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Localization of Increased Hepatic Vascular Resistance in Liver Cirrhosis

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To determine the localization of increased vascular resistance in cirrhotic liver, blood pressures were measured by a direct cannulation method at several key points in the hepatic vascular pathway in normal and cirrhotic rats. Cirrhosis was produced by feeding a choline-deficient diet. Blood pressures in normal rats were 110 mm H_2O in the portal vein, 68 mm H_2O in the terminal portal venule, 28 mm H_2O in the terminal hepatic venule and 20 mm H_2O in the inferior vena cava. In cirrhotic rats, blood pressures in the portal vein and the terminal portal venule were 173 and 100 mm H_2O, respectively, while those in the terminal hepatic venule and the inferior vena cava were elevated only slightly above normal. These hemodynamic data suggest that an increase in vascular resistance in cirrhotic liver is present in the intrahepatic portal vein and sinusoids, but not in intrahepatic hepatic vein. In cirrhotic liver, stenosis and distortion were found in peripheral branches of the portal vein, and sinusoidal stenoses and a decrease in sinusoidal space were recognized. Accordingly, it is suggested that the increase in vascular resistance in the intrahepatic portal vein and sinusoids correlate with these structural changes. Although severe stenoses and distortion were found in hepatic vein branches, it was thought that they do not contribute to portal hypertension because of lack of increase in vascular resistance in the intrahepatic hepatic vein.

The causes of portal hypertension in cirrhosis have been widely discussed and include increased vascular resistance in the liver (1–8), increased hepatic arterial blood flow and increased the number of arterio-portal anastomoses inside the liver (3, 4, 6, 7, 9–11), increased splenic blood flow (12–14) and increased vasoconstrictive substance in the portal blood (15). Increased intrahepatic vascular resistance, especially due to obstruction and stenoses of the intrahepatic hepatic vein, has been thought to be most important; however, the exact localization of increased vascular resistance is not known.

We determined the site of increased vascular resistance in cirrhotic liver by performing blood pressure measurements at several key points in the hepatic vascular pathway. The relation between the localization of increased vascular resistance and morphological changes in the liver was also examined.

MATERIALS AND METHODS

Male Wistar rats (weighing initially 100 to 150 gm) were fed a choline-deficient diet for 1.5 and 6 months to produce fatty liver and cirrhosis, respectively. As controls, intact rats were fed a standard diet. Each group contained 10 rats.

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The animals were anesthetized with sodium pentobarbital (60 mg per kg body weight, i.p.).

The apparatus for animal restraint consisted of two parts: a plastic plate to which the rat was attached on its back and a plastic dish (120 mm in diameter, 20 mm in depth) with a hole (30 mm in diameter) in the center (Figure 1). The rat's abdomen was transversely cut with an incision of about 20 mm below the xiphoid process. The abdominal wall was attached to the bottom of the dish by a paste to avoid leaking of physiological saline solution. The left lateral lobe of the liver was exposed through the hole and was immersed in circulating physiological saline solution (37°C). To minimize respiratory movement of the liver, the liver surface was covered with a thin plastic plate which provided a hole (10 mm in diameter).

Incident light illumination was used for microscopic observation and micropuncture of minute blood vessels of the liver because cirrhotic liver did not allow transillumination of the thickened parenchyma. Consequently, visible blood vessels were limited to the superficial layer of the liver. Light was conducted by fiber glass optics (Figure 1) from a light source (halogen lamp, 15 V, 150W, Phillips) to an illuminator (Figure 1). The illuminator was designed to illuminate the field from outside a water immersion objective lense (Leitz, × 11).

