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ANATOMICAL LESIONS BEING RESPONSIBLE FOR DEVELOPMENT OF PORTAL HYPERTENSION IN CARBON TETRACHLORIDE-INDUCED RAT LIVER CIRRHOSIS

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The hemodynamics of carbon tetrachloride-induced rat liver cirrhosis were examined by means of the perfusion experiment and the anatomical lesions being responsible for the development of portal hypertension were evaluated. The portal blood flow of isolated rat liver was 3.07±0.53 ml/g/min. in intact rats, 1.94±0.24 ml/g/min. in fibrosis rats and 1.75±0.23 ml/g/min. in cirrhosis rats under the condition of perfusion pressure 13.5 cmH_2O and of Ht 36%. The fibrotic liver as well as cirrhotic liver showed marked elevation of hepatic vascular resistance, and more than 20 cmH_2O perfusion pressures were necessary for the fibrotic liver as well as the cirrhotic liver to hold the normal level of hepatic blood flow. There was no perceptible stenosis or distortion of the intrahepatic portal tree and of the hepatic venous tree, but there was marked constriction of the sinusoid resulting from enlargement of the hepatic cell in the fibrotic liver as well as in the cirrhotic liver. The intimate correlation between the hepatic blood flow reduction and the extent of sinusoidal constriction suggested that vascular resistance of cirrhotic liver was located in the sinusoidal bed. ACTA PATH. JAP. 22: 625~635, 1972.

Introduction

Portal hypertension and decrease in hepatic blood flow, the usual clinical manifestations of liver cirrhosis, have been assumed to result from constriction of pre- and post sinusoidal vessels as well as of sinusoidal bed. There were, however, only few direct informations about the site of vascular resistance inside the liver and about the anatomical lesions of the liver being responsible for development of portal hypertension. The purpose of the present investigation is to analytically evaluate the significance of hepatic vascular lesions of carbon tetrachloride-induced rat liver cirrhosis, in contrast with the hemodynamical data. It is quite difficult to resolve this question by *in vivo* experiment and in human beings, because various extrahepatic factors may alter the hepatic blood circulation and make unable to examine the relationship between the morphological changes and hemo-

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dynamics of hepatic blood circulation. Thus, the perfusion experiment of the isolated rat liver, with which measurement of the exact hepatic blood flow was available without extrahepatic influences, was employed in the present experiment, and the relationship between the hemodynamical changes and anatomical lesions of the cirrhotic liver was examined.

Materials and Methods

Carbon tetrachloride (0.05 ml per 100 g body weight) was subcutaneously administrated to 70 Wistar strain rats, weighing 350 g at initiation of the experiment, twice a week for four to six months. Among 48 rats which survived, 10 rats showed liver cirrhosis, 14 rats showed a moderate grade of liver fibrosis, and the rest showed a mild grade of liver fibrosis or a nearly intact appearance. Twenty intact rat livers surved as control. The cirrhotic liver and fibrotic liver of moderate grade were isolated and perfused with a heparinized whole fresh rat blood (Ht 36%). The perfusion system as well as the technique for perfusion of isolated rat liver employed in the present experiment have been described in detail elsewhere[2,3]. The hepatic blood flow/perfusion pressure relation was measured. After perfusion experiment, a barium gelatin solution (mixed in a ratio of water 50 ml: barium 50 g; gelatin 10 g) was injected into the portal vein of one half of the cases of the perfused livers and into the hepatic vein of the remaining cases, then the liver was fixed in a 10% formalin solution. Thereafter, X-ray photographs of the left lateral lobe were taken, and distributions of the intrahepatic portal venous tree as well as of the hepatic venous tree were examined, and also their diameters up to the 8th branch of the portal vein and up to the 7th branch of the hepatic vein were measured. The left lateral lobe was embedded in paraffin and stained with hematoxylin-eosin and with silver impregnation method. The histology of the liver was also examined and the size of the hepatic cell was measured. The relative dimensions of hepatic cells, of sinusoid, of portal vein branch as well as hepatic vein branch, and of connective tissue occupying a unit square of the liver parenchyma were measured by means of an integrating eyepiece plate II and III (Carl Zeiss). The correlation between the hemodynamical data and the anatomical lesions was examined.

