*) is stenotic and microvasculatures are rarely present. UEA-I (ABC method) and haematoxylin, × 100

Fig. 9. A non-sclerotic portal tract in idiopathic portal hypertension. The portal venous radicle is obliterated. There are a small number of microvasculatures adjacent to the limiting plate (*arrows*) and also within the portal tract, particularly around bile ducts (*arrowheads*). An aberrant vascular channel is present (*double arrows*). UEA-I (ABC method) and haematoxylin, × 100

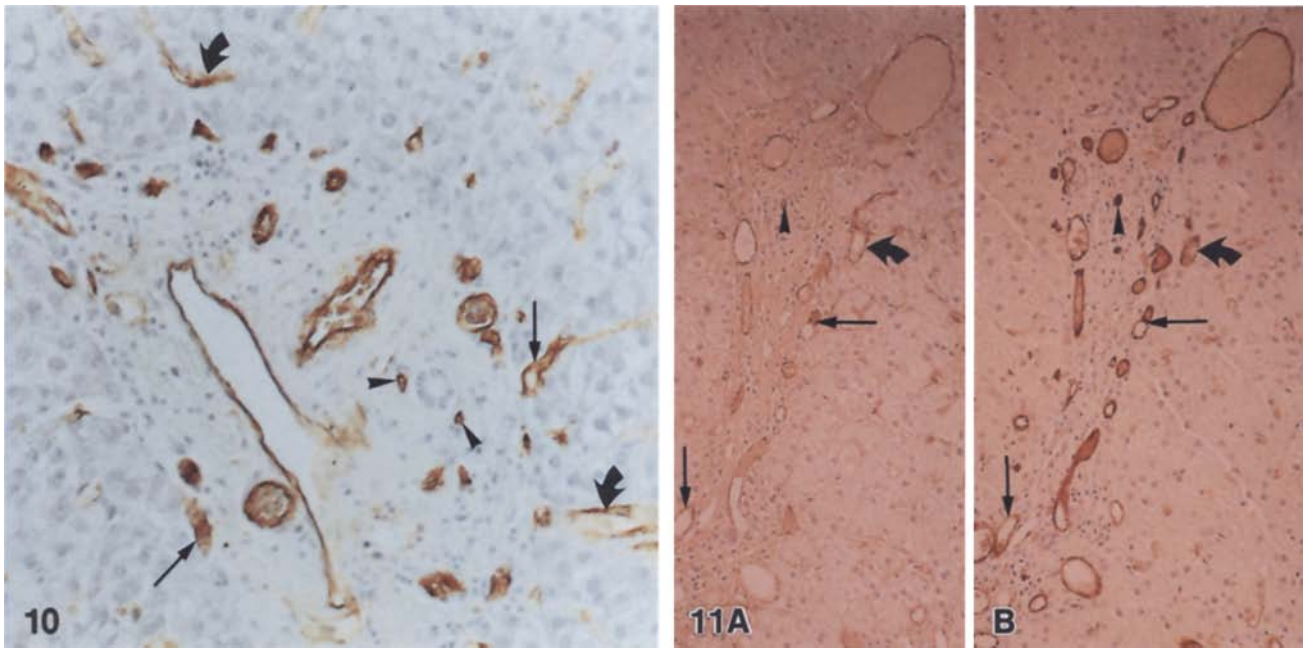


Fig. 10. A small portal tract in extrahepatic portal venous obstruction. There are UEA-I-positive small vessels adjacent to the limiting plate (*arrows*) and within the portal tract (*arrowheads*). Some of the sinusoidal lining cells are positive for UEA-I (*curved arrows*). UEA-I (ABC method) and haematoxylin, ×100

Fig. 11. Serial sections of the portal tract and fibrous septum in chronic active hepatitis. The small vessels abutting on the limiting plate (*straight arrows*) and within the portal tract (*arrowhead*) are positive for factor VIII-related antigen and UEA-I. Some of the periportal sinusoidal lining cells are positive for factor VIII-related antigen and UEA-I (*curved arrows*). **A** Factor VIII-related antigen (ABC method) and haematoxylin, ×40. **B** UEA-I (ABC method) and haematoxylin, ×100

