Experimental Perfusion of Isolated Liver to Study the Bile Secretion and Bromsulfalein Excretion

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Hepatic blood flow, endocrine factors, metabolic products and nervous control as well as functional states of the liver cells participate in the bile secretion. In the past, studies were conducted in our laboratory on the influence of many drugs on bile secretion and excretion of exogenous pigment such as bromsulfalein (BSP).[1] However, these results were not based on exclusion of one factor after another of these factors on bile excretion, and it has not been possible to decide on which factor a drug mainly influences to cause increase of bile secretion or BSP excretion. In order to study the influence of drugs on these factors individually, perfusion experiment was conducted on isolated rat liver to study the relationship between hepatic blood flow and bile excretion or BSP excretion into bile, as well as the drug effect on these.

As the test drug, sodium dehydrocholate (DHC), sodium salicylate and sodium hippurate were selected. DHC is known to be the most potent cholagogue and its administration to rats in adequate dose induces a marked increase of bile secretion and BSP excretion. At this time, the maximum value of BSP transport into bile (BSP-Tm) rises, capacity of the BSP-glutathione conjugation of the liver increases, and binding of serum albumin and BSP is inhibited.

Sodium salicylate increases bile excretion in rats mildly and BSP excretion markedly. Binding between serum albumin and BSP is markedly inhibited, but no influence is noted on BSP-Tm and capacity of BSP conjugation.

Sodium hippurate mildly increases bile excretion in rats and markedly increases BSP excretion, along with the rise of BSP-Tm, and mild inhibition of binding between serum albumin and BSP.

METHODS

Method of liver perfusion: The perfusion apparatus of Brauer et al[2] was used.

Male Wistar rats of 180–250g body weight were anesthesized by intraperitoneal injection of 10mg Na-thiopental. Laparotomy at the midline is followed by ligation of the lower part of the common bile duct and abrasion of this portion from the surrounding tissue. After placing a small incision on the common bile duct, a vinyl tube of 0.31 mm in inside diameter was inserted. Inferior vena cava was then doubly ligated between the right kidney and the liver and a canula filled with physiological saline with outside diameter of 1.9 mm was inserted into the portal vein. After ligation, physiological saline was allowed to stream into the liver immediately for perfusion. The thoracic cavity was then opened and the inferior vena cava was sectioned below the heart, and the liver and diaphragm were removed along with the stomach to install in the perfusion apparatus.

Composition of the perfusion fluid: Forty milliliter of whole heparinized blood of rabbit was diluted 3 times with Ringer solution to make 120 ml and 70 mg glucose was added to make the perfusion fluid. BSP, 6 mg, was further added. As the test drug, 100 mg DHC was added in DHC group, 30 mg sodium salicylate in Na-salicylate group, and 30 mg sodium hippurate in Na-hippurate group.

Conditions of perfusion: Temperature of the perfusing fluid was 38–40°C and pressure was 13–20 cm H_2O. Oxygen with addition of 5% CO_2 was added to the fluid. The hepatic blood flow was controled through regulating the lumen of the tube immediately before the entrance into the liver. After the end of the perfusion, the hepatic blood flow in the experiment was measured.

Bile sampling: After 15 minutes of the infusion of bile, constant hepatic blood flow was obtained. Bile was sampled 30 and 60 minutes thereafter. The total amount of bile over the period of 60 minutes was used to express the experimental result.

Estimation of BSP in the bile: Amount of bile was measured and 0.05 ml of bile was diluted 1000–3000 times with 0.1 N KOH. Then, the concentration of BSP was measured spectrophotometorically at 575 and 620 mμ wave length. In order to avoid the error due to turbidity, true absorbency was expressed as O.D. 575 mμ—O.D. 620 mμ × 1.2.[3] The concentration was caluculated by the standard curve.

RESULTS

1. Hepatic blood flow and amount of bile (Control group)

As shown in Fig. 1. when hepatic blood flow exceeds 1 ml/g liver tissue/min., the amount of bile secreted over a period of 60 minutes increa-

sed along with the rise of hepatic blood flow. However, the maximum amount of bile was 0.53 ml per 60 minutes.

2. Hepatic blood flow and BSP excretion in bile (Control group)

As shown in Fig. 2, BSP concentration in bile rose along with the increase of hepatic blood flow gradually below the hepatic blood flow of 1.5 ml/g liver tissue/min. and rapidly above this level of hepatic blood flow. Consequently, BSP excretion in bile rose as shown in Fig. 3. At the hepatic blood flow of 1.5 ml/g liver tissue/ min., BSP excretion stayed as low as 8 % in 60 minutes, but reached 72% at the hepatic blood flow of 2.3 ml/g liver tissue/min..

