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Bile Constituents in Blood and Lymph During Biliary Obstruction

I. The dynamics of absorption and transport of ions and colloid molecules

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Summary

$Na^{125}I$ and ^{131}I-labeled albumin was infused in dogs into the common bile duct at pressures of 20 to 25 and 40 mmHg. At 40 mmHg, the amounts of the iodide ion and labeled albumin in circulating plasma were, after correction for the secondary loss from the circulation, nearly identical. At 20-25 mmHg more iodid than labeled albumin was found in the circulation. In thoracic duct lymph the same fraction of the infused amount of albumin was recovered at both pressures. Lymphatic concentrations of albumin were in both types of experiments substantially higher than plasma concentrations. It is concluded, that at increased pressure fluid leaks first from the small biliary ducts into the Mall's spaces. In consequence of water absorption and the diffusion of ions and small molecules into the blood capillaries the concentrations of protein or protein bound molecules in this part of the hepatic interstitial fluid increases. This is reflected in their high concentration in the lymph. If bile pressure rises further, fluid leaks also into the Disse's spaces. This leads to a bulk flow of solvent and solutes into the sinusoids and to the near disappearance of the differences in the venous transport of ions and colloids.

In obstructive jaundice bile regurgitates into the venous system and into the lymphatics of the liver. The mechanism of the hyperbilirubinemia has been extensively studied. Less is known about the fate of the other bile constituents. Even in the case of bilirubin, an unsettled controversy still exists about the relative importance of the venous and lymphatic routes. (2, 3, 4, 5, 6, 8, 10, 11, 13, 16). The analysis of the transport of bile constituents is rather difficult because their concentrations in plasma and lymph are influenced by several factors, i.e. by the rates of hepatic formation and excretion, the enterohepatic circulation, the leakage accross the wall of the bile ducts, the diffusion into the liver sinusoids and lymphatics, the velocity and rate of lymph flow, the secondary escape from circulating plasma into the interstitial fluid and finally, their extrahepatic uptake, binding and elimination.

In the present study, in order to eliminate most of these complicating factors, known amounts of foreign substances were introduced into the biliary tract at a constant rate, and their concentration was measured in blood plasma and thoracic duct lymph (9). The introduced substances were $Na^{125}I$ and ^{131}I-albumin.

Material and Methods

The experiments were done on mongrel dogs with a body weight of 16,1(15-18) kg in pentobarbital (30 mg/kg) general anaesthesia. The animals received 8 hours before the experiment 10 mg/kg unlabelled sodium iodide. The abdomen was opened by a midline incision. The cystic duct was ligated and the common bile duct cannulated with a plastic tube. Catheters were introduced into both ureters and the thoracic duct was cannulated in the neck. Through the cannula fluid was infused into the common bile duct by means of a rotary pump. The fluid contained about 5 μCi $Na^{125}I$ and 5 μCi ^{131}I human albumin, diluted in 30 to 120 ml physiological saline solution. The total protein content of the infusate was 2 to 10 mg per 100 ml. The constant delivery pump was adjusted in the first type of experiments (9 animals) to maintain a pressure of 40 mmHg. This was attained at infusion rates of 1,1 to 2,0 (avg. 1,5) ml/min. In most of these animals, at constant inflow rates, 5 to 10 minutes after the onset of the infusion the pressure dropped by 5 to 10 mmHg. (7). In the second type of experiments (7 dogs)

the fluid was infused at a pressure of 20 to 25 mmHg. This is the level to which bile pressure usually rises after complete extrahepatic biliary occlusion. Incidentally, this is also the pressure at which in infusion experiments the fluid begins to flow into the biliary tract (1, 7, 9, 14, 15). The infusion rates were in these animals 0,1 to 0,3 (avg. 0,2) ml/min. In all animals lymph and urine was collected in 15 minute periods for 90 minutes. Blood samples were withdrawn at the end of each collection period.