A micropipette (Figure 1) was made from a glass tube (1.6 mm in diameter, 100 mm in length, Kimax 51*). One end of the tube was made to be 10 to 20 μm in

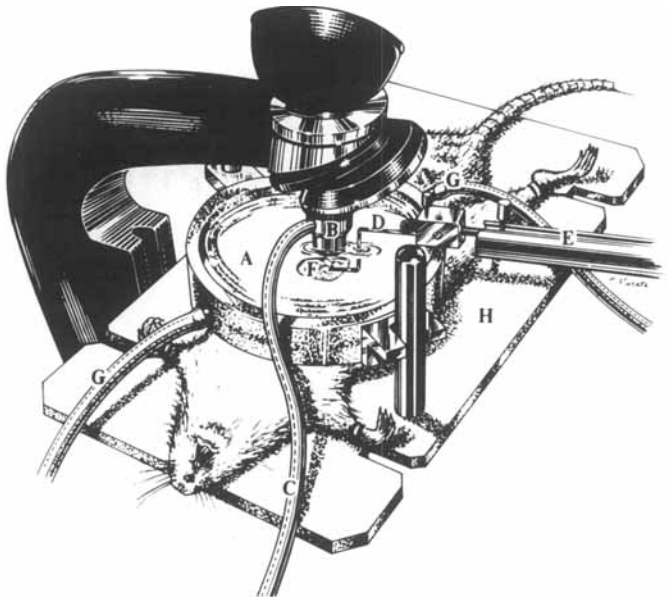

FIG. 1. Apparatus for microscopic observation and micropuncture of minute vessels in the liver: A, plastic dish; B, water immersion lense and incident light illuminator; C, fiber glass optics conducting light; D, micropipette; E, micromanipulator; F, liver; G, rubber tube for circulation of physiological saline solution; H, plastic plate for animal restraint.

diameter. The other end was connected by polyethylene catheters to a water column manometer (Figure 2) and to a hand-operated pressure controller (Figure 2). The pressure controller consisted of a rubber ball filled with mercury and a screw to compress or dilate the ball. The entire tubing system was filled with physiological saline solution colored with Evans blue.

Under the microscope (× 110), the tip of the micropipette was inserted into a minute blood vessel (20 to 30 μm in diameter) as nearly perpendicularly as possible, with the aid of the micromanipulator (Figure 2). The pressure in the punctured blood vessel was determined by the manometer when the blood and the colored physiological saline solution balanced in the tip of the micropipette; i.e., no flow into or out of the micropipette (the level of the punctured blood vessel = 0 mm H_2O). Blood pressures in the terminal portal venule and the terminal hepatic venule were each measured at three different branches in one rat. No significant variation (less than 5% of the mean) was found among the three blood pressures. The terminal portal venule and the terminal hepatic venule were reliably identified by direction of blood flow. Bleeding after puncturing the liver stopped spontaneously by gently pulling out the micropipette.

The dish was removed and the liver replaced into the abdominal cavity. Blood pressures in the portal vein at the liver hilum and inferior vena cava at the hepatic vein orifice were measured by catheterization (the level of the liver hilum = 0 mm H_2O). The height difference between the baseline of extrahepatic and intrahepatic blood vessels was negligible. All procedures were completed within 90 min without occurrence of any remarkable circulatory changes. Arterial blood pressures were not monitored in the present study.

Thereafter, blood in the liver was washed out with physiological saline solution, and 50% barium sulfate solution was injected either into the portal or the hepatic vein. The liver was extirpated, and an angiogram was taken using a super soft X-ray apparatus (Softex[R], Softex Co., Ltd., Tokyo, Japan).

For histological study, the liver was fixed in 10% neutral formalin for 3 days.

RESULTS

The minute blood vessels and sinusoids at the superficial layer of the liver were clearly visible using the present methods. Microcirculatory findings of normal and fatty liver were essentially the same as described by Rappaport et al. (16). Sinusoidal blood flow in fatty and cirrhotic liver was disturbed by sinusoidal stenoses resulting from hepatic cell swelling due to accumulation of fat droplets. However, we could not see the arterial sinusoids in the three groups. No pseudolobule supplied purely by the arterial blood was observed in the cirrhotic liver. No shunt vessel connecting the portal vein and the hepatic vein was confirmed in the cirrhotic liver. Distortion and stenoses of the most peripheral branches of the hepatic vein were seen in the cirrhotic liver, but such deformation of the portal vein was slight. No definite obstruction of these branches was found.

