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Quantitation and serial section observations of focal venocclusive lesions of hepatic veins in liver cirrhosis

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Summary. The pathogenesis and functional significance of the venocclusive (VO) lesions in small hepatic veins occurring in liver cirrhosis, remain controversial. The present study, using quantitative examination and serial sections has disclosed that these lesions are present in 71.7% of 106 autopsy livers with alcoholic, HBsAg-positive, biliary or cryptogenic cirrhosis. The lesions were usually focal: their number in a liver section (10 cm^2) was below 15 in 86.7% of the livers having them. The incidence and morphology of the lesions appeared similar in cirrhotic livers with different aetiology. Serial sections disclosed that the affected veins disappeared within the fibrous stroma at one side and were directly connected with the patent larger hepatic veins at other side, indicating that these veins had lost their function as a draining vein of the hepatic parenchyma. In addition, there was frequent recanalization within the VO lesions, and the recanalized vessels frequently communicated with neighboring thin-walled veins in cirrhotic stroma, suggesting an intrahepatic vein to vein anastomosis. In conclusion, VO lesions, when focal, may themselves be responsible to a lesser degree for obstruction of hepatic venous outflow in liver cirrhosis.

Key words: Liver cirrhosis – Hepatic venous occlusion – Intrahepatic venous anastomosis

There have been several reports on venocclusive (VO) lesions confined to small tributaries of the hepatic veins (Bras et al. 1954; Fajardo and Colby 1980; Goodman and Ischak 1982; Nakanuma et al. 1980; Shulman et al. 1980). For example, "venocclusive disease of the liver", which involves central and sublobular veins extensively, is associated with ascites and hepatomegaly and is causally related to ingestion of certain alkaloids or drugs, or to irradiation (Bras et al. 1954; Fajardo and Colby 1980; Shulman et al.

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1980). Recently, Goodman and Ishak (1982) have reported on another form of VO lesion as one of the vasculopathies in alcoholic liver disease. Further, a similar VO lesion is encountered routinely in our laboratory not only in alcoholic liver cirrhosis but also in liver cirrhosis with other aethiologies. The exact incidence, functional significance and pathogenesis of these VO lesions have not been reported to date.

In the present study, using quantitative examinations and serial sections, we tried to find out how often the lesions occur in several types of cirrhosis and whether there are any differences in morphology and distribution with different aetiologies. In addition, we also tried to evaluate the functional significance of the lesions.

Materials and methods

The material consisted of 27 livers with alcoholic cirrhosis, 15 livers with HBsAg positive cirrhosis, 24 livers with biliary cirrhosis (22 were primary and 2 secondary) and 40 livers with cryptogenic cirrhosis. All were adult autopsy livers without primary or metastatic tumors and all but primary biliary cirrhosis (PBC) livers were collected from our recent autopsy cases. Livers with PBC were kindly given from many Pathological Departments in Japan, including our University Hospital.

An autopsy liver with hepatic venocclusive disease after allogeneic bone marrow transplantation was used as a control. This patient, a female 20 years of age, had suffered from acute lymphatic leukaemia for about one and a half years, and then repeated relapses had occurred despite chemotherapy and autologous bone marrow transplantation. Finally, allogeneic bone marrow transplantation from her HLA identical sister was performed. Two weeks later, jaundice, ascites, hepatomegaly and abdominal pain developed, and she died of hepatic failure 42 days later after the allogeneic transplantation.

Liver blocks were obtained from the right and the left hepatic lobes in each case. These blocks were fixed in 10% formalin and embedded in paraffin, and histological sections were stained with H&E, elastica van Gieson (EVG), Gomori's reticulin stain and Shikata's orcein stain (Sano 1976; Shikata et al. 1974). The area of each section was about 5 cm^2.

About 200 serial sections were cut from each paraffin block of one case of alcoholic cirrhosis and one of PBC, respectively, and were stained with H&E and EVG, alternatively.