In 7 dogs a fluid of a similar composition was infused intravenously at the rate of 1,1 ml/min. Lymph, urine and blood samples were collected in the same way as in the previous groups.

Finally, in another group of 8 dogs the disappearance rate from the blood plasma and the distribution volume of $Na^{125}I$ was measured. These animals received 5 μCi $Na^{125}I$ by a single intravenous injection. Blood samples were obtained at 5, 15, 30, 60, 90 and 120 minutes. In all animals, at the end of the experiment, circulating plasma volume was measured with the Evans-blue dilution method.

The radioactivity of the lymph, plasma and urine samples was measured in a scintillation well detector, with an energy selective counter (GAMMA NK-150) at 1080 V (channel width 50 V). To avoid the quenching effect of ^{131}I, the ^{125}I activity was measured after protein precipitation with trichloroacetic acid.

Results

During its infusion into the biliary tract, both at high and at low pressure and flow rates, the concentration of ^{131}I-albumin in thoracic duct lymph rose rapidly. Lymph flow rates did not change in either type of experiments. The increase of the plasma concentrations was much slower than the rise of lymphatic concentrations. Accordingly, the average L/P ratio for labelled albumin rose in 1 hour to 5,6 (high infusion pressure experiments) and 12,3 (low pressure and flow experiments). The lymphatic concentration of the iodide ion increased only to about 1,65 and 1,25 times plasma concentration (Fig. 1 and 2). The lymphatic and plasma concentrations are expressed as fractions of the concentrations in the infused fluid, i.e.: *concentrations in lymph or plasma/concentration in the infusate.*

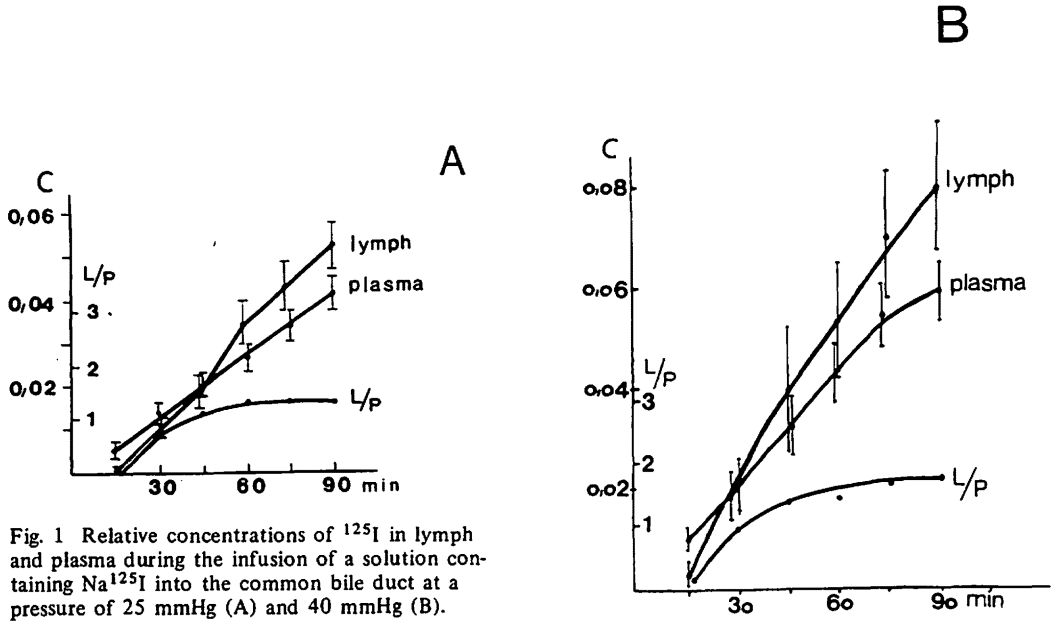

Fig. 1 Relative concentrations of ^{125}I in lymph and plasma during the infusion of a solution containing $Na^{125}I$ into the common bile duct at a pressure of 25 mmHg (A) and 40 mmHg (B).

