Regeneration in Cirrhosis of the Liver
Following Partial Hepatectomy

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Distribution of regenerative hepatocytes was observed by autoradiographic study in
_at liver after partial hepatectomy in order to investigate the relationship between hepatic
blood flow around the pseudolobule and regeneration in cirrhosis of the liver. In cirrhotic
liver, pseudolobules were mostly supplied by hepatic arterial flow as most of portal blood
flow were bypassed around the pseudolobule resulting in marked decrease of sinusoidal
blood flow. Regenerative rate of liver remnant was not increased in cirrhotic hepatectomized
group during the experimental period, while it was increased to $105.0\pm21.2\%$ at 48 hours
in normal hepatectomized group after operation ($P<0.01$). Unelevated regenerative rate
in cirrhotic liver may have been induced by marked decrease of sinusoidal portal blood
flow caused by intrahepatic portavenous shunt. As judged by H^3-thymidine incorporation,
DNA synthesis was increased to a maximum of 8335.1 ± 1668.8 dpm/mg/min. at 36 hours
in normal liver, and of 6538.9 ± 2898.5 dpm/mg/min. at 48 hours in cirrhotic liver after
partial hepatectomy ($P<0.01$). Average labeled hepatocyte number was also significantly
increased to a peak of 48.0 ± 9.1 at 36 hours in lobules of normal liver ($P<0.001$) and
of 12.9 ± 4.8 at 48 hours in pseudolobules of cirrhotic liver after partial hepatectomy ($P<0.01$). Dissociation of unelevated regenerative rate of liver remnant from increase of DNA
synthesis in cirrhotic hepatectomized group may indicate that cell volume stimulating factor
is entirely different from cell number stimulating factor, and each factor plays a role in-
dividually. Direction of spreading of regeneration in normal liver was from peripheral
zone to central zone, while spreading of regeneration in cirrhotic liver did not have specific
direction in the pseudolobule. Direction of spreading of regeneration in normal hepatecto-
mized group may substantiate the relation between direction of intralobular blood flow and
liver regeneration. Nonspecific distribution in cirrhotic liver which has no direction of
regeneration, indicates that regeneration does not always need the portal blood flow and
can be supported by hepatic arterial flow which is expected to contain the portal blood
factor after systemic circulation.

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INTRODUCTION

Factors which control the liver regeneration following partial hepatectomy have been studied by many workers. Hepatic blood volume (16) and humoral factors (2)(4)(8)(24) (29) were experimentally pointed out as extracellular factors which regulate the liver regeneration in normal liver. However, such regulatory factors in cirrhotic liver has not been well investigated, except some reports regarding capacity of regeneration. (5) (10) (11)(15)(17)(23)(25)(27). Liver cell carcinomas are frequently associated with cirrhosis of the liver (7)(12)(18) and have been resected successfully due to advances of diagnostic and surgical technics and postoperative management. Therefore, it seems important to study the regulatory mechanism in relation to specific environment such as vascular system in cirrhotic liver. In the present report, distribution of regenerative hepatocytes in pseudolobules was examined by autoradiographic study in cirrhotic rats in order to clarify the relationship between intralobular regeneration and intrahepatic vascular system around the pseudolobule.

MATERIALS AND METHODS

(1) Experimental Animals

Male Wistar albino rats, weighing 230-260 gm were used. Cirrhosis of the liver was produced by i. m. injection of 1 ml per 100 gm body weight of 20 per cent CCL4-olive oil twice a week for 3 months. Sixty-three animals which showed obvious cirrhosis of the liver microscopically which consisted of fibrous connective tissue and pseudolobule, were operated on at 8 days after discontinuation of oil injection. Normal 48 rats, weighing 250-350 gm, were used as control.

(2) Operation

Following fasting for 5 hours, either left lateral lobectomy of the liver or sham operation in which the liver was exposed and abdomen was closed without hepatectomy, was performed under ether anesthesia. After operation, the animals were offered food and water freely up to the time when they were sacrificed. Each rat was given intraperitoneally 6-methyl-H^3-thymidine (specific activity 5 Ci/mmol) in dose of 1 μCi/gm body weight at 1 hour before sacrifice. Experimental animals were divided into four groups ; normal hepatectomized, normal sham, cirrhotic hepatectomized, cirrhotic sham. The animals were sacrificed at 12, 24, 36 and 48 hours and 4 and 7 days after operation, respectively. Remaining liver was excised from each rat and the weight was recorded. In sham operation groups, left leteral lobe was removed and weighed. Small liver samples from each rat were weighed in wet condition and immersed in 1 ml of tissue solubilizer (Soluene 350).