The VO lesions were considered present if prominent intimal thickening by fibrosis with or without oedema was seen along almost all circumferences of the affected veins, the lumena of which were narrowed or occluded. Segmental intimal thickening, phlebosclerosis, which was recognized by a widening of the periadventitial fibrous tissue rather than the intimal and medial thickening, and aggregates of elastic fibers in the vascular walls were not regarded as the VO lesion. Using 2 liver sections per case, the number of VO lesions in a certain area of liver section (10 cm^2) was calculated. The shortest caliber between the external surfaces of the intima of the affected veins was measured.

Autopsy protocol and clinical summary were surveyed: Patients who had a history of excessive alcohol intake (over 80 g/day for more than 10 years) were regarded as alcoholics. According to Goodman and Ishak, portal hypertension was considered to be present if the autopsy revealed oesophageal varices, ascites ($\geqq$300 ml), or splenomegaly ($\geqq$400 g).

Association between variables was tested using the x^2-test and the frequency of positive ratio was compared with Fisher's exact test. $p < 0.05$ was considered significant.

Result

1. Morphology of the VO lesions in liver cirrhosis

The VO lesions with luminal narrowing or occlusion, when stained with either EVG or Shikata's orcein method, were clearly demonstrated (Figs. 1–

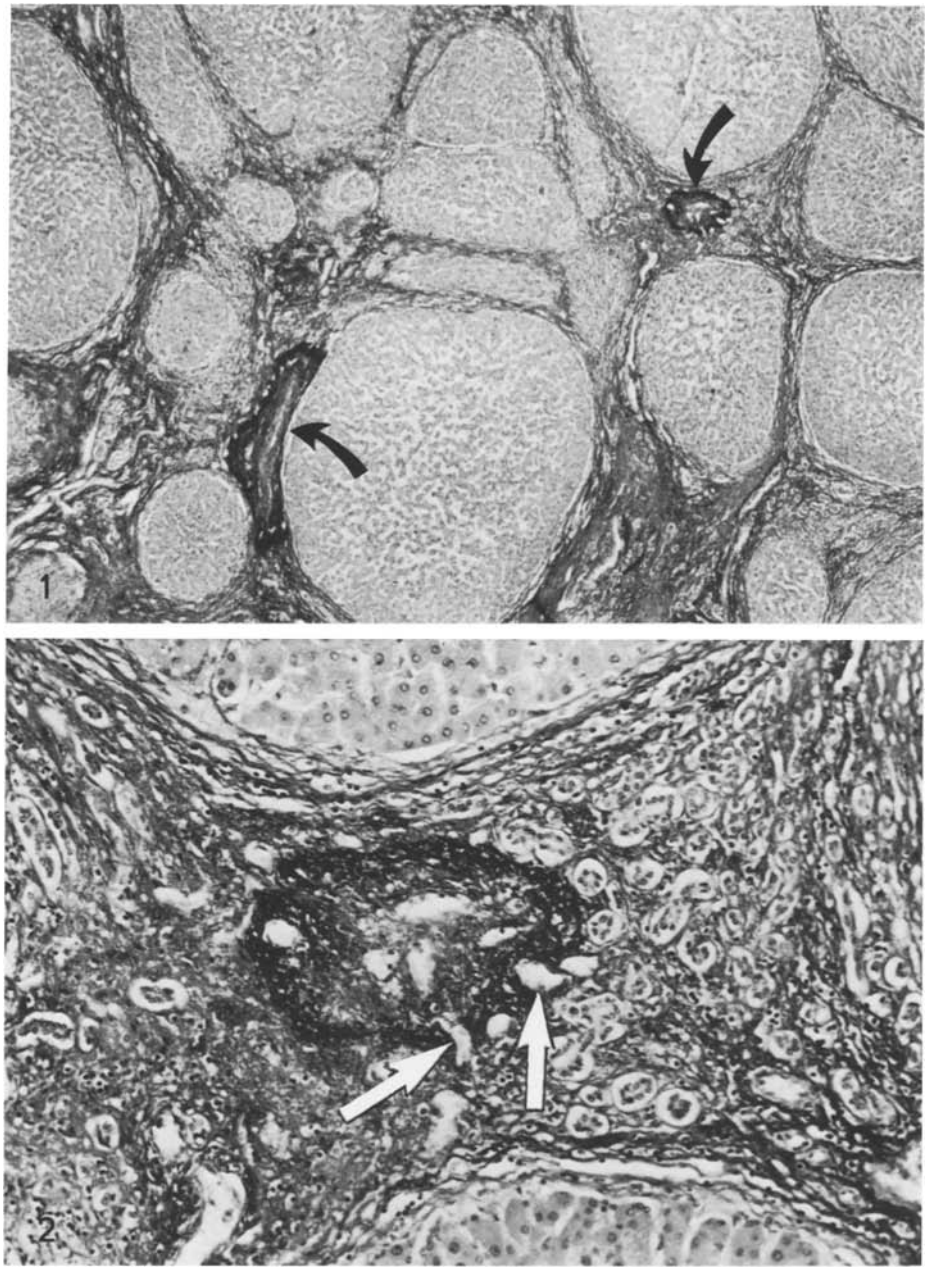
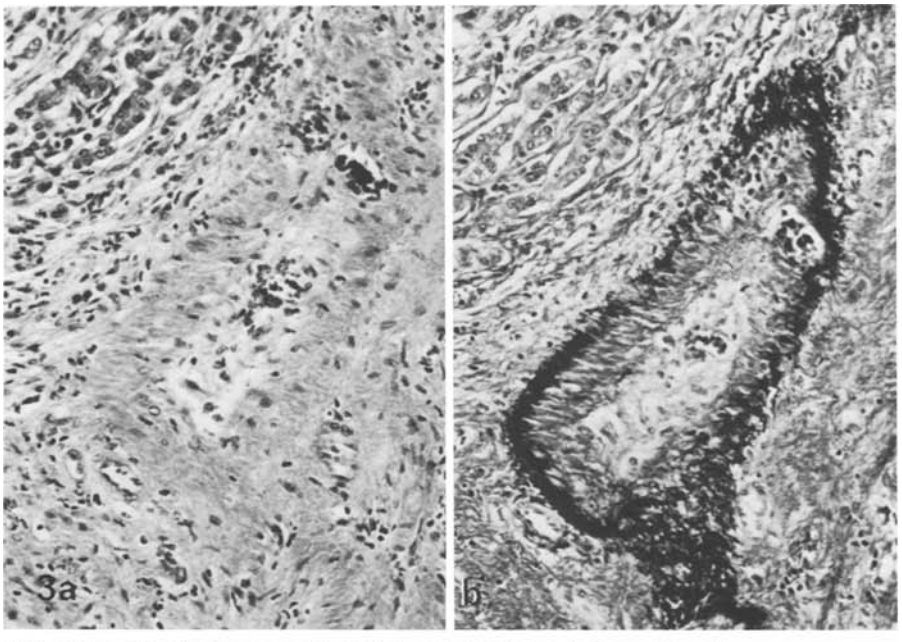


Fig. 1. Two venocclusive lesions of small hepatic veins (*arrow*) are seen in fibrous stroma of alcoholic cirrhosis. Autopsy liver. Elastica van Gieson. × 65

Fig. 2. Higher magnification of one of venocclusive lesions in Fig. 1. The lumen is completely occluded by fibrous tissue, and several recanalized vessels are seen. It is noted that some recanalized vessels are penetrating the thickened venous walls (*arrow*). Elastica van Gieson, × 375