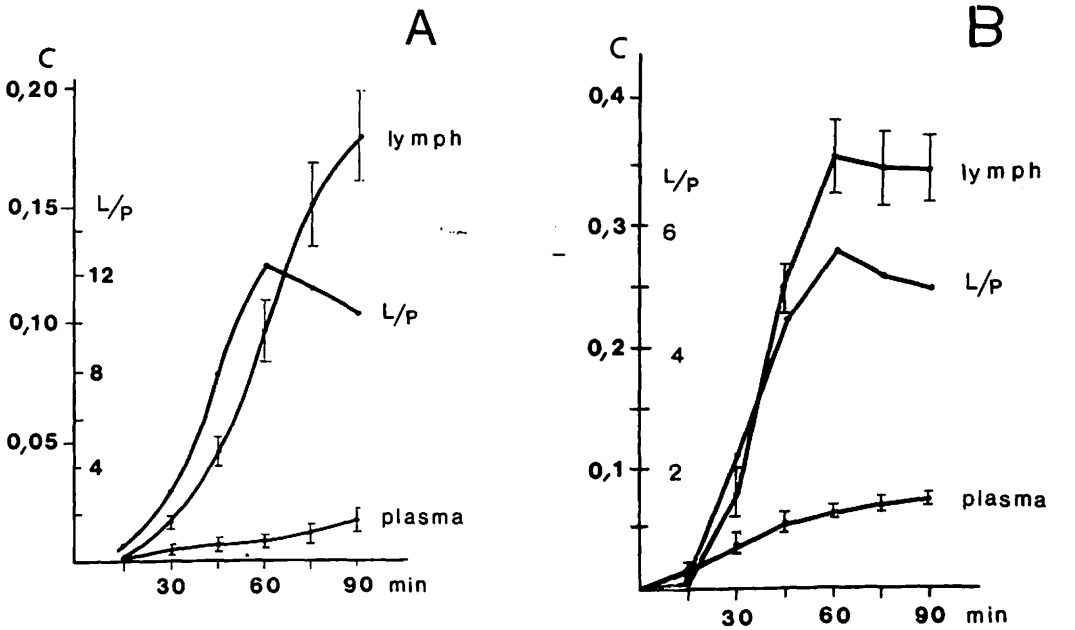

Fig. 2 Relative radioalbumin concentrations in lymph and plasma during the infusion of ^{131}I-albumin into the common bile duct at a pressure of 25 mmHg (A) and 40 mmHg (B).
In figs. 1 and 2 the concentrations in the biological fluids are expressed by the quotient: radioactivity in plasma or lymph/radioactivity in the infused fluid.

The amounts of the infused substance transported by the lymphatics were calculated by multiplying the volume of the collected lymph with the lymphatic concentrations. Similarly, the amounts present in circulating plasma by multiplying plasma volumes with the respective plasma concentrations and adding to this the amounts of the isotope excreted in the urine. Finally, to gain a common denominator for all experiments, the above values were divided by the total amount of the isotope infused in the respective experiments during the same period. It was established, that the amounts of the infused substances gaining access directly to blood plasma are much greater than those which are transported by the lymphatic system. After the lapse of an equilibration period, in circulating plasma, at high as well as at low infusion pressures, a constant fraction of the infused radioactivity was found both for Na^{125}I and ^{131}I-albumin (Fig. 3). The plasma fraction of Na^{125}I was at high infusion pressure on the average 36,5 and at low pressure 40,0 per cent of the infused ($p > 0,05$). For ^{131}I-albumin the same values were 54,5 and 42,0 per cent ($p < 0,05$).

Up to the end of the experiments (in 1 1/2 hours) 11,4 ± 1,6 and 11,5 ± 2,5 per cent of the infused albumin and 1,8 ± 0,2 and 1,2 ± 0,1 per cent of the iodide ion was transported with the lymph.