3. Amount of bile and rate of BSP excretion in bile (Control group)

As shown in Fig. 4 indicating the relationship between amount of bile and rate of excretion of BSP into bile, within the range of 0.5 ml of the amount of bile excreted in 60 min. slight increase in the amount of bile resulted in a marked increase of the rate of excretion of BSP in bile.

4. Hepatic blood flow and amount of bile (DHC group)

As shown in Fig. 5, in the DHC group below the hepatic blood flow of 1 ml/g liver tissue/min., the amount of bile was increased to 3–5 times the level in the control group. Above the hepatic blood flow of 1 ml/g liver tissue/min., the amount of bile reached the maximum value of 1.5 ml per 60 minutes. Despite further increase of hepatic blood flow, however, the amount of bile failed to increase. The amount of bile secretion apparently has a plateau.

5. Hepatic blood flow and BSP excretion into the bile (DHC group)

As shown in Fig. 6 and 7, in the DHC group the BSP concentration in the bile markedly fell, compared to the control group. The increase of BSP concentration in bile due to the increase of hepatic blood flow was not as distinct as in the control group, and there was apparently no change. Below the hepatic blood flow of 1 ml/g liver tissue/min., increase above the control level was noted. Above this level of hepatic blood flow, however, no change occurred and the rate of excretion was lower than in the controls.

6. Amount of bile and rate of BSP excretion into bile (DHC group)

As shown in Fig. 8, in the DHC group, within the range of increases of the amount of bile to 1.5 ml per 60 minutes, rate of BSP excretion was increased as the amount of bile increases. As the amount of bile reaches the maximum, rate of BSP excretion was increased as the amount of bile increases. As the amount of bile reaches the maximum, rate of BSP excretion became constant. In the DHC group, however, BSP excretion

was less than that in the control group, despite a marked increase in the amount of bile, suggesting the predominance of bile excretion by DHC than BSP excretion.

7. Hepatic blood flow and amount of bile (Salicylate group)

As shown in Fig. 9, in the salicylate group, amount of bile increased along with the increase of hepatic blood flow. At each level of hepatic blood flow, slightly higher values than in controls were noted. In the salicylate group, however, as the hepatic blood flow exceeds 1.5 ml/g liver tissue/min., the perfused liver became edematous and perfusion became entirely impossible, so that no results of further perfusion are available.

8. Hepatic blood flow and BSP excretion in bile (Salicylate group)

As shown in Fig. 10, BSP concentration in bile was higher in the salicylate treated group than in the control groups. As the hepatic blood flow increases, the value also rose as in the controls. As shown in Fig. 11, consequently, rate of BSP excretion into bile increased as the hepatic blood flow increased, as in the control group. The rate of BSP excretion was 3–7 times the control level at each level of hepatic blood flow.

9. Amount of bile and BSP excretion rate in the bile (Salicylate group)

As shown in Fig. 12, until the amount of bile reaches 0.6 ml per 60 min., slight rise in the amount of bile results in a marked rise of BSP excretion, as in the controls.

10. Hepatic blood flow and amount of bile (Hippurate group)

As shown in Fig. 13, in the hippurate group, amount of bile increased along with the increase of hepatic blood flow, as in the control group in the degree of increase.

11. Hepatic blood flow and BSP excretion in the bile (Hippurate group)

As shown in Fig. 14, in the hippurate group, BSP concentration in the bile was as high as 5 mg/dl even when hepatic blood flow is low. These are much higher than in the controls. As the hepatic blood flow reaches 2 ml/g liver tissue/min., however, the concentration becames similar to that in the controls. BSP excretion into bile, as shown in Fig. 15, stayed around 20% regardless of the increase or decrease of hepatic blood flow. Above the hepatic blood flow of 2 ml/g liver tissue/min., however, lower values than in controls were obtained.

12. Amount of bile and rate of BSP excretion in the bile (Hippurate group)

As shown in Fig. 16, rate of BSP excretion in bile tended to increase along with the increase in the amount of bile, in the range of the amount of bile less than 0.5 ml, in the hippurate group as well as the control group.