Results

A small amount of ascites was seen in the peritoneal cavity, and slight splenomegaly as well as moderate dilatation of the common portal vein were noted at surgery of the cirrhotic rat. Histology of the cirrhotic liver revealed that the liver parenchyma was divided into numerous pseudolobules surrounded by a narrow septum of connective tissue proliferating around the central vein. The Glisson's capsule, without remarkable change, was located in the center of the pseudolobule (Fig. 1). There was neither perceptible constriction nor distortion of the terminal portal vein (Fig. 2) and of the central vein, while a moderate to severe grade of fibrosis was present around the central vein (Fig. 3). The liver cell inside the pseudolobule, in general, was apparently larger than the liver cell of intact rat. The mean hepatic cell diameter was 16.44 ± 1.27 microns in the intact rat liver, 17.86 ± 1.80 microns in the fibrotic liver and 18.35 ± 2.06 microns in the cirrhotic liver. In contrast to the intact liver, the sinusoidal lumen of

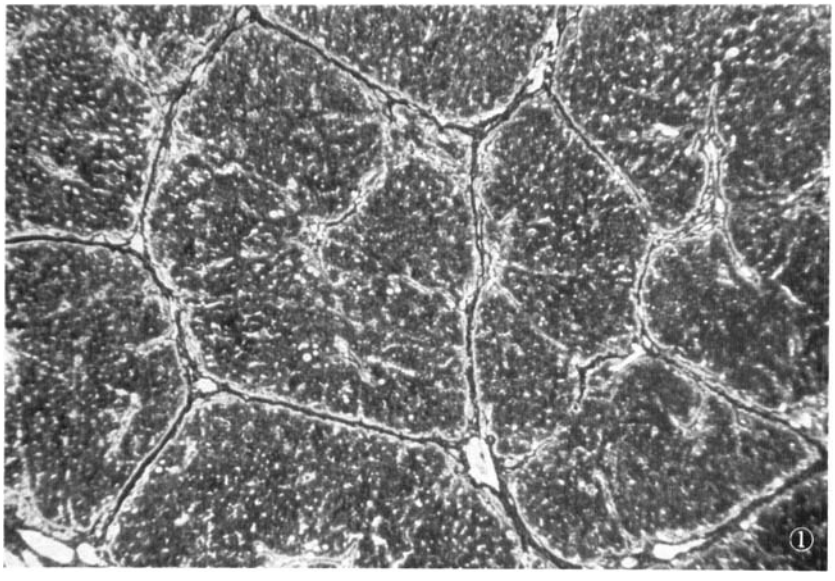

Fig. 1. Liver cirrhosis produced by carbon tetrachloride administration for six months. The liver parenchyma is subdivided into pseudolobules surrounded by narrow septum of connective tissue. Hepatic blood flow in this liver was 1.52 ml/g/min.. Silver impregnation, ×40.

