Effects of Hct and norepinephrine on segmental vascular resistance distribution in isolated perfused rat livers

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The responsiveness of the hepatic longitudinal vascular segments differs depending on vasoactive agents (3, 23, 24). The differences in the response of these vascular segments to a variety of stimuli are, in part, due to the intrinsic vasomotor properties of the blood vessels as well as their passive viscoelastic properties. In addition, the microhemodynamic behavior of blood may vary in the segments of the hepatic vasculature. Blood apparent viscosity has been shown to change depending on the different size vessels in the systemic circulation (13, 14). As blood vessels decrease in diameter between 300 and 30 μm, blood apparent viscosity decreases due to Fahraeus-Lindqvist effect (1, 6). Hematocrit (Hct) is an important determinant of blood viscosity and can affect resistance to blood flow in the circulation (4, 17). In the liver, little is known about the effect of changes in blood Hct on the longitudinal distribution of vascular resistance. Thus we examined the effect of different Hct on the longitudinal vascular resistance distribution in the rat liver.

Norepinephrine constricts predominantly the presinusoidal vessels over the postsinusoidal vessels of hepatic veins (3, 15, 22, 24). Maass-Moreno and Rothe (15), by using the double-lumen catheter inserted through the caval wall into hepatic vein in anesthetized dogs, reported that an infusion of norepinephrine caused a large increase in portal venous pressure but little change in pressure gradient from the large hepatic vein to vena cava. However, the side port of the catheter that was used to

LIVER AND LUNG circulation is analogous (19). Both the pulmonary artery and the portal vein are unique in their ability to carry large flows of venous blood under low hydrostatic pressures. The pulmonary arterioles and the portal venules have a similar anatomy (19). The vascular arrangement of the hepatic units is analogous to that of the lung in that both lobules have a central inflow (pulmonary artery and portal vein) and a peripheral outflow (pulmonary vein and hepatic vein) (18). In addition, embryologically, the tracheo-bronchial tree and the biliary ductal system originate from the gut.

The longitudinal distribution of pulmonary vascular resistance has been extensively studied using vascular occlusion techniques, and the pulmonary vasculature can be represented by a simple hydrodynamic model consisting of three segments in series, each with a characteristic resistance and compliance (10, 12). The middle segment, which contains the capillaries, has relatively low resistance and high compliance, and the other two segments have relatively low compliance and high resistance. The pulmonary arterial and venous occlusion technique (9–11) allows partitioning of the pulmonary vasculature into these three segments. Theoretically, if the flow is stopped across one segment, the pressure gradient across that segments becomes zero, and the total pressure gradient would decrease accordingly. In other words, the rapid changes in pulmonary arterial pressure and pulmonary venous pressure with inflow and outflow occlusion, respectively, represent the pressure drops across the arterial and venous relatively indistensible vessels (10). The total arteriovenous pressure difference minus the sum of these two pressure drops gives the pressure drop across the vessels in the middle that are much more distensible. Although hepatic circulation is analogous to pulmonary circulation in several aspects, as described above, no investigations have been performed to adopt this inflow and outflow occlusion technique to partition the hepatic vasculature into the three segments.

The responsiveness of the hepatic longitudinal vascular segments differs depending on vasoactive agents (3, 23, 24). The differences in the response of these vascular segments to a variety of stimuli are, in part, due to the intrinsic vasomotor properties of the blood vessels as well as their passive viscoelastic properties. In addition, the microhemodynamic behavior of blood may vary in the segments of the hepatic vasculature. Blood apparent viscosity has been shown to change depending on the different size vessels in the systemic circulation (13, 14). As blood vessels decrease in diameter between 300 and 30 μm, blood apparent viscosity decreases due to Fahraeus-Lindqvist effect (1, 6). Hematocrit (Hct) is an important determinant of blood viscosity and can affect resistance to blood flow in the circulation (4, 17). In the liver, little is known about the effect of changes in blood Hct on the longitudinal distribution of vascular resistance. Thus we examined the effect of different Hct on the longitudinal vascular resistance distribution in the rat liver.