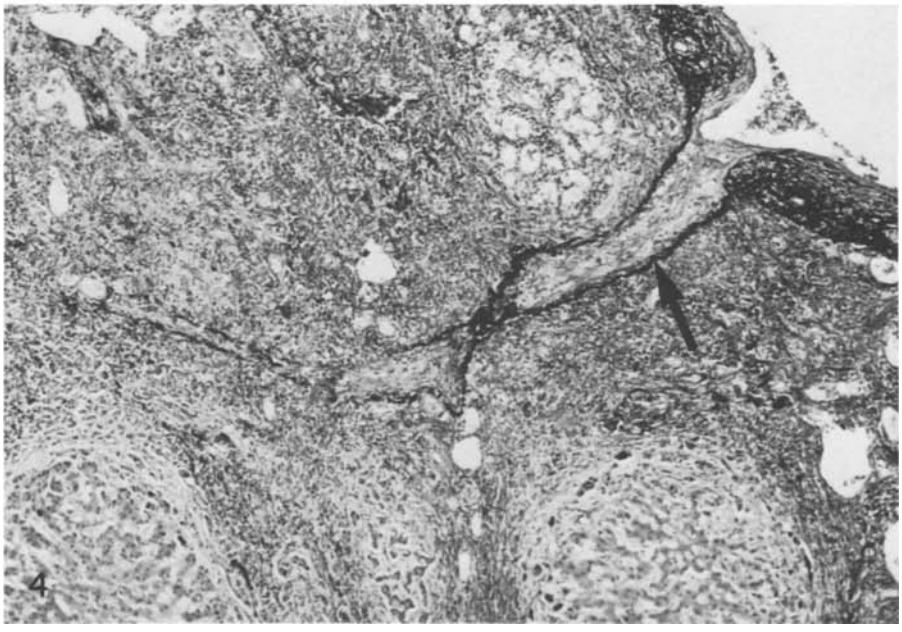


Fig. 3a, b. Serial sections of a vein showing venocclusive lesions. Make a comparison between HE stain (**a**) and Elastica van Gieson stain (**b**) in which this lesion is clearly identified. Collagenous fibrosis is seen in the outer part of this lesion and oedematous changes in the inner part. An autopsy liver of primary biliary cirrhosis. **a**. HE., ×375, **b**. Elastica van Gieson, ×375

Fig. 4. Venocclusive lesion (*arrow*) is noted at the confluence of a branch to a larger hepatic vein and continues to smaller levels throughout. Autopsy liver of primary biliary cirrhosis. Elastica van Gieson, ×130