The blood pressures in the portal vein, the terminal portal venule, the terminal hepatic venule and the inferior vena cava are shown in Table 1.

The blood pressure difference between the portal vein and the terminal portal venule, between the terminal portal venule and the terminal hepatic venule, and between the terminal hepatic venule and the inferior vena cava are shown in Table 2.

The relationship between blood pressures and the hepatic vascular path lengths, i.e., blood pressure gradients in hepatic vascular pathway, are shown in Figure 3. The hepatic vascular path lengths were measured from the angiograms.

Angiograms were carefully studied under the microscope (× 50). The contrast medium adequately filled the

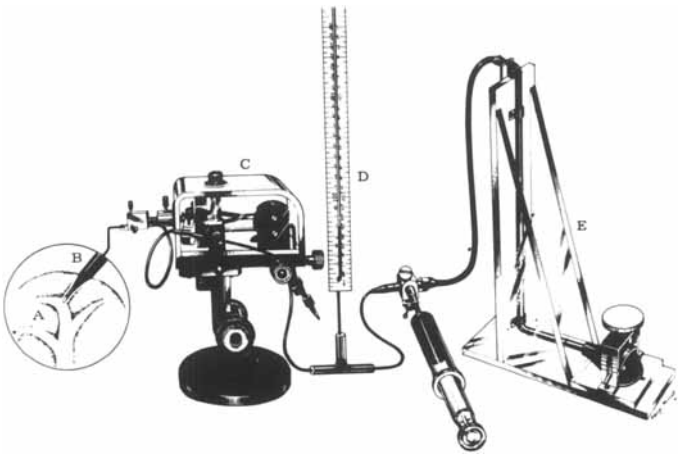

FIG. 2. Apparatus for blood pressure measurement of minute vessels in the liver: A, liver; B, tip of micropipette; C, micromanipulator; D, manometer; E, pressure controller.

TABLE 1. BLOOD PRESSURES AT KEY POINTS IN HEPATIC VASCULAR PATHWAY[a]

	Portal vein at liver hilum	Intrahepatic terminal portal venule	Intrahepatic terminal hepatic venule	Inferior vena cava at hepatic vein orifice
Normal	109.6 ± 3.0	68.1 ± 1.0	27.5 ± 1.2	20.3 ± 1.0
Fatty liver	142.9 ± 3.8[b]	82.0 ± 1.3[b]	34.3 ± 2.1[b]	22.2 ± 1.0
Cirrhosis	172.8 ± 6.5[b,c]	100.0 ± 4.2[b,c]	30.9 ± 1.3	24.8 ± 1.2[b]

[a] mm H_2O; mean ± S.E.; n = 10.
[b] $p < 0.05$; compared to normal.
[c] $p < 0.05$; compared to fatty liver.

TABLE 2. BLOOD PRESSURE DIFFERENCES BETWEEN TWO KEY POINTS IN HEPATIC VASCULAR PATHWAY[a]

	PVP-TPVP	TPVP-THVP	THVP-IVCP
Normal	41.5 ± 3.5	40.6 ± 2.0	7.2 ± 1.5
Fatty liver	60.9 ± 3.1[b]	47.7 ± 2.3[b]	12.1 ± 2.4
Cirrhosis	72.8 ± 4.6[b,c]	69.1 ± 4.7[b,c]	6.1 ± 1.0[c]

The abbreviations used are: PVP, portal vein pressure at liver hilum; TPVP, intrahepatic terminal portal venule pressure; THVP, intrahepatic terminal hepatic venule pressure; IVCP; inferior vena cava pressure at hepatic vein orifice.
[a] mm H_2O; mean ± S.E.; n = 10.
[b] $p < 0.05$; compared to normal.
[c] $p < 0.05$; compared to fatty liver.