Aside from the above experiment, investigation of the distribution of portal vein and hepatic artery in both normal and cirrhotic liver by dye injection technic was performed. About 7 ml of India ink was infused into portal vein after ligation of hepatic

artery, and into hepatic artery by direct heart injection after ligation of portal vein, using wings-equiped needle under ether anesthesia. After fixation by 10% neutral formalin, liver specimens were embedded in paraffin, cut 25μ thick and mounted.

(3) H^3-thymidine Incorporation into DNA

Nine ml of Instagel was used scintillator and incorporation of isotope was measured in Aloka LSC 602 liquid scintillation spectrometer. Value of the activity was corrected by external standard method.

(4) Autoradiographic Study

After fixation in 10% neutral formalin, liver specimens were embedded in paraffin and cut 4μ thick. These sections were deparaffined, dipped in the photographic emulsion (Sakura NR–M2), dried and stored in darkness at 4°C for 5 weeks. Then, each slide was developed and stained with hematoxylin and eosin by Harris' method.

Labeled hepatocyte number was counted in the lobules of normal liver and in the pseudolobules of cirrhotic liver. To determine the distribution of labeled hepatocytes, such cells were marked on the paper sketching the lobule in normal liver or the pseudolobule in cirrhotic liver. Circles, the diameter of which equaled one-third and two-third of the distance between portal area and central vein of lobule, and between perilobular area and central point of pseudolobule, were drawn on the map, with the use of an ocular reticule under a microscope. Thus, lobules and pseudolobules were divided into three areas, namely peripheral, middle and central zones. Labeled hepatocytes in all three areas were totaled and the numbers in each zone were expressed as per cent of the total number.

(5) Regenerative Rate of Liver Remnant

Average percentage of weight of left lateral lobe to whole liver in normal and cirrhotic sham groups, were 29.7 per cent, and 36.0 per cent respectively. Regenerative rate was estimated by percentage of weight of liver remnant to calculated whole liver weight. Change of body weight during experimental period was not taken into consideration.

(6) Statistical Analysis

The statistical comparison for significance was carried out utilizing the Student t test, and a P value of less than 0.05 was considered to be significant.
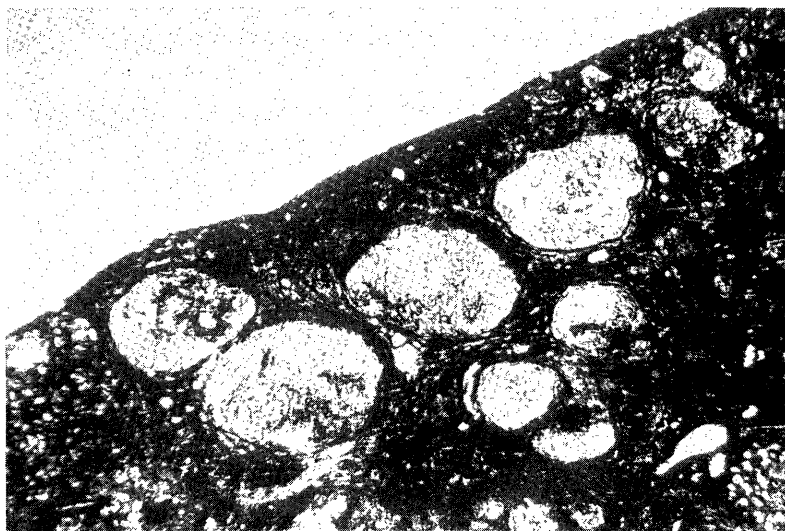
RESULTS

(1) Distribution of portal vein and hepatic artery shown by dye injection technic (Fig.1)

In normal liver, the india ink was diffusely distributed into the lobule, whichever route, arterial or portal venous, was selected. However, in cirrhotic liver, the india ink which was infused into portal vein, was distributed only slightly into the pseudolobules, but abundantly at the perilobular spaces. While, the dye which was infused into the hepatic artery, was distributed into the pseudolobules diffusely.