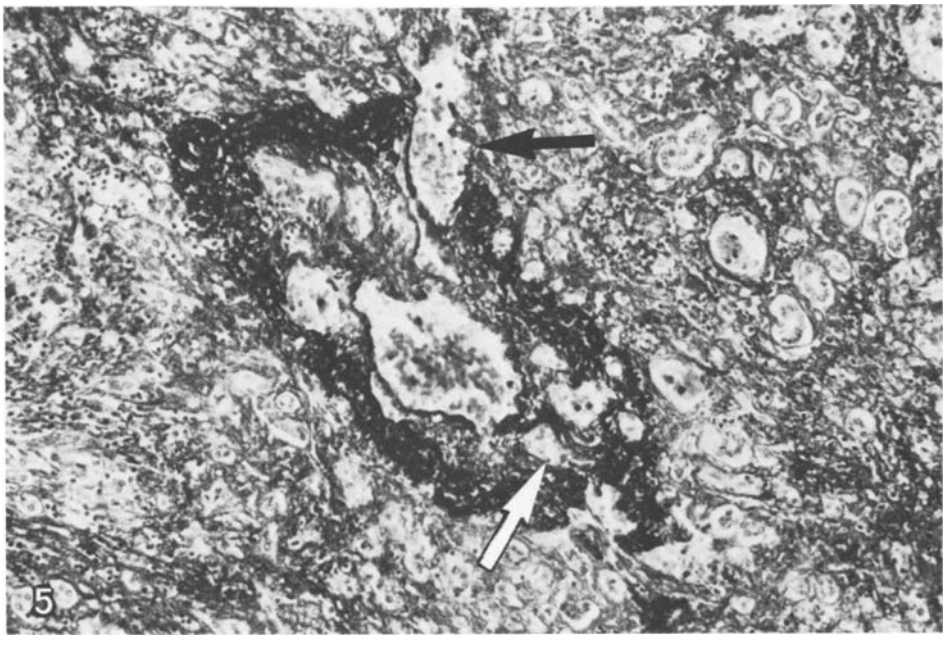


Fig. 5. There are several recanalized vessels in venocclusive lesion, and some (*arrow*) are in communication with thin-walled vessels in the fibrous stroma. Autopsy liver of alcoholic cirrhosis. Elastica van Gieson, × 300

4), while they were hardly detectable in HE-stained preparations. Observations of two serial sections stained with H&E and EVG, respectively (Fig. 3), disclosed that the VO lesions were composed of oedematous intimal thickening and collagenosis, leading to severe luminal narrowing. Elastic fibers also participated in these thickened areas in varying degrees. There was occasionally a mild infiltration of lymphocytes and macrophages, and a proliferation of fibroblast-like cells in the thickened intima (Fig. 3). An extravasation of erythrocytes was rare. Thin-walled vasculature reflecting recanalization were frequent within the VO lesions (Figs. 2, 3 and 5). There was, however, no demonstrable fresh thromboembolic material in these stenotic lumena. There was neither congestive changes nor haemorrhage in either the stroma or parenchyma around the VO lesions. None of the cirrhotic livers examined was of congestive origin, as seen in Budd-Chiari disease. The affected veins generally appeared shrunken, and their sizes and locations were recognizable as sublobular or central veins. There was no difference in the morphology of the VO lesions between all cirrhotic livers of varying aetiologies.

Serial section observations disclosed that a majority of the affected veins were usually small in size and often communicated directly with the patent larger hepatic vein tributaries (Fig. 4). The affected veins were quite long and often had a few smaller branches which were also occluded (Fig. 4). They terminated in fibrous tissue in the stroma, or on occasion were transformed into elastic fiber coils without lumena. A direct link between these

Table 1. Occurrence of venocclusive lesion of small hepatic vein branches in liver cirrhosis of various aetiologies

Presumed aetiology of liver cirrhosis	No. of venocclusive lesions in liver section (10 cm^2)		
	0~5	6~15	16~
Alcoholic cirrhosis	16 cases (53.9%)	6 cases (22.2%)	5 cases (18.5%)
HBsAg positive cirrhosis	8 (53.3%)	5 (33.3%)	2 (13.3%)
Biliary cirrhosis	11 (45.8%)	8 (33.3%)	5 (20.8%)
Cryptogenic cirrhosis	21 (70%)	6 (20%)	3 (10%)

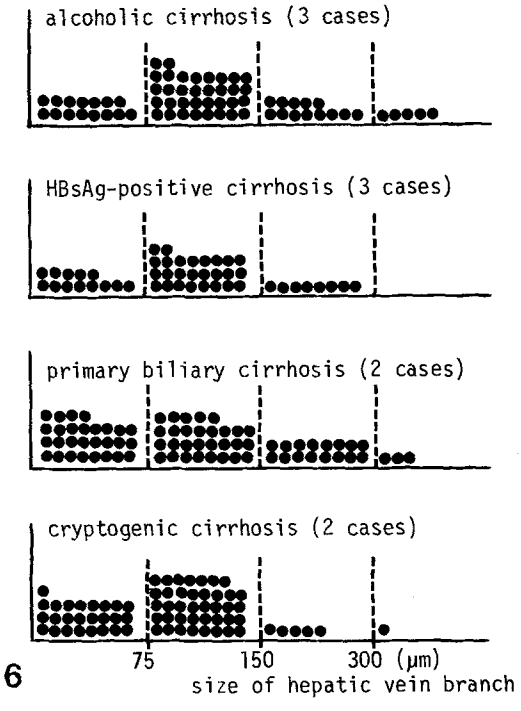


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Fig. 6. Distribution of each size of the hepatic vein branches (●) with venocclusive lesions in cirrhosis of four aetiologies