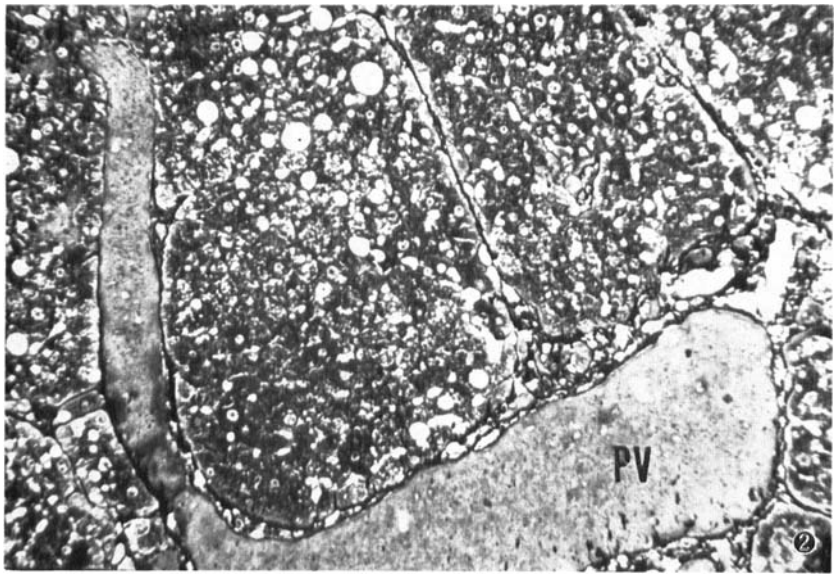

Fig. 2. No perceptible constriction or distortion was seen in the terminal portal vein (PV) of the cirrhotic liver in which a barium-gelatin solution was injected via the portal vein. Silver impregnation, ×100.

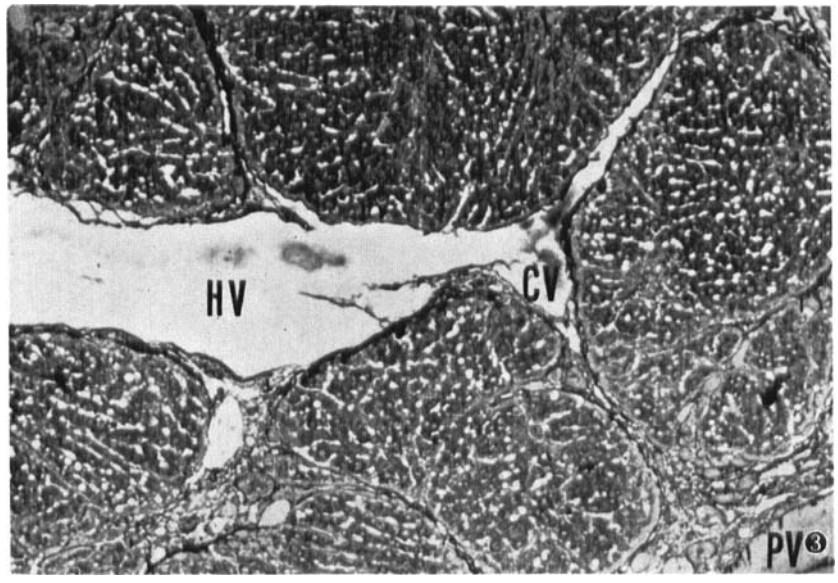

Fig. 3. There was no perceptible constriction or distortion of central vein (CV), although a moderate grade of fibrosis was seen around it. H.V.=hepatic vein. Silver impregnation, ×100.

cirrhotic liver was markedly compressed by enlarged liver cells (Fig. 5). The silver impregnation specimen showed mild proliferation of argyrophilic fiber at the sinusoidal wall. X-ray examination of the intrahepatic portal- and hepatic vein of the cirrhotic liver showed a less smoother course (Figs. 7, 9), in contrast to their smooth course in intact liver (Figs. 6, 8). Neither marked stenosis nor distortion of these vessels, however, was found. Diameter of the intrahepatic portal- and hepatic venous tree indicated that their decreasing rate following branching in the cirrhotic liver and in the intact liver was quite similar (**Table 1**). The mean relative dimension of the intrahepatic portal vein in a unit square of the cirrhotic liver parenchyma was 0.74 per cent, in contrast to 0.43 per cent in the intact liver; that is, the lumen of the terminal portal vein of the cirrhotic liver was rather dilated. On the other hand, the mean relative dimension of the central vein of cirrhotic liver was 1.04 per cent and that of intact liver was 0.95 per cent, i.e. there was no significant difference in the space of terminal hepatic vein between cirrhotic liver and intact liver. The mean hepatic blood flow of isolated intact rat liver was 3.07 ± 0.53 ml/g/min. under the condition that the perfusion pressure was 13.5 cmH_2O and the hematocrit was 36.4 per cent. The mean hepatic blood flow under the same condition was reduced to 1.94 ± 0.24 ml/g/min. in the fibrotic liver, and to 1.75 ± 0.23 ml/g/min. in the cirrhotic liver. The hepatic blood flow/perfusion pressure relation of the intact liver was approximately linear and steep