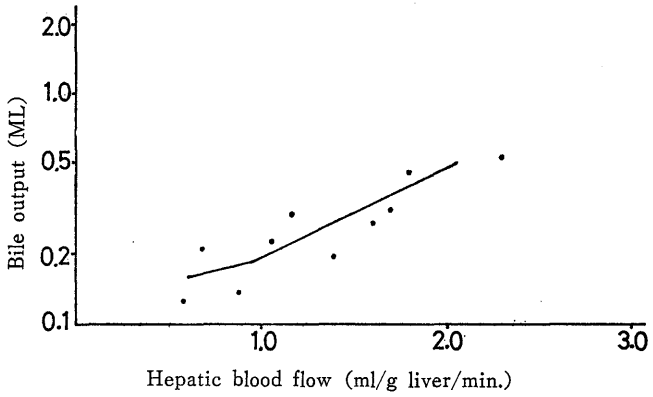

Fig. 1. Hepatic blood flow and amount of bile (Control group)

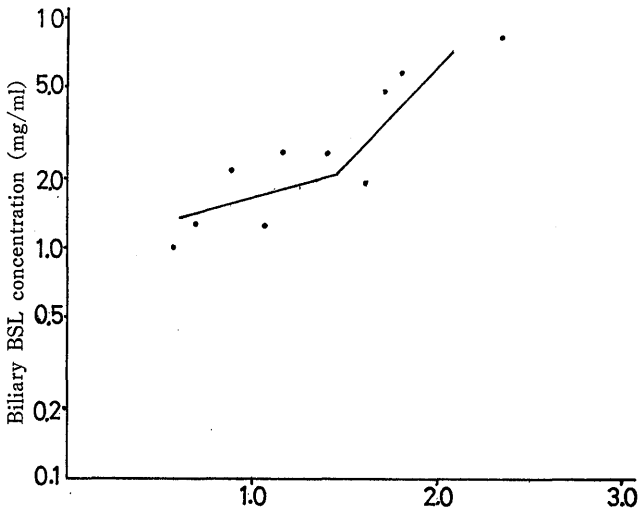

Fig. 2. Hepatic blood flow and BSP concentration in the bile (Control group)

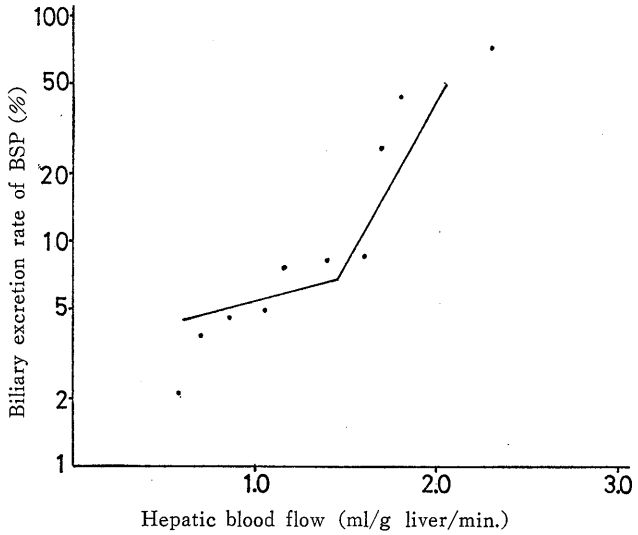

Fig. 3. Hepatic blood flow and rate of BSP excretion in the bile (Control group)

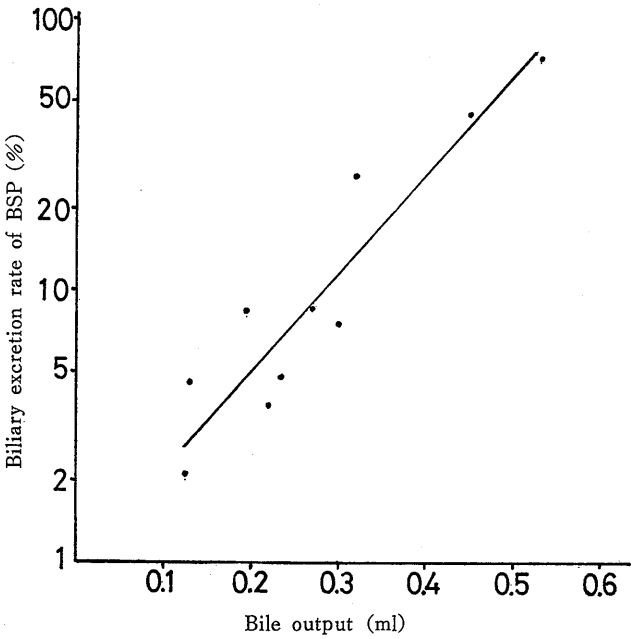

Fig. 4. Amount of bile and excretion in the bile (Control group)