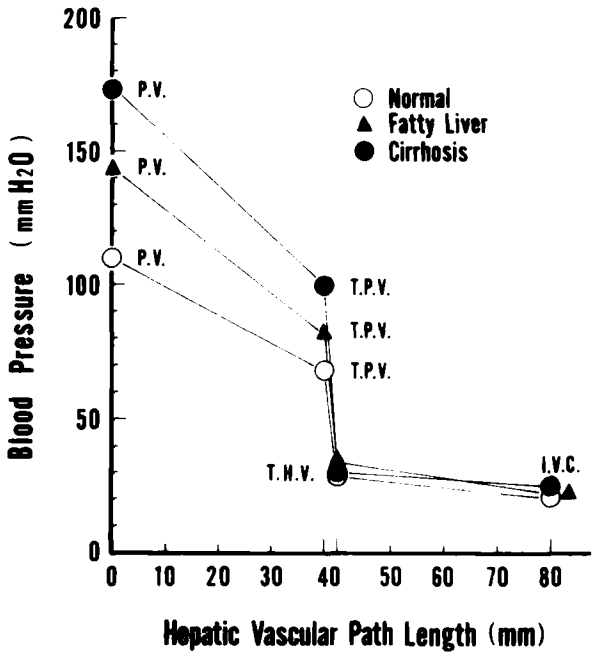

FIG. 3. Blood pressure gradient in hepatic vascular pathway: P.V., portal vein at liver hilum; T.P.V., terminal portal venule; T.H.V., terminal hepatic venule; I.V.C., inferior vena cava at hepatic vein orifice. In the fatty liver and the cirrhotic liver, the gradients in the intrahepatic portal vein and the sinusoids are steeper than the normal. No difference among the three groups is seen in the intrahepatic hepatic vein.

most peripheral branches of the portal or the hepatic vein in the three groups (Figure 4, A to F). Of the large branches of the portal and hepatic veins, no remarkable change in number or distribution was found in the three groups. In cirrhotic liver, however, there was severe distortion with stenoses of the peripheral branches of both the veins; such deformation of the hepatic vein branches was more severe than that of the portal vein branches (Figure 4, E and F). The deformation of the peripheral branches as seen in the cirrhotic liver was also found in the fatty liver, but those were milder than those of the cirrhotic liver (Figure 4, C and D). In unit field, no decrease in number of the peripheral branches of either vein was seen in the fatty liver and the cirrhotic liver. No remarkable porto-hepatic anastomosis was found in the three groups.

The fatty liver was large and usually yellow. Histologically, almost all hepatic cells were markedly swollen by accumulated fat droplets; consequently, the sinusoids were severely narrowed, and the sinusoidal space was decreased (Figure 5, C and D). No fibrosis, deformation of the lobular structure or remarkable change in the branches of the portal and hepatic veins were seen. The cirrhotic liver was hard and the surface nodular (Figure 6). All cirrhotic rats had signs of portal hypertension, ascites and collateral blood circulation, i.e., dilatation of veins surrounding the esophagus and veins of the ligamentum between the liver and diaphragm. Histologically, the cirrhotic liver revealed that the parenchyma was subdivided into pseudolobules surrounded by connective tissue septa (Figure 5E). Most of the hepatic cells contained fat droplets and were markedly swollen. In contrast, the sinusoids were narrowed, and the sinusoidal space was extremely decreased (Figure 5F). Slight sinusoidal fibrosis was recognized with staining for reticulin and collagen fibers. No obstruction of the branches of the portal and hepatic veins was found, although some of the peripheral hepatic vein branches were distorted by regenerated nodules. Congestion in the sinusoids suggesting postsinusoidal block was not found.

DISCUSSION

An increase in blood pressure difference between two given points means an increase in vascular resistance at an intermediary site, unless the blood flow increases. It is presumably true in the present study that the blood flow of the cirrhotic liver would not have been above normal, since a decrease in the blood flow in the cirrhotic liver has been confirmed in both human beings (Myers, J. D. and Durham, N. C., *J. Clinic Invest.* 1950; 29:836–837, Abstract; 17–19) and animals (20–23). Accordingly, the present study suggests an increase in vascular resistance in the intrahepatic portal vein and sinusoids, but not in the intrahepatic hepatic vein.