Norepinephrine constricts predominantly the presinusoidal vessels over the postsinusoidal vessels of hepatic veins (3, 15, 22, 24). Maass-Moreno and Rothe (15), by using the double-lumen catheter inserted through the caval wall into hepatic vein in anesthetized dogs, reported that an infusion of norepinephrine caused a large increase in portal venous pressure but little change in pressure gradient from the large hepatic vein to vena cava. However, the side port of the catheter that was used to
measure the hepatic venous pressure was far downstream from the hepatic venules. Their subsequent study (22), which used the micropuncture method, revealed that norepinephrine caused significant increases in microhepatic venous (venule) pressure and the resistance between the sinusoids and the vena cava. However, a more detailed investigation on the constrictive site of norepinephrine has not been reported.

Recently, Hakim et al. (11) showed that the double occlusion conducted in isolated perfused lungs can be analyzed to provide three segments without performing the single occlusion of pulmonary arterial or venous occlusion independently. We examined whether resistance of the three segments of portal-hepatic venous vessels can be determined by one double occlusion maneuver lasting 3 to 4 s in isolated perfused rat livers. This approach has the advantage of being able to partition the vasculature into three segments when the vasculature is not in a steady state.

The first purpose of this study was to obtain the segmental resistances of portal veins and middle and hepatic veins by using both the portal (inflow) and hepatic venous (outflow) occlusion techniques in isolated rat livers perfused via the portal vein with the hepatic artery ligation. In the liver, the highest compliant and most distensible segment of the middle segment, which could be obtained by the present vascular occlusion methods, corresponds to the sinusoidal bed, but not the small portal and hepatic veins, because the sinusoids are the locus of the high compliance in the liver (2, 8). This is based on the evidence that the sinusoids comprise the majority of the vasculature of the liver and that the vascular compliance of the liver is ~10 times higher than that of the body as a whole (2). Thus one of the most important purposes of the present study is to measure the resistance of the middle segment of the sinusoids. The second purpose was to describe the effects of Hct and blood flow on the distribution of vascular resistance. The third goal was to determine the effect of norepinephrine on the vascular resistance distribution. The final purpose of the present study was to determine whether one double occlusion technique could provide portal or hepatic venous occlusion pressure, either of which was obtained individually by occlusion of the corresponding vessel.

MATERIALS AND METHODS

Isolated liver preparation. Twenty-nine male Sprague-Dawley rats weighing 270–370 g [328 ± 32 g (SD)] were anesthetized with pentobarbital sodium (50 mg/kg iv) and mechanically ventilated with room air. The experiments conducted in the present study were approved by the Animal Research Committee of Shinshu University School of Medicine. A catheter was placed in the right carotid artery. After laparotomy was performed, the hepatic artery was ligated. Before the cannulation of the portal vein, the perfusion circuit was filled with diluted blood of a donor rat, as described below. At 5 min after the injection of 500 units of heparin into the carotid artery, the portal vein was cannulated with a stainless cannula (2.1 mm ID, 3.0 mm OD) and then portal perfusion started. The rat was rapidly bled through the carotid arterial catheter just before the portal cannulation. After thoracotomy, the suprachlavicular portion of the inferior vena cava (IVC) was cannulated with a stainless cannula (2.1 mm ID, 3.0 mm OD), and the IVC above the renal veins was ligated. The perfused liver was then transferred to a weighing pan, which was suspended from an electric balance (model LF-6, Murakami Koki; Osaka, Japan), and the initial wet liver weight [8.8 ± 1.1 g (SD)] was recorded.