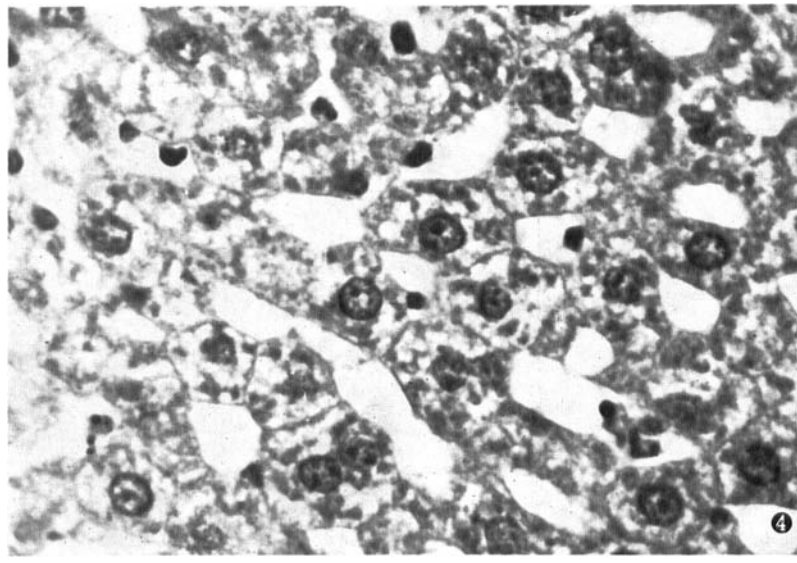

Fig. 4. The liver parenchyma of intact rat perfused for 90 minutes. Sinusoidal lumen was well preserved. Hepatic blood flow 3.03 ml/g/min.. H.E. stain, ×420.

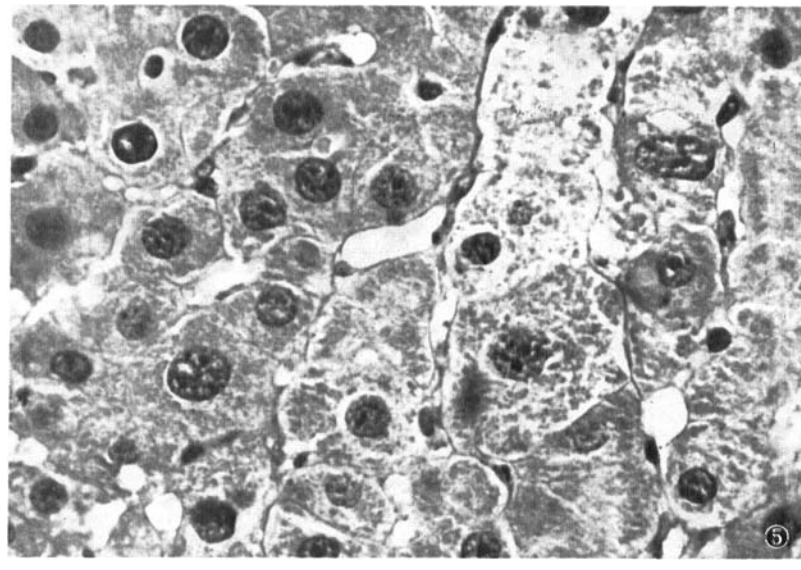

Fig. 5. The liver parenchyma of cirrhotic liver of which blood flow was 1.52 ml/g/min.. The sinusoidal lumen was markedly compressed by swollen liver cells. H.E. stain, ×420.