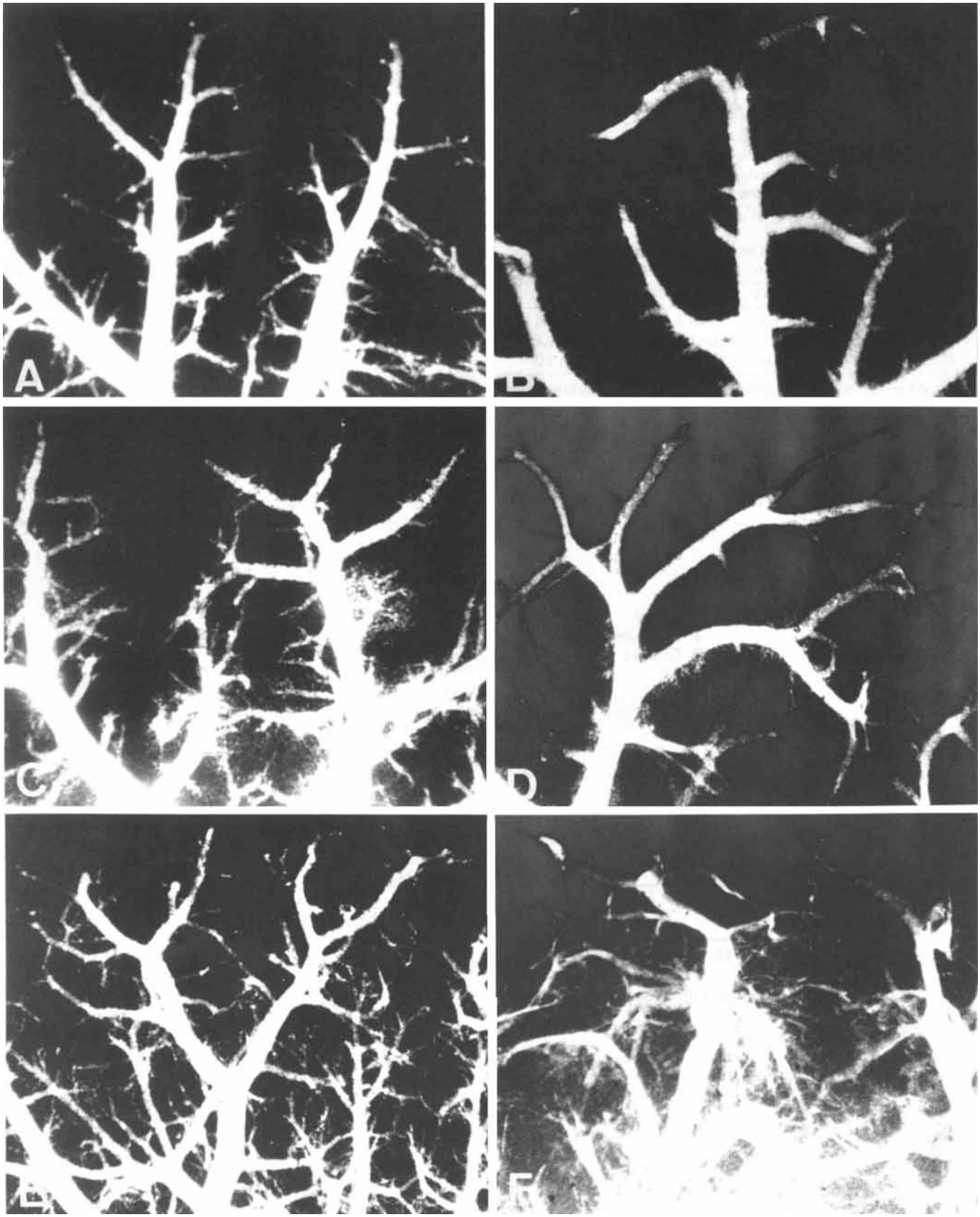

FIG. 4. Angiogram of the liver (× 20): (A) portal vein branches in the normal liver; (B) hepatic vein branches in the normal liver; (C) portal vein branches in the fatty liver; (D) hepatic vein branches in the fatty liver; (E) portal vein branches in the cirrhotic liver; (F) hepatic vein branches in the cirrhotic liver. In all livers, the most peripheral branches are adequately filled with contrast medium. Distortion and slight stenoses of the peripheral portal vein branches are seen in the cirrhotic liver; these deformations are slight in the fatty liver. Severe distortion and stenoses of the peripheral hepatic vein branches are seen in the cirrhotic liver; there is no remarkable change in the fatty liver.