The perfusing blood was obtained by exanguination of an intact donor rat that was anesthetized and heparinized, and this blood was diluted with 5% bovine serum albumin (Fraction V powder-A2153, Sigma) in a Krebs solution composed of (in mM) 118 NaCl, 5.9 KCl, 1.2 MgSO4, 2.5 CaCl2, 1.2 NaH2PO4, 25.5 NaHCO3, and 5.6 glucose at the following Hct levels: 30% (n = 8), 20% (n = 7), 10% (n = 8), and 0% (n = 6). The blood (50 ml) was recirculated at a constant flow rate using a Masterflex pump through a heat exchanger and a bubble trap in the portal line. The perfusing blood in the reservoir was warmed with a water bath (37°C). The portal (Ppv) and hepatic venous (Phv) pressures were continuously measured with pressure transducers (Gould) attached to a sidearm placed just proximal to the perfusion cannula. The zero reference was set to the level of the hepatic hilus. The flow rate and height of the venous reservoir could be adjusted independently to maintain Phv at 0 to 1 cmH2O, with Ppv a dependent variable. The perfusion flow rate (Q) was measured with an electromagnetic flow meter (model M1V 1200, Nihonkohden), and the flow probe was positioned in the portal inflow line. To occlude the portal or hepatic venous line instantaneously for measurement of the portal occlusion pressure (Ppo) or hepatic venous occlusion pressure (Ppfo), two solenoid valves were placed around the perfusion tubes upstream from the Ppv sidearm cannula and downstream from the Phv sidearm cannula. The hemodynamic variables were continuously monitored and displayed on a thermal physiograph (model 8K23, NEC Sanei; Tokyo, Japan).

Measurement of Ppv and Phv by single occlusion maneuver. When a steady state of a constant Ppv was reached, the single occlusion maneuver of portal or venous occlusion was performed, and the signals were sampled at 100 Hz and stored in a computer. Portal occlusion was accomplished for 3 s by closing the solenoid valve set in the portal line. Hepatic venous occlusion was accomplished for 1.5 s by closing the solenoid valve set in the hepatic venous line while inflow continued. Portal and hepatic venous pressure tracings were displayed and analyzed independently. An example of portal occlusion was shown in Fig. 1A. A stretch of data on Ppv between 0.3 and 1.8 s after portal occlusion for 3 s was fitted to a single exponential and extrapolated back to time 0 (instant of occlusion). This extrapolated pressure was then used as Ppo. An example of hepatic venous occlusion tracing was shown in Fig. 1B, and a stretch of data (0.3–1.0 s) on Phv was fitted to a straight line and extrapolated back to time 0 (instant of occlusion). This extrapolated pressure was used as Ppfo.

Estimation of Ppv and Ppfo from double occlusion. We estimated Ppv and Ppfo from the double occlusion tracing, as shown in Fig. 1C. Double occlusion was accomplished for 3 s by closing both of the inflow and outflow valves simultaneously. Ppv during double occlusion was analyzed to determine Ppv in the same manner as the portal occlusion maneuver. The stretch of Ppv data between 0.3 and 1.8 s was selected and fitted to a monoeponential. The fitted curve was extrapolated back to the time of occlusion, and the time 0 pressure was designated as the double occlusion-derived Ppv (Ppv-do). Likewise, data between 0.3 and 1.8 s on Phv tracing during double occlusion was fitted to an exponential and extrapolated back to the time of occlusion. This time 0 pressure was designated as the double occlusion-derived Ppfo (Ppfo-do).

Experimental protocol. Hepatic hemodynamic parameters were observed for at least 30 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting flow rate and the height of the reservoir at a Ppv of 0 to 1 cmH2O, and at a highest Q. After this baseline measurement, the flow rate was increased or decreased by 5 ml/min ranging from 5 to 40 ml/min with keeping Ppv constant at 0 to 1 cmH2O. At each steady state of a given flow rate, all three occlusion maneuvers of portal occlusion, hepatic venous occlusion, and double occlusion were performed at a random venous flow rate was then increased to 25 ml/min for the 30% Hct group, 30 ml/min for the 20% Hct group, 35 ml/min for the 10% Hct group, and 40 ml/min for the 0% Hct group. After stabilization of vascular pressures, the effect of two doses of norepinephrine on the
vascular resistance distribution was studied. Norepinephrine (Sigma) was infused continuously at the low dose of 1 μg/min into the portal vein until P pv increased and stabilized. Under this steady state, portal, hepatic venous, and double occlusions were performed. The effect of the high dose of 10 μg/min was then examined in a similar manner.