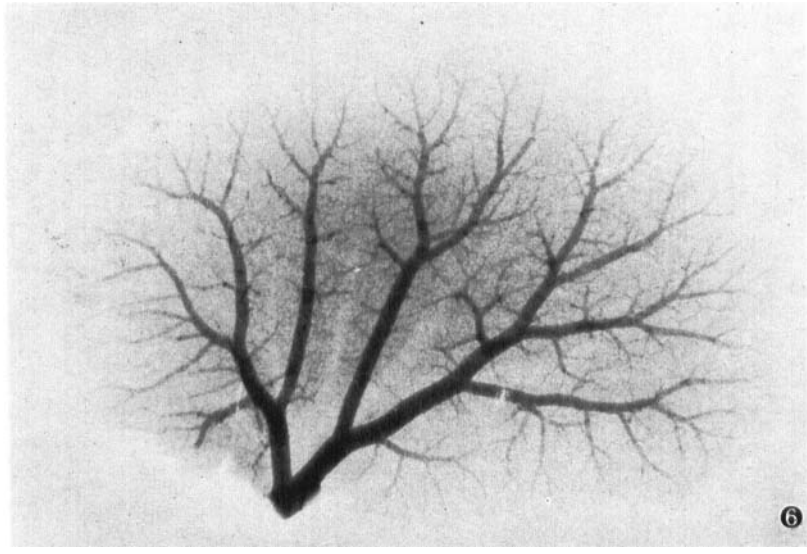

Fig. 6. Radiograph of intrahepatic portal venous tree of left lateral lobe of intact rat liver.

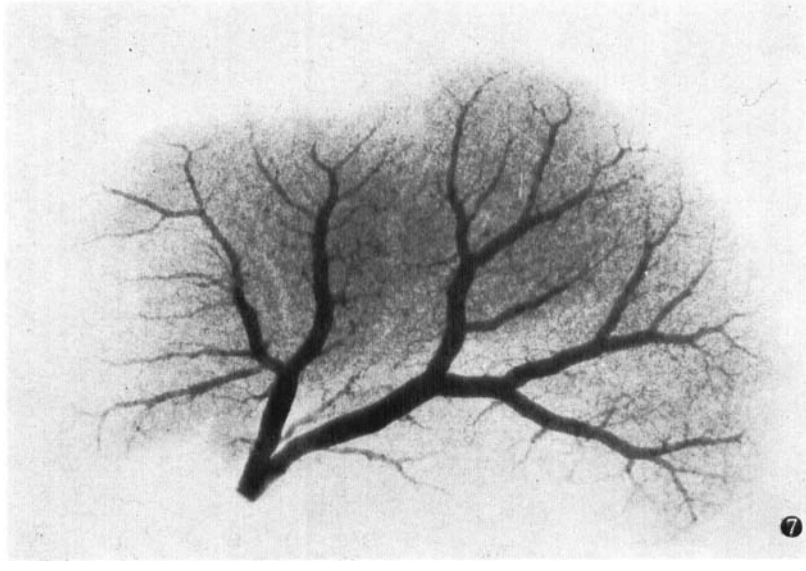

Fig. 7. Radiograph of intraheptic portal venous tree of cirrhotic liver. The course of vasculature was somewhat irregular, but neither marked stenosis nor distortion was seen.