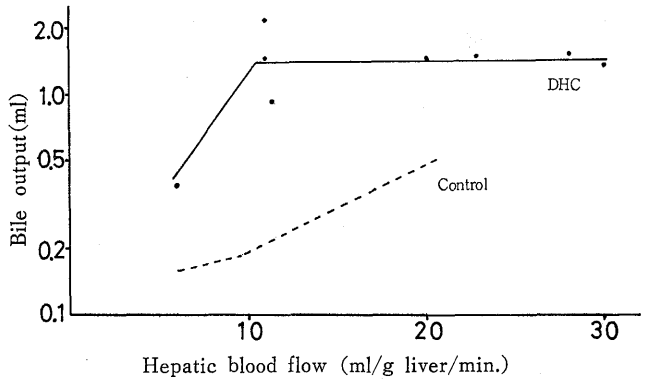

Fig. 5. Hepatic blood flow and amount of bile (DHC group)

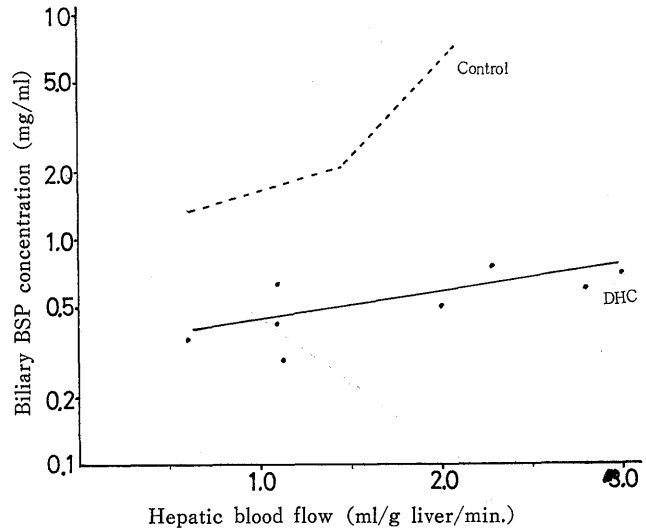

Fig. 6. Hepatic blood flow and BSP concentration in the bile (DHC group)

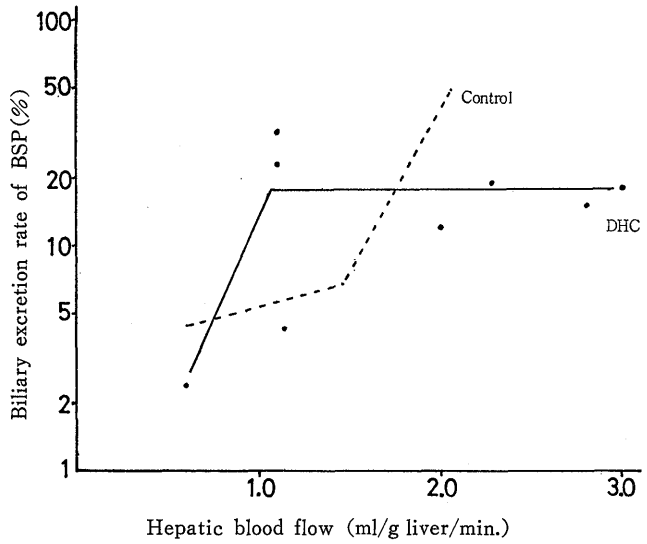

Fig. 7. Hepatic blood flow and rate of BSP excretion in the bile (DHC group)

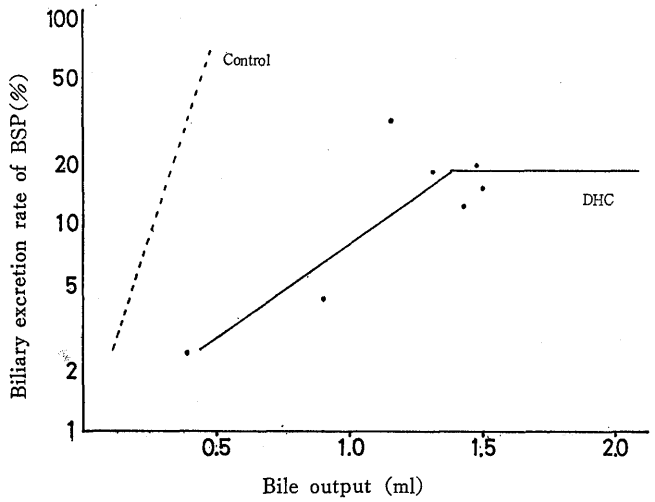

Fig. 8. Amount of bile and excretion in the bile (DHC group)