Experiments in the present study were carried out in livers perfused with blood of 30%, 20%, 10%, and 0% Hct. In each preparation, the effect of only one fixed Hct was examined: the perfusate Hct was not changed throughout the experimental period, once the perfusion started at a given Hct. Blood flow rate was expressed as ml/min 1-10 g liver wt -1; and the blood flow groups were assigned to eight groups from 5 ml/min (2.5-7.5 ml/min 1-10 g liver wt -1) to 40 ml/min (37.5-42.5 ml/min 1-10 g liver wt -1) group.

For determination of hepatic segmental vascular resistances, the total portal-hepatic venous (R t), portal venous (R pv), sinusoidal (R sinus), and hepatic venous (R hv) resistances were calculated as follows:

\[ R_t = \frac{(P_{pv} - P_{hv})}{Q} \]  
\[ R_{pv} = \frac{(P_{pv} - P_{hv})}{Q} \]  
\[ R_{sinus} = \frac{(P_{pv} - P_{hvo})}{Q} \]  
\[ R_{hv} = \frac{(P_{hvo} - P_{hv})}{Q} \]

For calculation of these segmental vascular resistances, we adopted the mean value of P pv for 5 s before the initial vascular occlusion maneuver as the P pv in the equations. The P pv before the subsequent occlusion maneuver did not differ by 0.2 cmH 2 O from the P pv of the initial occlusion maneuver.

Statistics. All results are expressed as the means ± SD, unless mentioned otherwise. Comparisons of a given variable between the groups were performed using analysis of variance, followed by Bonferroni’s test. A P value <0.05 was considered significant. For the correlation between P pv and P pv-do or between P hvo and P hvo-do, least-square linear regression analysis was used. Statistical significance of correlation of the linear regression was tested with analysis of variance. A paired Student’s t-test was used to compare the mean pressures obtained with the single occlusion method and the double occlusion method.

RESULTS

Effects of blood flow rate on hepatic vascular pressures and resistances. Figure 2, left, shows the mean data of hepatic vascular pressures, including P pv and P hvo obtained from analysis of tracings of portal occlusion and hepatic venous occlusion, respectively, in different Hct groups. For example, in the Hct 30% and 15 ml/min group, where the livers were perfused with blood of the highest Hct of 30% at 15.6 ± 1.6 ml/min 1-10 g liver wt -1, P pv was 9.1 ± 1.4 cmH 2 O, which was similar to the P pv levels observed in in vivo rats (3), P pv 5.3 ± 1.1 cmH 2 O, P hvo 2.0 ± 0.4 cmH 2 O, and P hv 0.4 ± 0.11 cmH 2 O. On the basis of these data, the calculated segmental vascular resistances of R t, R sinus, and R hv were 0.251 ± 0.044, 0.212 ± 0.071, and 0.105 ± 0.022 cmH 2 O ml -1 min -1 10 g liver wt -1, respectively, as shown in Fig. 2, left. This indicates that R pv comprises 44% of R t, 37% of R sinus, and 19% R hv in livers perfused with blood of 30% Hct at the physiological portal pressure.

Within each Hct group, when the flow rate increased at a constant outflow pressure of P hv, P pv and P hvo increased, whereas P pv did not change significantly, as shown in Fig. 2, left. This results in the flow-dependent increases in both P pv-to-P pv and P hvo-to-P hv gradients, and the flow-dependent decreases in the P pv-to-P hvo gradients. On the basis of these pressure gradient changes, R sinus decreased, and either R pv or R hv did not change when the blood flow increased within each Hct group, as shown in the right panel of Fig. 2. Actually, R sinus at 30% Hct comprised 60 ± 12% of R t in the minimal flow group of 5 ml/min, 41 ± 11% in 10 ml/min, 36 ± 11% in 20 ml/min, 28 ± 8% in 15 ml/min, and 25 ± 6% in 25 ml/min. An interesting finding was that the sensitivity of the inflow pressure of P pv to changes in flow rate was low: in the 30% Hct groups, only a twofold increase in P pv from 6 to 12 cmH 2 O was observed as blood flow was increased fivefold from 5 to 25
Representative recordings of hepatic vascular pressures in various flow rates of a liver perfused with 30%, 20%, 10%, and 0% Hct in the blood flow groups. Right, summary of segmental hepatic vascular resistances of portal venous (Rpv), sinusoidal (Rsinus) and hepatic venous resistance (Rhv) of isolated rat livers perfused with blood of Hct 30%, 20%, 10%, and 0% in the blood flow groups. Values are means ± SD; *P < 0.05 vs. 5 ml/min group; **P < 0.05 vs. 10 ml/min group; ***P < 0.05 vs. 15 ml/min group; †P < 0.05 vs. 20 ml/min group; ‡P < 0.05 vs. 25 ml/min group; and ‡P < 0.05 vs. 30 ml/min group.