Substances introduced into the circulating blood are lost to the interstitial fluid at a constant exponential rate. In 8 dogs the T 1/2 of the decrease of plasma concentration for injected Na^{125}I was 20,4 ± 8,6 min, the iodide space was found to be 27,2 ± 2,2% of body weight. In another group of dogs (8) with thoracic duct fistula T 1/2 of ^{131}I-albumin was 318 ± 29 min and albumin space 11,2% of body weight. Finally, during the intravenous infusion of a solution containing Na^{125}I and ^{131}I-albumin at a constant rate the activities in plasma and lymph rose rapidly. Lymphatic concentrations of both substances remained however below plasma concentrations. In Fig. 4 concentrations in plasma and lymph are shown as fractions of the respective concentrations in the infused fluid.

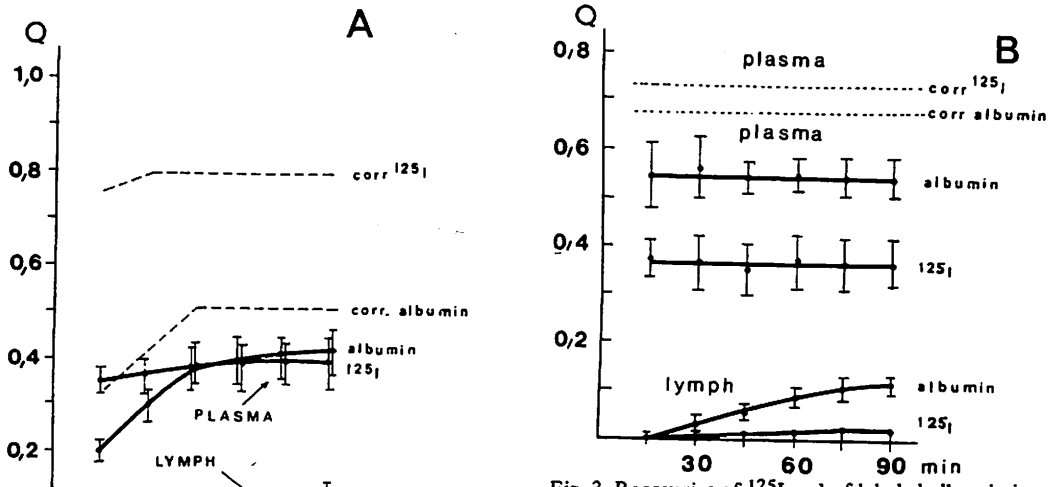

Fig. 3 Recoveries of ^{125}I and of labeled albumin in circulating plasma and in collected thoracic duct lymph during the infusion of Na^{125}I and ^{131}I-albumin into the common bile duct at a pressure of 25 mmHg (A) and 40 mmHg (B).

Corr. ^{125}I and corr. albumin: Values corrected for the secondary loss from the circulating plasma. The recoveries are expressed as fractions of the infused amounts. *(Total activity in circulating plasma or collected lymph/total infused activity).*