(2) Regenerative rate of liver remnant (Fig. 2)

Regenerative rate was increased in normal hepatectomized group to $105.0\pm21.2\%$ at 48 hours after partial hepatectomy, while it was not increased in cirrhotic hepatectomized group for as long as seven days after operation. Regenerative rate in normal hepatectomized

Distribution of portal vein
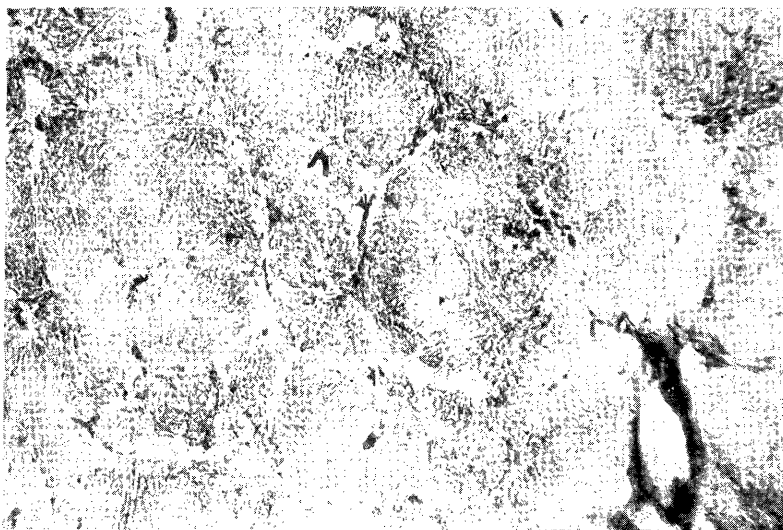
Distribution of hepatic artery

Fig. 1 Distribution of portal vein and hepatic artery shown by dye injection technic
India ink which was infused into portal vein, was distributed only slightly into
the pseudolobules, but abundantly at the perilobular spaces. While, the dye
which was infused into the hepatic artery, was distributed into the pseudolobules
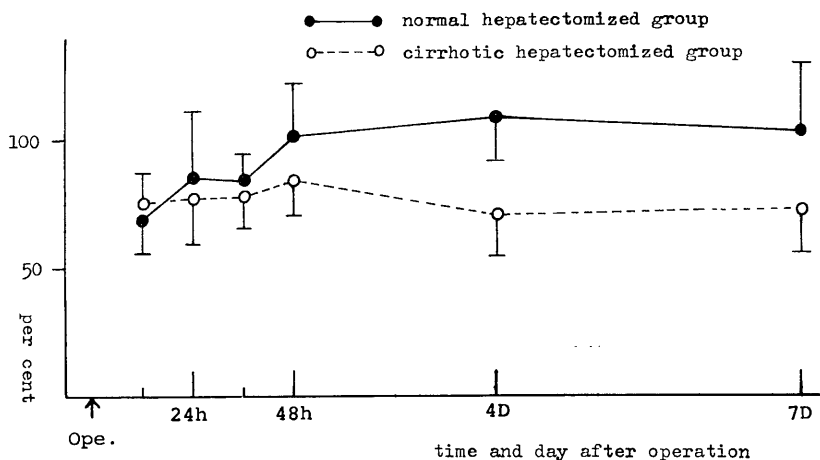diffusely.

Fig. 2 Regenerative rate of liver remnant
Regenerative rate was increased in normal hepatectomized group to 105.0±21.2%
at 48 hours after partial hepatectomy, while it was not increased in cirrhotic
hepatectomized group for as long as seven days after operation. Regenerative
rate in normal hepatectomized group was significantly higher than that in cirrhotic
hepatectomized group (P<0.01). Mean ±S. E. (vertical lines), normal liver
group : n=4 at 12hrs, n=5 at 24hrs, n=5 at 36hrs, n=5 at 48hrs, n=4 at 4
days, n=5 at 7days, cirrhotic group : n=6 at 12hrs, n=7 at 24hrs, n=5 at 36hrs,
n=7 at 48hrs, n=5 at 4days, n=5 at 7days.

group was significantly higher than that in cirrhotic hepatectomized group (P<0.01).
(3) H³-thymidine incorporation into DNA (Fig. 3)

As shown by Fig. 3, levels of DNA synthesis in both hepatectomized groups were higher than sham operation groups at 12 hours after operation, and reached to a maximum of 8335.1±1668.8 dpm/mg/min. at 36 hours in normal hepatectomized group (P<0.01) and of 8538.9±2898.5 dpm/mg/min. at 48 hours (P<0.01) in cirrhotic hepatectomized group. The peak DNA synthetic response in cirrhotic hepatectomized group was lower than normal hepatectomized group.
(4) Autoradiographic study (Fig. 4, 5 and 6)