Effects of Hct on hepatic vascular pressures and resistances. Figure 4 shows the hepatic vascular pressures (left panel) and segmental vascular resistances (right panel), as a function of perfusate Hct. At all blood flow rates studied, Ppv, Ppo, and Phvo were significantly greater in 30% Hct groups than those in 20% Hct groups. Thus increases in Hct from 20% to 30% resulted in significant increases in all three segmental resistances of Rpv, Rsinus, and Rhv at blood flow >15 ml/min. However, at the low blood flow of 5 and 10 ml/min, only Rsinus did not change significantly whereas Rpv and Rhv significantly increased as Hct increased from 20% to 30%. All three segmental vascular resistances of any blood flow became significantly greater at
30% Hct than at 10% Hct and 0%. Figure 5 shows representative recordings of livers perfused with blood of different Hct at the same blood flow of 25 ml/min. Ppv and Phv in this figure were not recorded at same time but were compositely shown in the same frame at each blood flow rate. White lines indicate the fitting curves for Ppv and Phv, after portal and hepatic venous occlusion, respectively. The top and bottom arrows indicate Ppv and Phv, respectively.

Effects of norepinephrine infusion on the hepatic vascular resistance distribution at various Hct and flow rates. Figure 6 shows the summary of the hepatic segmental vascular resistances in response to norepinephrine at the four levels of Hct. Although the basal levels of segmental vascular resistances differed depending on Hct, the trends of the response of segmental vessels to norepinephrine were similar; Rpv did not change significantly, whereas Rsv and Rsinus increased dose and Hct dependently. Actually, an infusion of 1 µg/min norepinephrine caused a 1.5- to 1.7-fold increase in Rpv and a 1.7- to 2.4-fold increase in Rsinus and caused no significant changes in Rsv among any Hct groups studied. Furthermore, the higher dose of 10 µg/min norepinephrine caused a 2.5-fold increase in Rpv and a 3.1- to 4.4-fold increase in Rsinus and again caused no significant changes in Rsv.

Estimation of Ppv, and Phvo by the double occlusion maneuver. Figure 1 shows a representative recording during portal occlusion, hepatic venous occlusion, and double occlusion in an isolated rat liver perfused with blood of 30% Hct at 25 ml/min. Ppv and Phvo obtained via portal occlusion and hepatic venous occlusion, respectively, were almost identical with the pressures obtained by analysis of the tracings of Ppv, and Phvo during the double occlusion maneuver. The agreement between the two methods was tested using all data obtained from both normal livers and livers with vasocstriction induced by norepinephrine, where Ppv ranged from 2.25 to 36.58 cmH2O. Actually 235 paired measurements of Phvvo and Ppv-do, and 225 paired measurements of Ppv and Ppv-do were analyzed. The agreement between these two methods was evaluated in two different ways. First, the mean values of each pressure with the two methods were compared using a simple Student’s t-test. There were no significant differences between Ppv and Ppv-do, and between Phvvo and Phvvo-do under all conditions studied. The agreement between the two methods was further tested by using regression analysis. Regression line equations for Ppv versus Ppv-do, and for Phvvo versus Phvvo-do are given in Fig. 7, A and B, respectively. There were strong positive relationships for Ppv and Ppv-do (r > 0.99). The slope for Ppv (1.009 ± 0.009, P = 0.99) and the y-intercept (−0.021 ± 0.042 cmH2O, P = 0.612), statistically, did not differ from the median slope and zero, respectively. Likewise, concerning the regression lines for Phvvo Versus Phvvo-do, the slope for Phvvo (0.996 ± 0.013, P = 0.62) and the y-intercept (0.009 ± 0.031 cmH2O, P = 0.775), statistically, did not differ from the median slope and zero, respectively (Fig. 7).