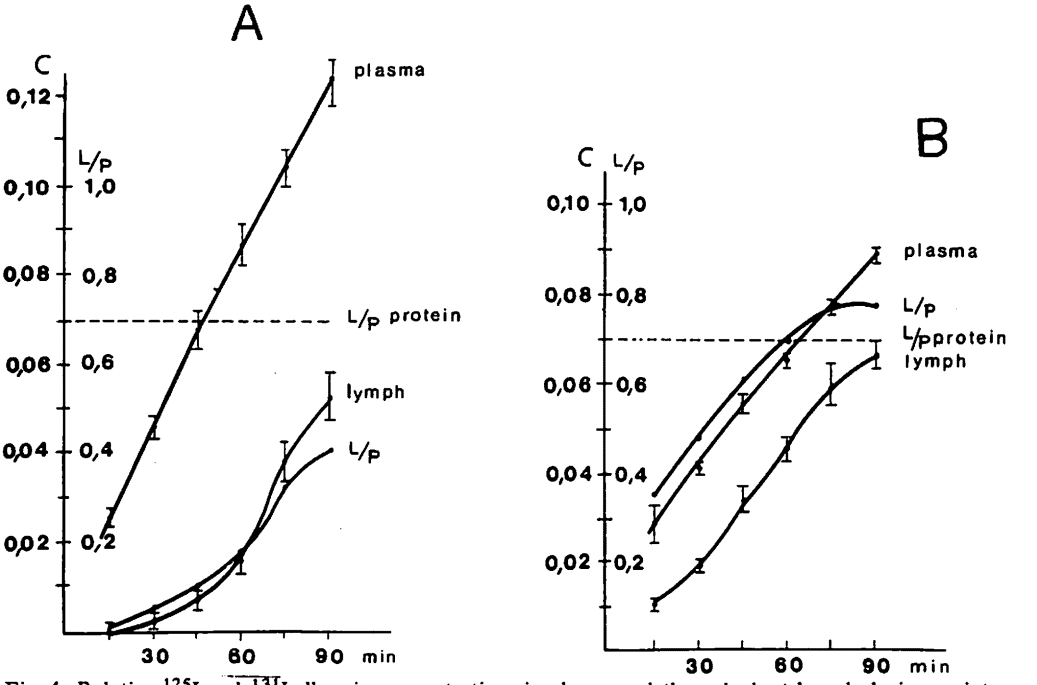

Fig. 4 Relative ^{125}I and ^{131}I-albumin concentrations in plasma and thoracic duct lymph during an intravenous infusion of both substances. (A: ^{131}I-albumin; B: ^{125}I).
L/P protein: protein concentration in thoracic duct lymph/protein concentration in blood plasma.
The lymphatic and plasma concentrations are expressed as fractions of the concentration present in the infused fluid.

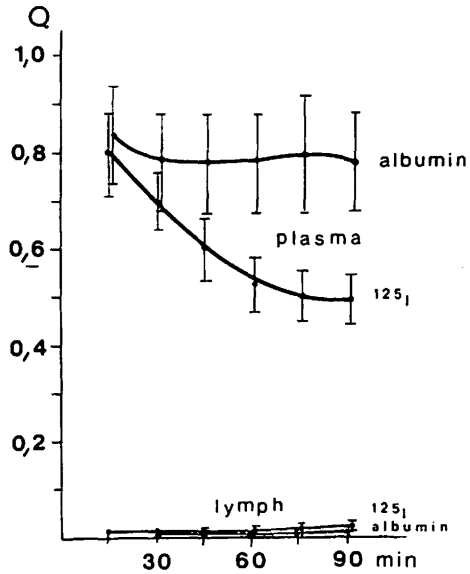

Fig. 5 Recoveries of labeled albumin and ^{125}I in plasma and lymph during their intravenous infusion.
The amounts present is the circulating plasma and in the excreted thoracic duct lymph are expressed as fractions of the infused amount. *(Total radioactivity in lymph or plasma/total infused activity).*

The fraction of the infused amount present in the circulating plasma (corrected for urinary loss) was calculated by the same methods as in the bile duct infusion experiments. After equilibration, about 50% of the ^{125}I and 80% the ^{131}I-albumin was found in circulating plasma. The amounts eliminated with thoracic duct lymph were negligible (Fig. 5). Accordingly, if it is assumed that in the bile duct infusion experiments, after the equilibration with the interstitial fluid of the liver, a constant fraction of the infused $Na^{125}I$ and ^{131}I-albumin regurgitates into the veins, then the amounts of ^{131}I-albumin found in plasma should be corrected by a factor of 1,25 and those of $Na^{125}I$ by a factor of 2.