affected veins and hepatic parenchyma was very rarely seen. On the other hand, there were frequent communications between recanalized vessels within the VO lesions and adjacent thin-walled veins in the fibrous stroma of cirrhosis (Fig. 5). Further communications of recanalized vessels were occasionally noted with lumena of patent larger hepatic vein tributaries, suggesting a vein to vein anastomosis.

2. Frequency and distribution of the VO lesions in cirrhotic livers of different aetiologies

Twenty two (81.5%) of the 27 livers with alcoholic cirrhosis, 12 (80%) of the 15 with HBsAg positive cirrhosis, 22 (91.7%) of the 24 livers with

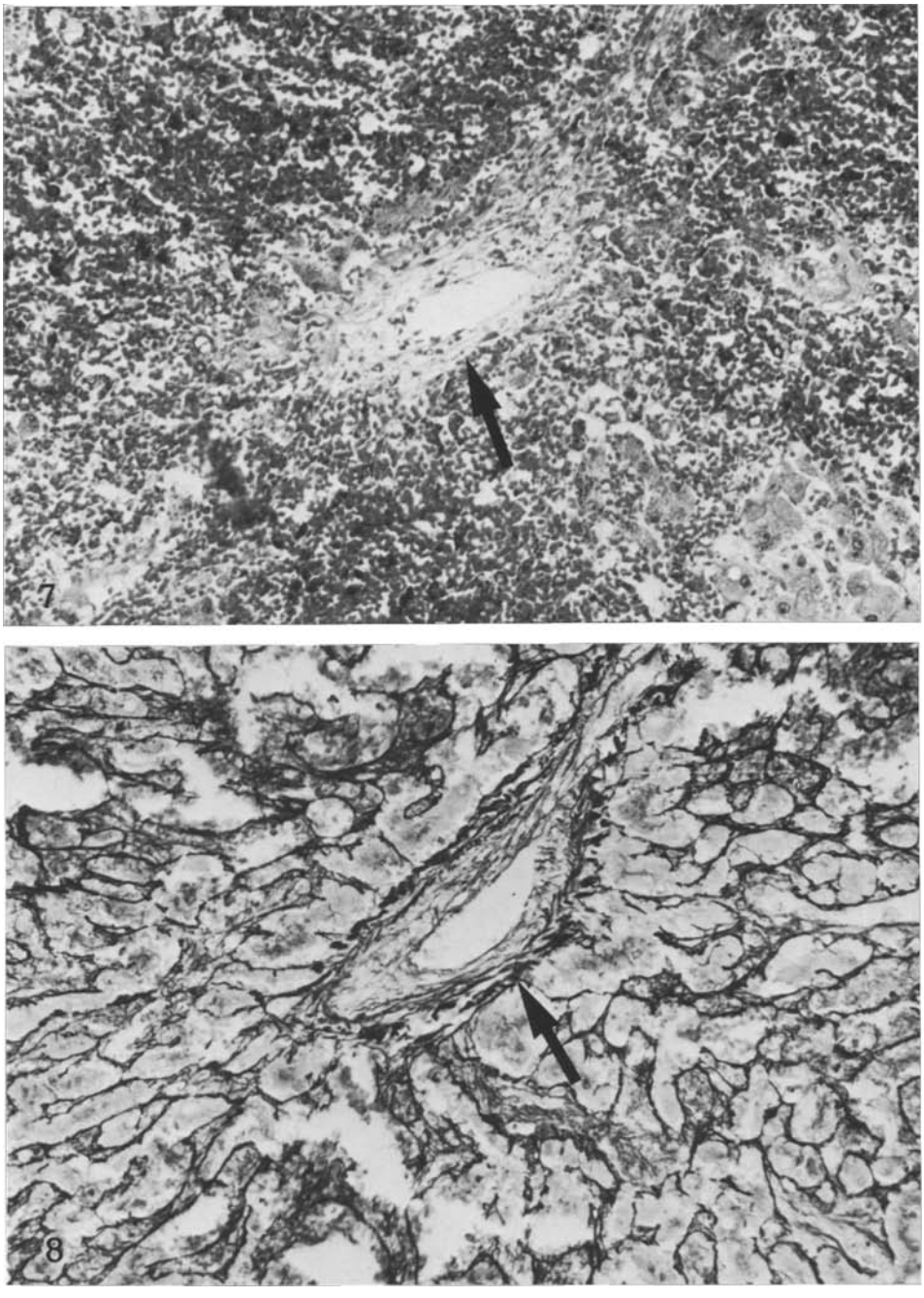

Fig. 7. Note marked centrolobular congestion with hepatocytic dropout. A sublobular hepatic vein (*arrow*) shows subintimal oedema and slight extravasation of erythrocytes. Autopsy liver with hepatic venocclusive disease after bone marrow transplantation. HE., × 375

Fig. 8. Luminal narrowing and intimal thickening of the terminal hepatic vein shown in Fig. 7, treated by reticulin stain of Gomori. There is centrolobular perisinusoidal fibrosis. Fig. 7 and 8 are serial sections of the same specimen. × 375

Table 2. Relation of presence of venocclusive lesion of small hepatic vein branches in livers to presence of portal hypertension

		Presence of findings suggestive of portal hypertension
No. of venocclusive lesions of small hepatic vein branches in liver section (10 cm^2)	0~5	38/53 cases (71.7%)
	6~15	21/25 cases (84%)
	16~	15/15 cases (100%)

biliary cirrhosis and 30 (75%) of the 40 with cryptogenic cirrhosis, exhibited the VO lesions. There was no statistical difference between them ($p > 0.05$). All cases examined were subdivided into three degrees according to the number of the VO lesions in a certain area (10 cm^2) of the liver section: mild (0–5), moderate (6–15) and severe degree (over 16). About half of the cases were mild degree, about 20 to 30% moderate, and about 10 to 20% severe, irrespective of the aetiology of liver cirrhosis (Table 1). The largest number of VO lesions was 54 in a liver with HBsAg positive cirrhosis. There were no statistical differences in quantitative distribution of the VO lesions among livers with each of four aetiologies ($p > 0.1$).