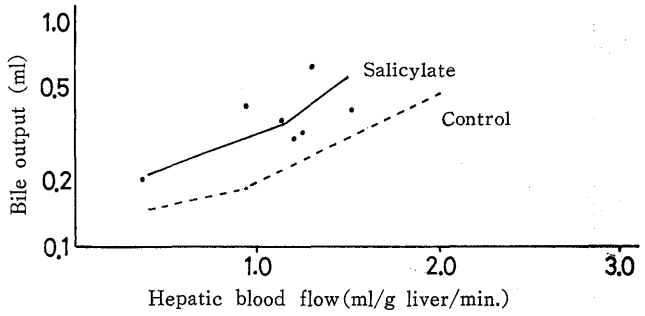

Fig. 9. Hepatic blood flow and amount of bile (Salicylate group)

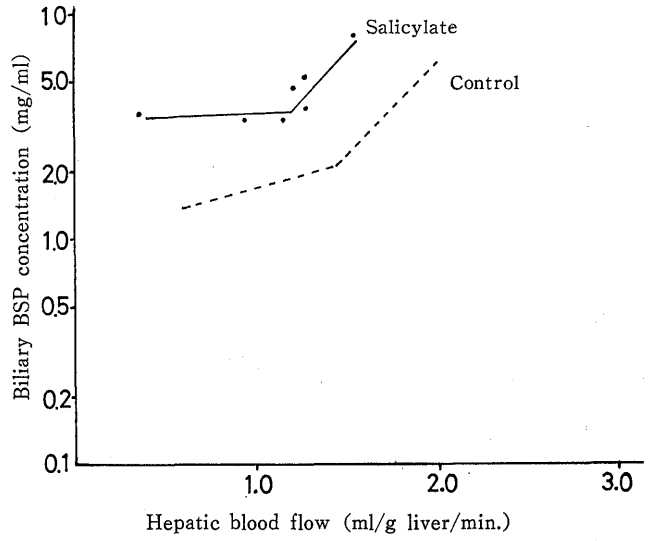

Fig. 10. Hepatic blood flow and amount of bile (Salicylate group)

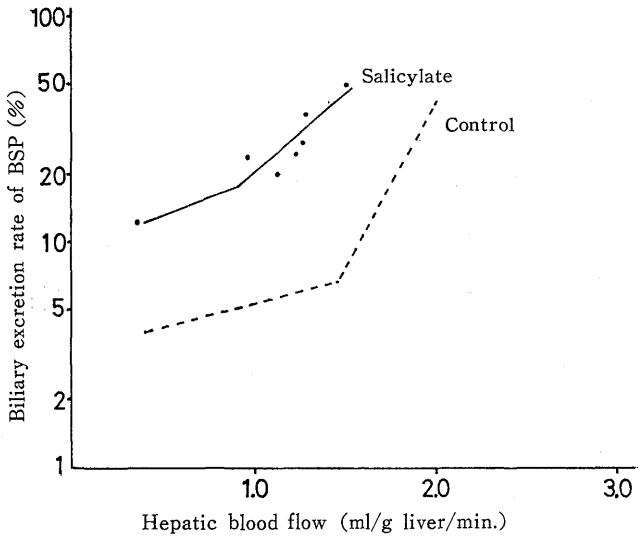

Fig. 11. Hepatic blood flow and rate of BSP excretion in the bile (Salicylate group)