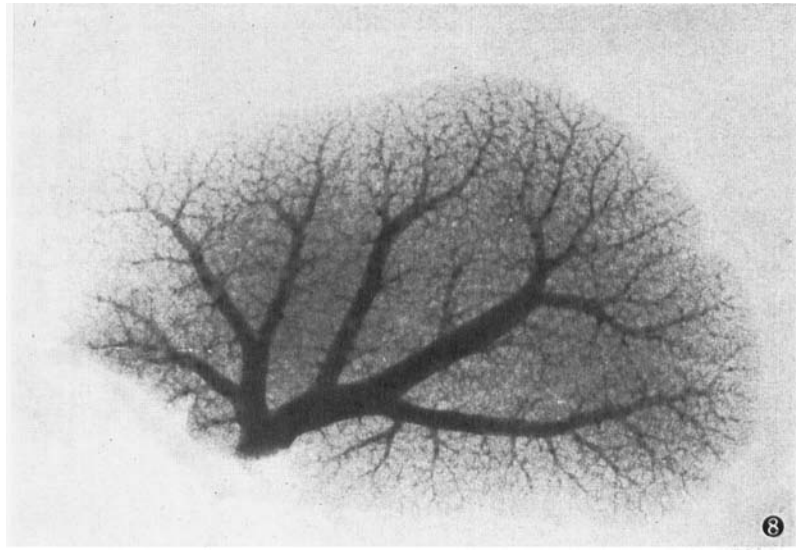

Fig. 8. Radiograph of hepatic vein of left lateral lobe of intact rat liver.

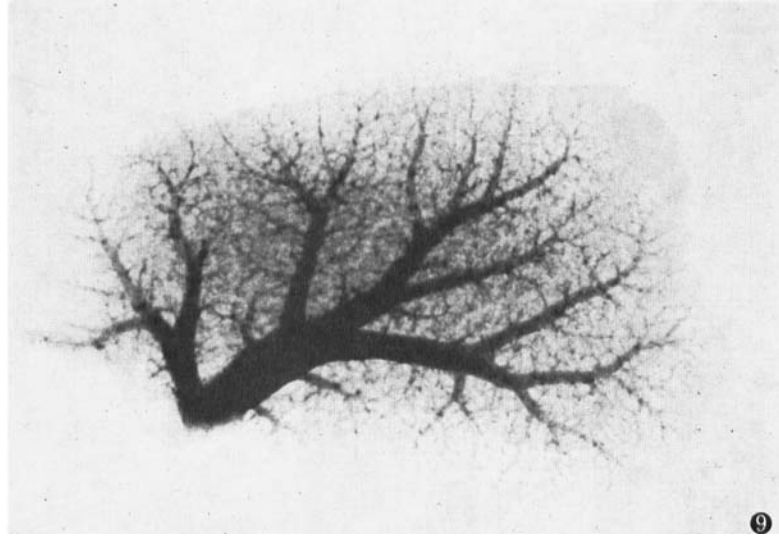

Fig. 9. Radiograph of hepatic vein of cirrhotic liver. There is neither marked stenosis nor distortion.

as shown in the upper line of Figure 10. This gradient became evidently low and flat in fibrotic liver as well as in cirrhotic liver as shown in the lower line of Figure 10. This figure indicates that the vascular resistence of fibrotic and cirrhotic

Table 1. *Relative Diameter of the Branches of Portal Vein as well as Hepatic Vein*

Order of Branch	Portal Vein (%)		Hepatic Vein (%)	
	Intact	Cirrhosis	Intact	Cirrhosis
1	100	100	100	100
2	82.9±7.7	81.2±9.7	92.5±2.1	88.0±4.4
3	61.9±5.2	59.5±6.5	64.2±4.7	66.6±5.4
4	48.5±7.2	45.0±6.9	47.3±4.4	42.2±4.8
5	31.4±6.7	32.2±5.1	31.6±2.9	27.6±2.3
6	22.7±3.1	21.6±4.1	18.6±1.7	19.5±3.1
7	13.9±3.1	15.6±3.7	11.1±1.6	12.2±1.2
8	7.7±1.0	9.7±2.3		