Size distribution of the affected veins examined in several representative cases of each aetiology of liver cirrhosis is shown in Fig. 6. A majority of the affected veins were below 300 μm in caliber, most often below 150 μm, and the greater the size, the less the veins were affected. This distribution pattern was the same for all groups of liver cirrhosis.

3. Correlation between the number of VO lesions and several pathological conditions

Table 2 shows that patients having larger number of VO lesions in a given area of liver section also have a higher association rate of portal hypertension ($p < 0.05$). There was, however, no correlation between the number of VO lesions and liver weight or type of liver cirrhosis, classified according to representative size of regenerative nodules.

4. Histological features of the liver with diffuse venocclusive disease after bone marrow transplantation

There was severe centrolobular congestion with hepatocytic dropout and atrophy (Figs. 7 and 8) around the occlusive central veins. Gomori's reticulin stain rather than EVG demonstrated clearly that the small hepatic veins were also severely involved (Figs. 7 and 8). There were a few differences in the morphology of the VO lesions between patients with bone marrow transplantation and those with cirrhosis: the intimal oedematous changes and intimal extravasation of erythrocytes were more prominent in livers from the former, and elastosis and recanalization were more frequent in those from the latter. In two liver sections examined, 97% of the 202 small veins with a diameter < 150 μm and 25.7% of the 26 small veins with a diameter $\geqq 150$ μm showed VO lesions. Veins with a diameter $\geqq 300$ μm

were not affected. The number of VO lesions in a 10 cm^2 liver section was 236.