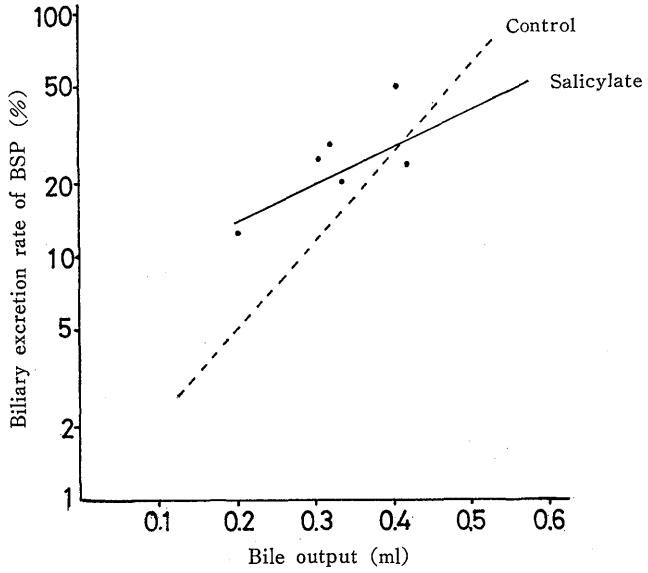

Fig. 12. Amount of bile and excretion in the bile (Salicylate group)

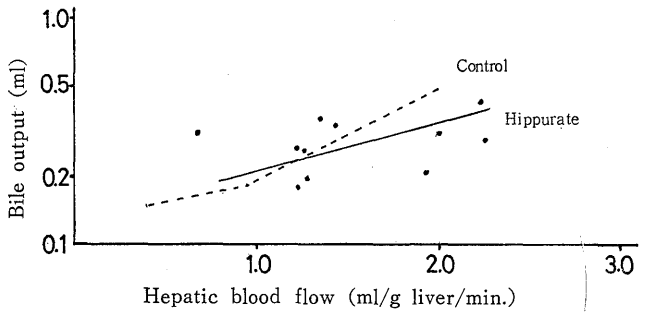

Fig. 13. Hepatic blood flow and amount of bile (Hippurate group)

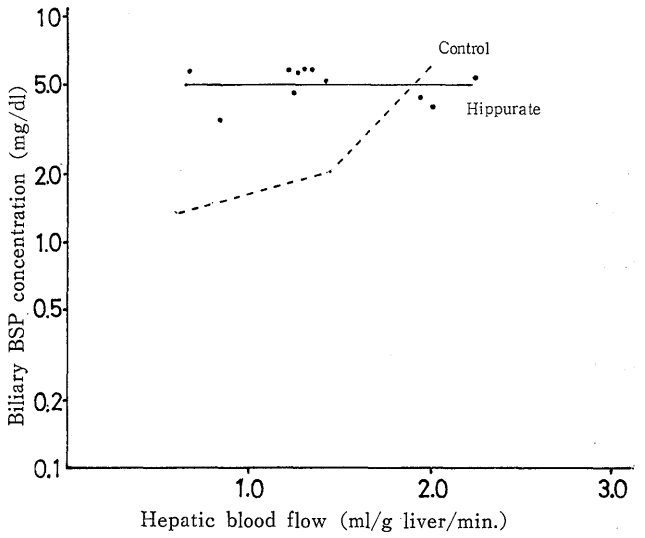

Fig. 14. Hepatic blood flow and BSP concentration in the bile (Hippurate group)

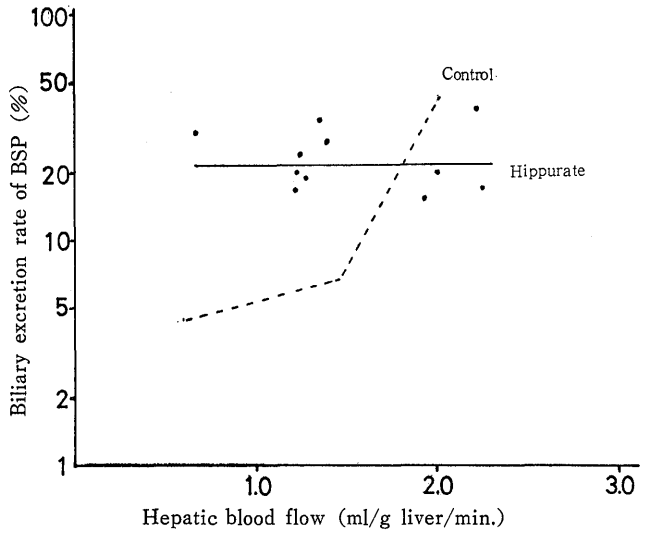

Fig. 15. Hepatic blood flow and rate of BSP excretion in the bile (Hippurate group)