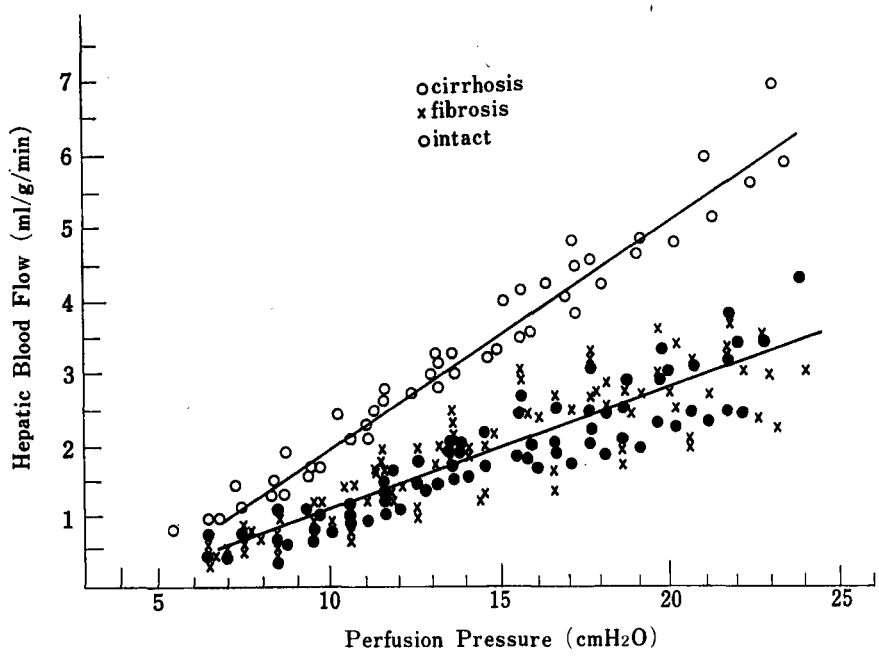

Fig. 10. Hepatic blood flow-pressure relation in the isolated rat liver.

liver increased to an extent by 1.5 to 2 times of the intact liver and also that the hepatic vascular bed became rigid. This figure also showed that 20 cmH_2O or much higher perfusion pressure (portal vein pressure) was necessary for the cirrhotic liver as well as the fibrotic liver to hold an equal hepatic blood flow as of the intact liver. The mean relative dimension of liver cell as well as sinusoidal lumen occupying a unit square of the intact liver lobule was 77:23. This ratio became 86:14 in the fibrotic liver and to 89:11 in the cirrhotic liver (Fig. 11). Since the amount of

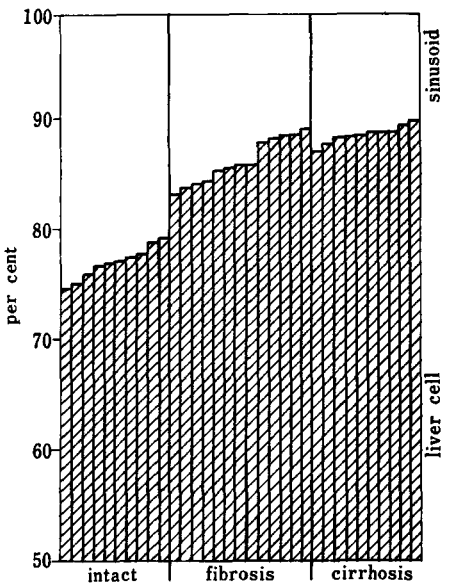

Fig. 11. Relative dimension of liver cell and sinusoid in a unit square of liver parenchyma.

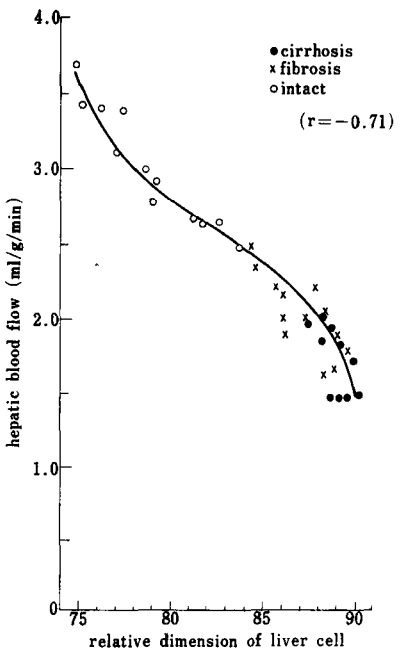

Fig. 12. Relationship between hepatic blood flow and dimension of liver cell occuping a unit square of liver parenchyma.