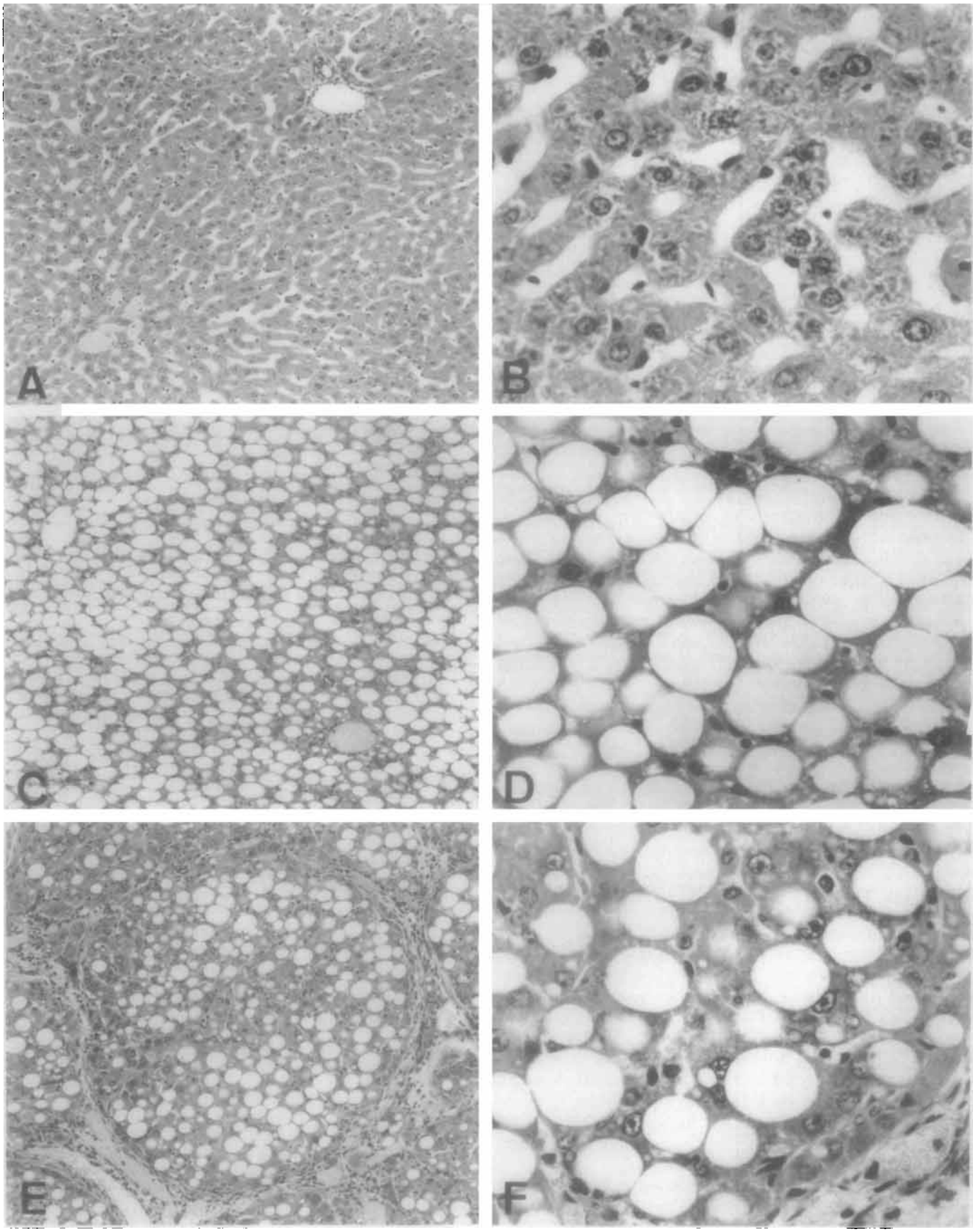

FIG. 5. Histologic findings of the liver (H & E): (A) normal liver (× 100); (B) normal liver (× 400); (C) fatty liver (× 100); (D) fatty liver (× 400); (E) cirrhotic liver (× 100); (F) cirrhotic liver (× 400). In the fatty and cirrhotic livers, the hepatic cells contain large fat droplets and are markedly swollen. Consequently, the sinusoids are severely narrowed, and the sinusoidal space is decreased.

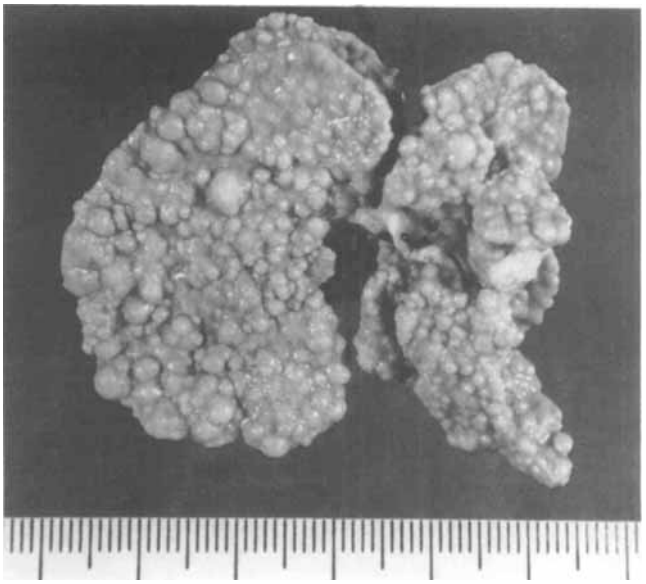

FIG. 6. Liver cirrhosis produced by feeding a choline-deficient diet for 6 months.

Postsinusoidal block due to obliteration or stenoses of the hepatic vein branches has been regarded as a cause of portal hypertension in liver cirrhosis (1–7). If these lesions disturbed the outflow of the sinusoidal blood, the increase in vascular resistance in the intrahepatic hepatic vein and congestion in the sinusoids ought to be found. In the present study, however, such evidence was not found, although distortion of the peripheral branches of the hepatic vein was found in the cirrhotic liver. If the cirrhotic liver in the present study had abundant portohepatic anastomoses, our results do not successfully refute the postsinusoidal block theory. However, we did not find any such anastomoses at the vital microscopic observation or by the examination of angiograms. Accordingly, in liver cirrhosis, the organic changes in the hepatic vein branches do not contribute to the increase in hepatic vascular resistance because the main resistance is already present upstream—in the intrahepatic portal vein and sinusoids—as discussed below.

Sinusoidal block has been suggested as a cause of portal hypertension in liver cirrhosis (8, 16, 24, 25). However, there is no direct proof. In the present study, the increase in sinusoidal vascular resistance was directly confirmed in the fatty and cirrhotic livers. We believe that the increase in resistance resulted from sinusoidal stenoses and the decrease in sinusoidal space due to hepatic cell swelling. Sinusoidal fibrosis may be considered the reason for the increase in sinusoidal vascular resistance. However, we think that the changes in sinusoidal lumen is more important for the increase in vascular resistance than sinusoidal fibrosis because sinusoidal fibrosis was slight in the present study.

These results suggest that increased vascular resistance in the intrahepatic portal vein and sinusoids is the main cause leading to portal hypertension in cirrhosis.

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