Discussion

In the patient with allogeneic bone marrow transplantation, presented here widespread VO lesions occurred in the small hepatic and central veins, resulting in severe congestion with necrosis of the centrolobular hepatocytes and its related clinical symptoms. These features are also known to occur in patients with intoxication by certain alkaloids or those receiving irradiation (Bras et al. 1954; Fajardo and Colby 1980; Shulman et al. 1980). In sharp contrast, the VO lesions in cirrhotic livers involving the small hepatic and central veins were usually focal. Hepatic congestion around the lesions was not demonstrated, and clinical symptoms related to the lesions were indistinct. Such differences between the two groups of patients might be explained by the extent, degree and rapidity of venous involvement. The focal venocclusive changes were commonly seen in all liver cirrhosis groups with different aetiologies: alcoholic, HBsAg positive, biliary and cryptogenic types. Higher frequency of the VO lesions suggests that they occur in relation to results of the cirrhotic process itself rather than the aetiology of cirrhosis.

Serial section observations disclosed that the occluded veins are usually connected directly with the patent larger hepatic veins at one side and disappear in the fibrous stroma at the other side. Therefore, the affected veins did not seem to function as efferent veins of hepatic parenchyma. These findings, and absence of congestion around the VO lesions, lead us to speculate that these VO lesions might be a secondary response to what ever causes a decrease or cessation of the blood flow in some vein tributaries. It is well known that a decrease of post-sinusoidal venous outflow occurs by fibrous obliteration of the hepatic sinusoids, compression of the sinusoids by regenerating hepatocytes, and deprivation of the blood flow from hepatic parenchyma via arterio-venous or porto-hepatic anastomoses in the septa, in all types of cirrhosis (Lehmann et al. 1982; Popper 1977). As a result, intimal proliferation might occur reducing the caliber to a size appropriate for the reduced blood flow. Further, mechanical compression of the small hepatic veins by expanding regenerative nodules might assist the intima proliferation.

These findings and the fact that the number of affected veins was small, all seem to be against an active participation of these lesions in the production of post-sinusoidal portal hypertension. However, there was a positive relationship between the amount of VO lesions and the association with portal hypertension. It might be possible that the presence of established VO lesions, especially in livers with the "severe" change, might contribute to the promotion of postsinusoidal portal hypertension to some degree. Goodman and Ishak (1982) also attributed the VO lesions in alcoholic liver disease, especially in precirrhotic condition, to a raised portal pressure.

Several hepatic venographic changes have been described in cirrhosis, such as stenosis or irregular caliber of the hepatic vein branches, numerical reduction and abrupt narrowing of small hepatic veins, and cut-offs of

their branches (Bookstein et al. 1975; Futagawa et al. 1981; Takayasu et al. 1978). A majority of such abnormalities have been presumed to be attributable to the compression or obstruction of the hepatic venous tributaries by regenerative nodules (Futagawa et al. 1981; Takayasu et al. 1978), although there are few studies on the relationship between venography and pathology (Bookstein et al. 1975). The present study implies that the VO lesions must also in part be responsible for some of the venographic changes, especially abrupt narrowing of small hepatic veins or cut-offs of their branches. In addition, communication between well-developed recanalized vessels in the VO lesions and thin-walled veins running in the cirrhotic fibrous stroma, and between the smaller hepatic veins with the recanalized vessels and the larger patent hepatic veins at their branching portions, suggest an intrahepatic vein to vein anastomosis. Presumably, the anastomosis may become fully developed, when it would function as a draining canal of the hepatic outflow. Such a bypass may be functionally a portal to hepatic venous anastomosis because recent venographic studies have documented a portal to hepatic rather than a hepatic to hepatic venous anastomosis in cirrhotic livers (Bookstein et al. 1975; Futagawa et al. 1981).

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