**DISCUSSION**

The present study determined the three segmental vascular resistances of isolated portally perfused rat livers by using both portal (inflow) and hepatic venous (outflow) occlusion techniques. When outflow from the liver was suddenly occluded while inflow continued at a constant rate, there was a virtually instantaneous increase in outflow pressure of Phv, after which outflow pressure rose more gradually. This initial increase in Phv was designated as Ppv. Infow occlusion at a constant outflow pressure caused a nearly instantaneous drop in inflow pressure of Ppv, followed by a more gradual decline. This initial decrease Ppv was designated as Ppv. The sudden increase in Phv on outflow occlusion and the sudden decrease in Ppv on inflow occlusion can be interpreted to result from cessation of flow across resistances provided by relatively noncompliant vessels on the hepatic venous and portal ends of the vasculature, respectively, as observed in isolated lungs (10). With the use of Ppv and Phvvo, the total hepatic vascular...
The pressure gradient of $P_{pv}$-to-$P_{hv}$ was divided into three pressure gradients of $P_{pv}$-to-$P_{pv}$, $P_{pv}$-to-$P_{hv}$, and $P_{hv}$-to-$P_{hv}$, which may correspond to the segmental vascular resistances of the relatively less compliant portal veins ($R_{pv}$), a middle compliant compartment of sinusoids ($R_{sinus}$), and the relatively less compliant hepatic veins ($R_{hv}$).

One of the major findings of the present study was that $R_{pv}$ comprises 44% of $R_t$, and $R_{sinus}$ 37%, and $R_{hv}$ 19% in livers.

**Fig. 4.** Left, summary of hepatic $P_{pv}$, $P_{pv}$, $P_{hv}$, and $P_{hv}$ pressures of isolated rat livers perfused at various flow rates in the different Hct groups. Right, summary of segmental hepatic $R_{pv}$, $R_{sinus}$, and $R_{hv}$ resistances of isolated rat livers perfused at various flow rates in the different Hct groups. Values are means ± SD; *P < 0.05 vs. Hct 30% group; **P < 0.05 vs. Hct 20% group; ***P < 0.05 vs. Hct 10% group.
perfused with 30% Hct at physiological Ppv of 9.1 cmH2 O. This finding indicates that almost one-half of Rt occurs in the presinusoidal vessels, one-third in the hepatic sinusoids, and only one-fifth of Rt in the postsinusoidal vessels in rat livers. These results on hepatic vascular resistance distribution are consistent with the findings from the hepatic micropuncture study (3, 16). Bohlen et al. (3), by puncturing surface hepatic venules (10–30 μm diameter), into which two adjacent acini drained, with servo-null micropipettes, reported that hepatic venule pressure was 5.1 ± 1.0 mmHg, and Ppv and vena caval pressure averaged 8.0 ± 1.4 and 3.4 ± 0.9 mmHg, respectively, indicating that the hepatic venule to vena caval pressure gradient comprised as small as 37% of the total Ppv-to-vena caval pressure gradient. Maass-Moreno and Rothe (16) demonstrated much more definitively that the Ppv-to-portal venule gradient comprises 53% of the total Ppv-to-Phv gradient, the portal venule-to-hepatic venule gradient, which may correspond to Rsinus of the present study, 25%, and the hepatic venule-to-Ppv gradient, which may correspond to Rpv, 22%.

Flow rates and hepatic segmental vascular resistances. It is well known that an increase in blood flow decreases vascular resistance in systemic circulation. The flow dependence of vascular resistance was also observed in isolated perfused rat livers of the present study. As shown in Fig. 3, when the flow rate at any given Hct was increased at a constant Phv, Rt decreased. This decrease in Rt was ascribed exclusively to a decrease in Rsinus but not in Rhv or Rpv. The mechanism for this decrease in Rsinus may be mainly due to microvascular distension and recruitment.