Average labeled hepatocyte number was significantly increased to a peak of 48.0± 9.1 at 36 hours in lobules of normal liver compared with the normal sham group (P<0.001) and of 12.9±4.8 at 48 hours in pseudolobules of cirrhotic liver compared with the cirrhotic sham group (P<0.01) after partial hepatectomy similar to the result of H³-thymidine incorporation. In normal hepatectomized group (Fig. 5), there was prominent localization of labeled hepatocytes in peripheral zone for 36 hours after operation, and from 48 hours such localization disappeared as the results of decrease of labeled hepatocytes in peripheral zone and increase in middle and central zones.

In cirrhotic hepatectomized group (Fig. 6), percentage of the labeled hepatocytes in each zone of the pseudolobules was the most prominent in peripheral zone, the least in central zone and in-between in middle zone. The rates of these localization in three zones
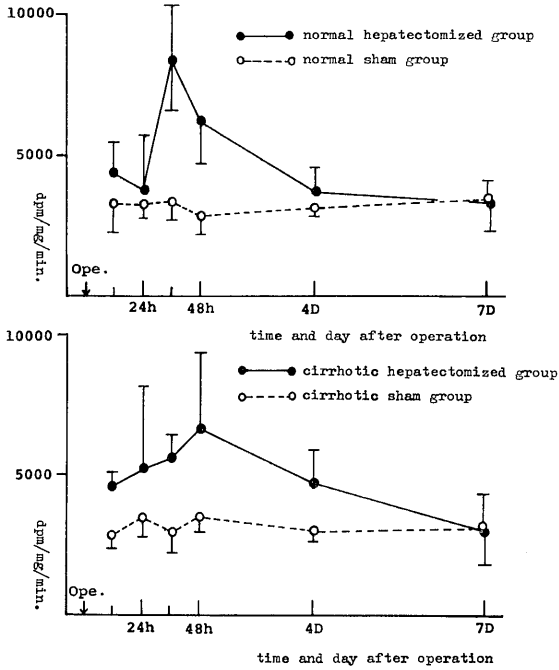
Fig. 3 H³-thymidine incorporation into DNA

As judged by H³-thymidine incorporation, levels of DNA synthesis in both hepatectomized groups were higher than sham operation groups at 12 hours after operation, and reached to a maximum of 8335.1±1668.8 dpm/mg/min. at 36 hours in normal hepatectomized group (P<0.01) and of 6538.8±2898.5 dpm/mg/min. at 48 hours in cirrhotic hepatectomized group (P<0.01). Mean±S.E. (vertical lines)
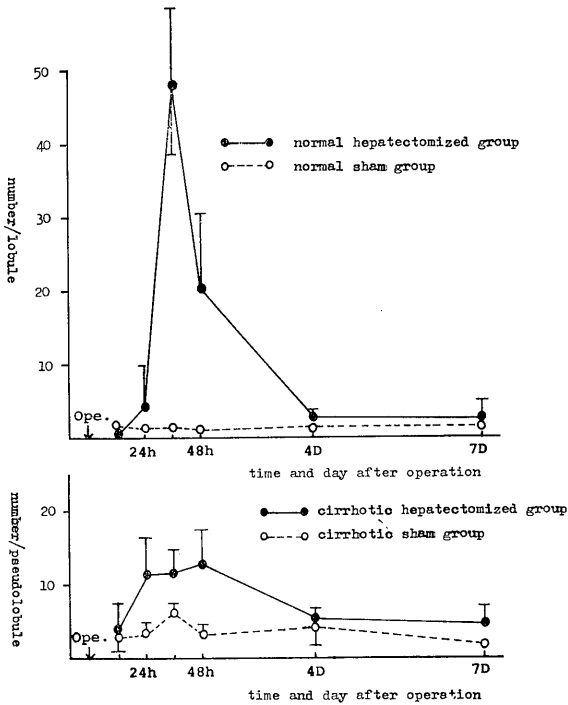
Fig. 4 Average labeled hepatocyte number in each lobule and pseudolobule