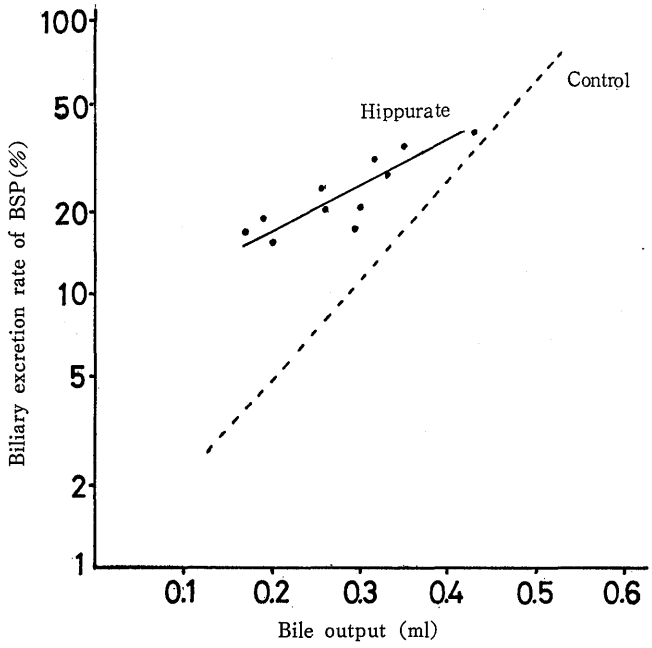

Fig. 16. Amount of bile and excretion in the bile (Hippurate group)

DISCUSSION

Since bile secretion immediately ceases when the hepatic blood flow is blocked, hepatic blood flow seems to be necessary in bile production[4)5)]. For the secretion of bile, portal blood does not participate and artery blood supply was emphasized[6)] and oxygen supply was considered to be significant[5)7)]. Even if the hepatic blood flow is markedly changed by means of vascular anastomosis, amount of bile does not change, so that only a small amount of arterial blood appears to be necessary for bile production[4)8-10)].

According to the transportal perfusion experiment of the isolated liver of the rat by the authors a small amount of hepatic blood flow is necessary for the secretion of a small amount of bile, and a positive correlation was found between the amount of bile and hepatic blood flow above certain hepatic blood flow (Fig. 1). Such relationship is seen not only in the control group, but also in the DHC group (Fig. 5), salicylate group (Fig. 9), and hippurate group (Fig. 13). It was, therefore, concluded that changes of hepatic blood flow participate as a factor in the bile secretion.

DHC caused a marked increase of bile acids. Under certain hepatic blood flow, increase of hepatic blood flow and amount of bile showed a positive correlation. After increase of the amount of bile above certain level, increase of hepatic blood flow no longer causes increase of the amount of bile (Fig. 5). In the bile secretion, as in the excretion of bilirubin and BSP, maximum value for transport appears to be present.

In the production of bile and secretion, bile acid is secreted from the liver cells to the bile ductule in high concentrations. Due to the osmotic pressure gradient within the bile duct, water and electrolyte are mobilized from the bile duct wall for bile production according to the concept of Sperber[11)].

Increase of bile acids excretion into bile by DHC[12)] is convenient for the explanation of choleretic action of DHC. Even if the hepatic blood flow stays the same, marked increase of the amount of bile acids is obtained in the DHC group compared to the control group. While increase of hepatic blood flow causes an increase of the amount of bile, factors other than increase of hepatic blood flow such as facilitation of bile acids secretion probably participate in the choleretic action of DHC.

It thus appears to be reasonable to assume that the maximum value of bile secretion represents the maximum value of bile acids secretion into bile. The degree of increase of bile acids in the bile upon administration of DHC, however, is mild compared to the increase of the amount of bile.

As was described above, problems still appear to remain in the explanation on choleretic action of DHC with increase of bile acids into the bile alone.

Administration of sodium hippurate in rats causes mild increase of the amount of bile[1], but no such phenomenon was noted in the experiment of isolated rat liver perfusion by the authors.

In the salicylate group, mild increase of the amount of bile is noted (Fig. 9), suggesting a mechanism similar to that in DHC group.

In the salicylate group, raising the speed of perfusion resulted in edematous change of the liver, making the perfusion difficult. Administration of sodium salicylate for a long time causes liver damage, but sodium hippurate belonging to the same aromatic compound was devoid of such action[13]. In agreement with this, the hepatic toxicity of sodium salicylate was suggested.

In the relationship between hepatic blood flow and BSP excretion into bile, increase of hepatic blood flow is generally associated with increase of BSP excretion according to the impression. This is supported by some[14], negative correlation was pointed out by others[15-17], and undetermined relationship was suggested by still others[6], and no conclusion is yet drawn.