An interesting finding was that the sensitivity of inflow pressure of Ppv to changes in flow rate is low: in the 30% Hct groups, only a twofold increase in Ppv from 6 to 12 cmH2O was
observed as the flow is increased fivefold from 5 to 25 ml/min. This small increase in P_pv, hence R_pv, in response to increased blood flow is ascribed to minimal changes in the pressure gradient of the sinusoids, that is P_po-to-P_hvo gradient. This indicates that hepatic sinusoids could accept more blood flow without greatly elevating P_pv probably through sinusoidal dis-tension and recruitment.

Hct changes and hepatic segmental vascular resistances. In the present study, an increase in Hct from 0% to 30% resulted in a significant increase in R_t. This increase in R_t seems to be due to increases in all three segments of R_pv, R_sinus, and R_hv, as shown in Fig. 4. However, as perfusate Hct increased from 20% to 30% at low flow rate of 5 and 10 ml/min, R_pv and R_hv significantly increased, whereas R_sinus did not change significantly. This lack of change in R_sinus may be ascribed to the Fahraeus effect, where Fahraeus and Lindqvist (6) first demonstrated that, as tube diameter is reduced <300 μm, the apparent viscosity of red blood cell suspensions decreases as a result of an actual decrease in small tube Hct. In the systemic microcirculation, in vivo studies indicate that the Hct starts decreasing in vessels with diameters <70 μm and reaches a value in the capillaries of <25% of the systemic Hct (14). If this finding of the systemic circulation could be applied to the hepatic circulation, the changes in the perfusate Hct, hence the perfusate viscosity, would be small in hepatic sinusoids due to the Fahraeus effect, compared with the large portal and hepatic veins, then the sinusoidal resistance may not change significantly.

In contrast, at blood flow >15 ml/min, R_sinus also increased as perfusate Hct increased from 20% to 30%. This seems to be contradictory to the Fahraeus effect. A possible explanation may be related to an increase in sinusoidal Hct due to rapid transcapillary fluid shifts, which could be caused by an increase in blood flow, hence the microvascular pressures (2). At a high blood flow rate, the microvascular pressures between P_po and P_hvo are high enough to cause enhanced transvascular cell-free perfusate filtration at the sinusoids, whose endothe-lium is not continuous and extremely leaky (8). During passage of blood through the sinusoids at high sinusoidal pressure, the apparent Hct might have increased due to enhanced transsinu-soidal filtration. This possible increasing Hct effect might have counteract the decreasing Hct effect of the Fahraeus effect, resulting in an increase in R_sinus at high flow rate. However, we did not measure the difference in Hct between inflow and outflow blood. Further careful study is required in this respect.

Effect of norepinephrine on hepatic segmental vascular resistances. The present study shows that norepinephrine increased primarily R_pv and, to the lesser extent, R_sinus, whereas it did not affect R_hv. The absence of vasoreactivity to norep-inephrine in the large hepatic veins is also reported in vivo rats. Bohlen et al. (3) showed that an infusion of norepinephrine 2.9 ± 1.3 μg·min⁻¹·kg⁻¹ caused a significant increase in P_pv by 2.96 ± 1.00 mmHg but no change in hepatic venule pressure (−0.22 ± 1.26 mmHg) with the decrease in portal flow rate 88% of the control. This finding suggests that the large hepatic veins distal to the hepatic venules do not respond to norepinephrine, and this is consistent with the present study. However, their subsequent study revealed that norepinephrine caused significant increases in the hepatic venule pressure and the resistance between the sinusoids and the vena cava in rabbits (22). The discrepancy might be ascribed to the species difference between rat and rabbit.