Average labeled hepatocyte number was significantly increased to a peak of 48.0±9.1 at 36 hours in lobules of normal liver (P<0.001) and of 12.9± 4.8 at 48 hours in pseudolobules of cirrhotic liver (P<0.01) after partial hepatectomy. Mean ± S.E. (vertical lines)
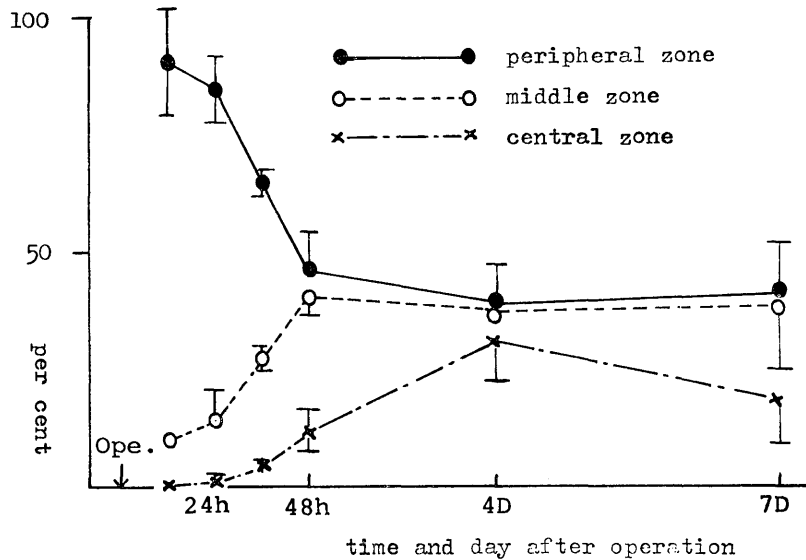
Fig. 5 The time course of distribution of labeled hepatocytes in lobules in normal hepa-
tectomized group
There was prominent localization of labeled hepatocytes in peripheral zone for 36
hours after operation, and from 48 hours such localization disappeared as the
results of decrease of labeled hepatocytes in peripheral zone and increase in
middle and central zones.
Mean±S.E. (vertical lines), n=4 at 12hrs, n=5 at 24hrs, n=5 at 36hrs, n=5 at
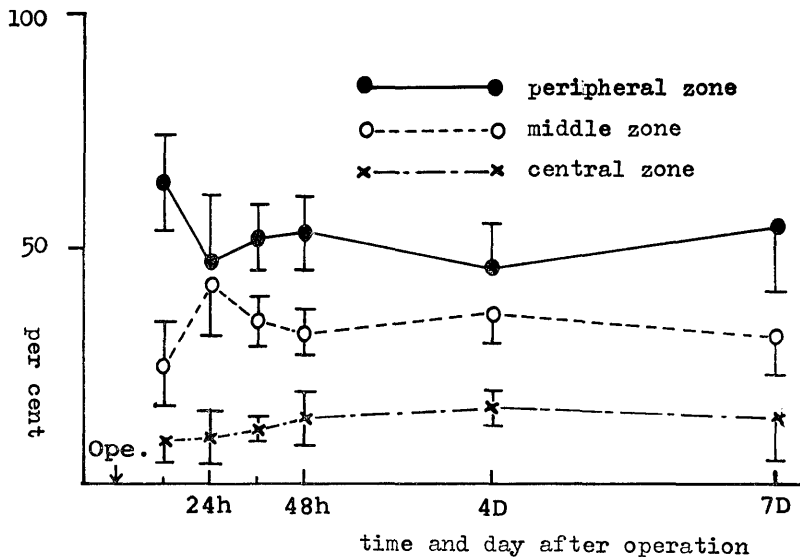48hrs, n=4 at 4days, n=5 at 7days.

Fig. 6 The time course of distribution of labeled hepatocytes in pseudolobules in cirrhotic
hepatectomized group
Percentage of the labeled hepatocytes in each zone of the pseudolobules was the
most prominent in peripheral zone, the least in central zone and in-between in
middle zone. The rates of these localization in three zones were almost stationary
during the experimental period.
Mean±S.E. (vertical lines), n=6 at 12hrs, n=7 at 24hrs, n=5 at 36hrs, n=7 at
48hrs, n=5 at 4days, n=5 at 7days

were almost stationary during the experimental period. The results which the percentage of labeled hepatocytes in three zones were not increased in spite of increase of average labeled hepatocytes in pseudolobules after partial hepatectomy in cirrhotic liver as shown in Fig. 4, implicate that rates of increased labeled hepatocytes in three zones were almost same.