In the perfusion experiment of isolated rat liver by the authors, above certain level of hepatic blood flow, hepatic blood flow is definitely associated with increase of BSP excretion (Fig. 3). This was also noted in salicylate group (Fig. 11). In the DHC group, such tendency was also noted when hepatic blood flow is small. When hepatic blood flow increases and the amount of bile reaches the maximum, BSP concentration in the bile does not change, so that the correlation between hepatic blood flow and BSP excretion is lost behind choleretic phenomenon (Fig. 7). In the hippurate group, BSP excretion into bile is extremely high even if the hepatic blood is low, and no correlation was found between these two (Fig. 15).

DHC is said to increase the excretion of exogenous pigment (BSP, PSP, Azorbin S) into bile[1)18], increase it only in pathological liver[19], or inhibit the excretion according to many report[20)21]. In the experiment of the authors, BSP excretion rate increased when hepatic blood flow is low and choleretic effect is small, while BSP excretion is lower than the conrols when the choleretic effect is pronounced. When BSP excretion into bile by DHC is discussed, dose of DHC becomes a problem but the time factor of bile sampling is also important, suggesting the complexity of the problem. Except for the DHC group (Fig. 6), increase of hepatic blood flow caused a rise of BSP concentration in the bile in the control group (Fig. 2), salicylate group (Fig. 10), and hippurate group (Fig. 14). This would indicate that the increase of BSP excretion into bile is not due to the increase of bile

flow, but probably due to the increase of liver cells, through the liver cells, leading to metabolic facilitation by the drug. Including DHC, sodium salicylate or sodium hippurate inhibits binding between plasma albumin and BSP[1)], so that such drug effect on the extrahepatic factor should also be considered.

As to the relationship between the amount of bile and rate of BSP excretion into bile, a positive correlation appears to be present between these two in each group (Fig. 4, 8, 12, 16). When hepatic blood flow increases, BSP concentration in the bile increases. This fact would indicate the presence of correlation between hepatic blood flow and BSP excretion, rather than the correlation between hepatic blood flow and BSP concentration in the bile, to explain such results.

These may be summarized as follows. In the control group, changes of hepatic blood flow was found to influence the bile excretion and BSP excretion in the bile. In the hippurate group, however, bile secretion and BSP excretion are definitely based on different mechanisms. Bile secretion or BSP excretion in the bile changes along with the change of hepatic blood flow, but this is probably not due to the direct effect of hepatic blood flow but based on the indirect effect of drugs on the metabolism of hepatic cells, or on the extrahepatic factor.

In the experiment on DHC group, presence of maximum value of bile excretion was demonstrated as a new fact.

Even under the same hepatic blood flow, DHC caused an increase of the amount of bile, sodium salicylate mildly increased the amount of bile and marked increase of BSP excretion in bile, and sodium hippurate caused no increase of amount of bile and a marked rise of BSP excretion in the bile.

CONCLUSION

In the perfusion experiment of isolated rat liver, mutual relationship between hepatic blood flow on one hand and amount of bile and BSP excretion in bile on the other was studied. Furthermore, influence of sodium dehydrocholate, sodium salicylate and sodium hippurate on these was studied. The following results were obtained.

1) In the liver isolated from normal rats (control group), increase of hepatic blood flow caused increase of bile secretion and BSP excretion into the bile.

2) In the experiments with sodium dehydrocholate administration, a positive correlation between hepatic blood flow and bile secretion was

obtained at the range of low hepatic blood flow but no increase of bile secretion was noted along with the increase of intrahepatic blood flow once certain level of bile secretion is reached. BSP excretion in bile was lower than in the control and constant. Consequently, rate of BSP excretion was higher than in the control in the area with low hepatic blood flow but became lower than the control as the bile secretion reached the maximum.

3) In the experiment of administration of sodium salicylate, positive correlation was noted between hepatic blood flow on one hand and bile secretion and BSP excretion on the other. BSP concentration in the bile was higher than in the control. Sodium salicylate caused liver damage.

4) A positive correlation was noted between hepatic blood flow and bile secretion in the experiment of sodium salicylate administration. But no correlation was noted at all between hepatic blood flow and BSP excretion in the bile.

5) These results would indicate that hepatic blood flow influences the bile secretion and BSP excretion into bile to some extent, but effect of drugs on liver cells was found to be more important.

Choleretic phenomenon was found to have the maximum value, suggesting the independence of bile secretion mechanism from BSP excretion mechanism.

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