An interesting finding of the present study is that norep-inephrine increased significantly the sinusoidal resistance. This suggests that norepinephrine causes sinusoidal constriction. Reilly et al. (20) reported that the small but significant decrease in sinusoidal diameter was observed when α-adrenergic receptors were stimulated. Although sinusoids do not contain smooth muscle cells, hepatic stellate cells, which are contractile and located around the sinusoidal endothelial cells, might reduce the diameter of sinusoids if it could contract in response to norepinephrine (25). However, there is no evidence that norepinephrine or α-adrenergic agonist contracts stellate cells (21). Zhang et al. (28), using intravital microscopy, measured the diameter of sinusoids in isolated perfused rat livers. They found no changes in sinusoidal hemodynamics in response to phenylephrine, α-adrenergic agonist, although the portal pres-sure increases. In addition, although activated stellate cells clearly exhibit enhanced contractility, the degree of contractil-ity of normal stellate cells remains controversial (5, 21). Another possible mechanism for the norepinephrine-induced increase in R_sinus may be due to contraction of vascular smooth muscle. Anatomic studies show that in rat livers, presinusoidal vessels of preterminal portal venules as small as 40 μm diameter contain a significant amount of smooth muscle (5).
Thus the middle segment in the present study might contain the portal venules.

**Estimation of P_{po} and P_{hvo} by double occlusion maneuver.**

We have shown that P_{po} and P_{hvo} obtained via the single-occlusion technique of portal occlusion and hepatic venous occlusion, respectively, were identical with P_{po-do} and P_{hvo-do} that were obtained by the double occlusion maneuver. The agreement between the two methods was verified in the wide range of P_{pv} from 2.25 to 36.58 cmH_2O. There were strong positive relationships between P_{po} and P_{po-do} and between P_{hvo} and P_{hvo-do}. More importantly, the slope and y-intercept of their regression lines, statistically, did not differ from the median slope and zero, respectively.

The shortcoming of portal occlusion and hepatic venous occlusion techniques is that measurement should be done during steady state to obtain the segmental vascular resistance distribution because each technique cannot be done simultaneously. When two occlusions are being performed, it is necessary to wait for the pressure to return to a steady value before the next occlusion can be performed. This may cause problems because vascular constriction is not always stable. This problem is totally solved by one double occlusion technique. The double occlusion technique makes it possible to measure P_{po} and P_{hvo} during an unsteady state, when hepatic vascular pressures are changing in response to experimental maneuvers such as a bolus injection of vasoactive substances.

**Limitation of present study.** There are limitations of the current experiment. The first is related to the lack of hepatic artery flow and a good source of oxygen, especially in the groups with a Hct of 0% and 10%. However, even in the perfused livers with 0% Hct, perfusate P_{O_2} could attain as high as 300 mmHg by bubbling the perfusate with 95% O_2-5% CO_2, as revealed by our previous study (26). Another criticism is that the tying off of the hepatic artery could be influenced by our previous study (26). There are strong positive relationships between P_{po} and P_{po-do} and between P_{hvo} and P_{hvo-do}. More importantly, the slope and y-intercept of their regression lines, statistically, did not differ from the median slope and zero, respectively.

In summary, we provided the hepatic vascular occlusion methods in isolated perfused rat livers to measure P_{po} and P_{hvo}, which enabled us to assign the portal hepatic R_t to the portal R_{pv}, R_{sinus}, and R_{hv}. It was demonstrated that R_{pv} comprises 44% of R_t, 37% R_{sinus}, and 19% R_{hv} in livers perfused at physiological P_{pv} and 30% Hct. We determined the effect of changes in blood Hct or blood flow rate on segmental vascular resistance distribution. As Hct increased at a given blood flow, all three segmental vascular resistances of R_{pv}, R_{sinus}, and R_{hv} increased at flow >15 ml/min. Because blood flow increased at a given Hct, either R_{pv} or R_{hv} did not change, but only R_{sinus} decreased presumably due to distension or recruitment of sinusoids. We also determined the preferential vasoconstrictive site induced by norepinephrine. Norepinephrine increased predominantly R_{pv} over R_{sinus}, but it did not affect R_{hv}. Finally, we demonstrated that P_{po} and P_{hvo} can be obtained by the double occlusion method in isolated perfused rat livers.

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