Table 3. Number of small vessels within the portal tract per 40000 μm^2 of the portal tract

Materials		No. of small vessels (m ± SD)
NL	($n=35$)	$2.66 \pm 1.30^{a, b, c}$
IPH	($n=40$)	2.07 ± 2.85^{a}
EHO	($n=4$)	4.47 ± 1.58^{b}
CAH	($n=9$)	4.59 ± 1.89^{c}
EHC	($n=4$)	5.12 ± 1.14^{c}

NL, normal liver. IPH, Idiopathic portal hypertension. EHO, extrahepatic portal obstruction. CAH, chronic active hepatitis. EHC, extrahepatic obstructive cholestasis. [a] not significant (NL vs IPH); [b] $p<0.01$ (NL vs EHO); [c] $p<0.005$ (NL vs CAH and EHC)

livers ($p<0.005$). In EHO, the number of both vessels abutting on the limiting plate and small vessels within portal tracts was larger than that in normal livers ($p<0.01$). In CAH and EHO, both the number of vessels abutting on the limiting plate and the small vessels within portal tracts were larger than those in normal livers ($p<0.005$).

Discussion

UEA-I-stained serial section observations of the normal liver disclosed that portal venous radicles frequently gave off distributing portal veins and inlet venules. The distributing portal veins were located within portal tracts, whereas inlet venules were situated in the vicinity of the limiting plate. Serial section observations also showed that hepatic arteries gave off capillary plexus within portal tracts. Thus, it seem likely that UEA-I-positive microvasculature near the limiting plate was largely composed of inlet venules and that within portal tracts originated from peribiliary capillary plexus and distributing portal veins. The scanning electronmicroscopic findings; that the portal venous casts gave off distributing portal veins (Elias 1946) and inlet venules connecting with the sinusoids, and that hepatic arterial casts showed a plentiful plexus probably corresponding to peribiliary capillary plexus (Murakami et al. 1974), supports the above-mentioned suggestion.

It is generally accepted that the endothelium of blood vessels is positive for FVIII-R-Ag and UEA-I (Little et al. 1986), but the majority of the vasculature in the small portal tracts was positive for UEA-I and only infrequently for FVIII-R-Ag. FVIII-R-Ag is known to be particularly prone to lose its antigenicity during fixation, which may have occurred here. A majority of UEA-I-positive

small vessels, with or without FVIII-R-Ag positivity, were shown to be derived from the portal vein or hepatic arterial branches in this study. Some of FVIII-R-Ag-negative and UEA-I-positive vessels may, however, be lymphatics.

It was noted in this study that inlet venules were decreased in IPH, and this decrease seems to lead to the reduction of portal venous vascular bed. Thus, the decrease in inlet venules may be one of the major factors in the development of sustained portal hypertension in IPH, together with the narrowing, destruction and phlebosclerosis of portal venous radicles. Scerlosis of the portal tract is clearly also important. However, it remains to be seen whether the changes in the inlet venules is a primary event causing portal hypertension or is secondary to the ischaemia caused by narrowing of the portal venous radicles. In EHO, inlet venules, distributing portal veins and peribiliary capillary plexus were increased in number, demonstrating that haemodynamics in the small portal tract is different between IPH and EHO and also that the peribiliary capillary plexus may play a part in hepatopetal collaterals as suggested from our laboratory (Ogawa et al. 1987; Terada et al. 1988). The increased inlet venules, distributing portal veins and peribiliary capillary plexus in CAH and EHC may be due to increased portal and arterial blood flow caused by portal tract inflammation.

In the present study, sinusoidal lining cells were negative for FVIII-R-Ag or UEA-I in normal livers, whereas they were focally positive in most of IPH and EHO and rather strongly positive in all of CAH and EHC. The expression of FVIII-R-Ag and UEA-I in the sinusoidal lining cells in IPH and EHO may imply that the hemodynamics of the liver are localy varied and that characteristics of sinusoidal lining cells become similar to the endothelium of capillaries or venules. In CAH and EHC, strong expression of FVIII-R-Ag and UEA-I was seen, mainly in the periportal sinusoidal lining cells. This suggests that lobular architecture had been disturbed in the periportal parenchyma, as indicated by Fukuda et al. (1986).

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