connective tissue inside the pseudolobule was ignorably minimal, these values indicated that the liver cell became enlarged and compressed the sinusoidal space. The reverse correlation was found between the extent of liver cell enlargement and the hepatic blood flow reduction as shown in Figure 12, in which correlation dots distributed around the S-shaped curve and the correlation coefficiency was –0.71. This curve became approximately linear when the value of the hepatic cell dimension 83 per cent, in other words the sinusoidal space 17 per cent, were regarded to be zero and variation from zero was raised to the second power. The correlation coefficiency was 0.85 in this relation. The relative dimension of the connective tissue occupying a unit square of the histological specimen, in which all tissue components of the liver; such as the Glisson's capsule, liver cells, sinusoids and the central veins were included, was 2 per cent in the intact liver, was 4.75 per cent in the fibrotic liver and was 9.06 per cent in the cirrhotic liver. These values showed that the cirrhotic liver contained as five times much amount of connective tissue as that of the intact liver. However, no correlation was found between the hepatic blood flow reduction and the amount of connective tissue, since the correlation coefficiency was merely –0.07.

Discussion

BONO and his coworkers[1] reported that the mean portal vein pressure of the rat with a severe grade of liver cirrhosis resulting from carbon tetrachloride administration, elevated to 21.67 ± 0.67 cmH_2O. This value coincides incidently with the data of the present experiment that more than 20 cmH_2O perfusion pressures (portal vein pressure) were necessary for the cirrhotic liver to hold as equal amount of blood flow as that of the intact liver. Thus, these results may suggest that portal hypertension is a compensatory phenomen in the sense of sending as possibly a normal amount of blood into the cirrhotic liver in which vascular resistance was elevated. That no remarkable stenosis or distortion was found in the entire course of the intrahepatic portal tree and the hepatic venous tree, except that the terminal portal vein became rather dilated in the cirrhotic liver, suggests that the cause of elevation of vascular resistance of carbon tetrachloride-induced liver cirrhosis is in elsewhere than these two vessels. KLUGE and his coworkers[3], and TANDON and his coworkers[8] reported that the presence of a large number of collagen bundles in the perisinusoidal area of human liver with portal hypertension, but no cirrhosis, might possibly be the anatomical lesion related to portal hypertension. The present experiment, however, proved that the fibrosis, though which lead to rigidity of the hepatic blood vessels as shown by the flow/pressure relation (Fig. 10), has no essential meaning for elevation of hepatic vascular resistance. RAPPAPORT *et al.*[7], based on the microcirculatory observation of fatty liver of mice and rats, described that sinusoidal blood flow was disturbed by the bulged liver cell, hence they considered that such circulatory changes in the sinusoidal bed would possibly lead to portal hypertension. NAKATA and his coworkers[4,5] have quantitatively proved that liver cell swelling produced by various toxic agents and by hypoxia resulted in a marked decrease in portal blood flow. NAKAMURA *et al.*[6] reported that the diameters of liver cell plate of human liver cirrhosis were significantly larger than those of intact liver cell and that the size of liver cell plate was directly proportional to the hepatic vein wedge pressures. The present experiment proved that decrease in hepatic blood flow of carbon tetrachloride-induced rat liver cirrhosis was intimately related to the extent of sinusoidal constriction resulting from enlargement of the liver cell and that the hepatic blood flow was proportional to the second power of the dimension of sinusoid. Thus, the conclusion that sinusoidal stenosis is possibly the most important causative anatomical lesion for the development of portal hypertension in the case of carbon tetrachloride-induced rat liver cirrhosis may be elicited.

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