DISCUSSION

The control of liver regeneration may be considered to be done by intracellular and extracellular regulation(31). Intracellular regulation is thought to exist and to be a control mechanism within the cell which initiate and mediate the change in nucleic acids and in protein synthesis associated with cell division(9).

Extracellular regulation relates to conditions arising outside of a liver cell which promote cell division and cell enlargement, and hepatic blood volume(6)(16) and humoral factor are proposed as extracellular factors. As humoral factor, for instance, insulin(3) (30), glucagon(3)(24)(34) and portal blood factor (P.B.F) (8) have been reported. In this study, in order to investigate the relationship between hepatic blood flow and regeneration in cirrhotic liver, distribution of regenerative hepatocytes was observed by autoradiographic method in both normal and cirrhotic livers in rats after partial hepatectomy.

As shown by Fig. 4, 5 and 6, there was increase of labeled hepatocyte after partial hepatectomy in both normal and cirrhotic livers. Direction of spreading of regeneration in normal liver was from peripheral zone to central zone, while in cirrhotic liver such direction of spreading was not seen. The rates of labeled hepatocyte in three zones were almost constant throughout the experiment, in spite of demonstrating an increase of average labeled hepatocytes after partial hepatectomy. These results indicate that rates of increased labeled hepatocytes in three zones in cirrhotic liver were the same.

Rabes(28) concluded from the proliferative differences in time and intensity within lobule in his autoradiographic study in normal rat liver after partial hepatectomy that microenvironment related to distribution of blood flow in liver lobules, would determine a topography of optimal condition for cell proliferation. Sigel(32) reported that localization of new hepatocyte formation could not be attributed to an inherently greater capacity of peripheral liver cell to divide but was probably related to their preferential exposure to blood constituent changes from his experiment which labeled hepatocytes were more prevalent about the central vein in the "reverse" blood flow graft.

In liver cirrhosis, portal blood flow circuits the pseudolobules via intrahepatic shunt which was reported to be specific finding of vascular change of liver cirrhosis with reduction of vascular bed (1) (13) (14) (19) (20) (26). Koo(13) and Kretz(14) reported that regenerative nodules in liver cirrhosis were mainly supplied by hepatic arterial flow. As shown by Fig. 1, portal blood flow perfuses mainly perilobular area of the pseudolobules resulting in marked decrease of sinusoidal portal blood flow, and pseudolobules are mostly supplied by hepatic arterial flow.

Weinbren(35) showed that portal blood diversion away from a remnant of liver tissue after partial hepatectomy did not impair regeneration. Fisher(8) reported that there was a portal blood factor (P.B.F.) which was capable of stimulating hepatic cells and was neither destroyed nor inhibited in systemic blood. Bucher(2) and Price(24) showed that hepatic artery alone was capable of supporting liver cell proliferation. Thus, the same increasing rates of labeled hepatocytes in three zones in cirrhotic liver after partial hepatectomy as shown by Fig. 4, may substantiate that liver regeneration is not always affected by contact with portal blood flow, and can be stimulated by hepatic arterial flow which is expected to contain the portal blood factor after systemic circulation. Because, if the portal blood flow only could stimulate the liver regeneration, the increasing rate of labeled hepatocyte in the peripheral zone should be higher than the other two.

There was no elevation of regenerative rate though there was increase of DNA synthesis in cirrhotic liver after partial hepatectomy. Fisher(8) reported that volume of the portal blood flow was related to hypertrophy and humoral factor was related to hyperplasia. Malamud(22) suggested that cellular hypertrophy and cellular hyperplasia were independent each other. Dissociation of unelevated regenerative rate of liver remnant from increase of DNA synthesis may indicate that cell volume stimulating factor is not identical with cell number stimulating factor, and each factor plays a role individually. Unelevated regenerative rate of liver remnant in cirrhotic liver may be induced by marked decrease of sinusoidal portal blood flow which is caused by intrahepatic portavenous shunt. Volume of sinusoidal blood flow in cirrhotic liver may affect liver regeneration and restoration of postoperative liver function in critical stage after partial hepatectomy. Then, clinically it may be useful for estimating the indication of hepatic resection of liver cell carcinoma associated with liver cirrhosis to calculate the intrahepatic shunting rate before operation. Furthermore, it may be recommended to perform periarterial neurectomy of the hepatic artery which was reported that it would increase the hepatic arterial blood flow(21), bring a good effect to postoperative course(36) and promate liver regeneration after hepatic surgery(33).

ACKNOWLEDGEMENT

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