Discussion

During the infusion into the bile duct albumin and iodide regurgitates rapidly into the circulating blood. The amounts of the iodide and of the colloid found in circulating plasma in the experiments with high infusion pressure were nearly identical. The values, corrected for secondary loss from the plasma, were 73% for $Na^{125}I$ and 68% for ^{131}I-albumin (Fig. 3 B).

At lower pressure the difference between the transport of the ions and of the colloid molecules increases. After equilibration the corrected values were for the iodide ion in plasma 80% and for albumin 52,5% of the infused amount (Fig. 3 A). It can be concluded that at high pressures colloid molecules and electrolyte ions are regurgitating into the circulation mainly through capillaries where the passage of the large colloidal molecules is not restricted, probably by bulk flow of the solvent and solutes. At lower pressure the bulk flow diminishes, but the small ions are also diffusing into the plasma, independently from the hydrostatic pressure gradient, at a site where the passage of colloid molecules is restricted. This must lead to an accumulation of the colloid molecules in some part of the interstitial fluid of the liver.

The composition of the lymph reflects essentially the composition of the interstitial fluid of the organ of its origin. About 1/3 to 1/2 of the thoracic duct lymph stems from the liver (12). At high infusion rates the lymphatic concentration of labeleld albumin attained 36 per cent of its concentration in the infusion fluid. Accordingly, in these experiments the concentration of the infused colloid in liver lymph and interstitial fluid must approach its concentration in the infusate. Not only did the concentration of labelled albumin in the lymph rise constantly

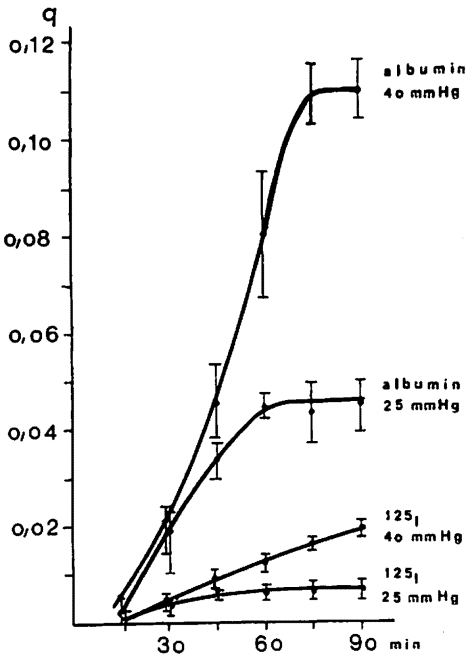

Fig. 6 Lymphatic transport of $Na^{125}I$ and ^{131}I-albumin during their infusion into the common bile duct at pressures of 25 and 40 mmHg.
The fractional quotients on the ordinata are the amounts of the label transported in the individual lymph collection periods divided by the total infused amount until the end of the respective period.
$\frac{q\ (lymph)}{S_q\ (infused)}$

during the infusion, but also the fraction transported by the lymphatics. On the other hand, if the amount of albumin eliminated during any given lymph collection period is divided by the total amount of the substance infused until the end of the respective period, then after the lapse of an equilibration time, a constant ratio was found between the two values (Fig. 6). These observations support again the assumption, that in some part of the interstitial fluid the concentration of albumin (and to some extent also of $Na^{125}I$) is progressively increasing. The amount of the label transported by the lymph is a function of the amount actually present in this pool.

The passage of electrolyte ions and of colloid molecules at high infusion pressure into the blood stream at a nearly identical rate points clearly to the sinusoids as the site of the regurgitation. To reach the sinusoids the fluid must leak into the Disse's spaces. In the Disse's spaces there are however no lymphatics. The terminal lymph vessels are situated in the portal tracts, in the periportal spaces of Mall (12). This is obviously the site of lymph formation, i.e. liver lymph reflects the composition of the interstitial fluid of the periportal spaces. In the periportal spaces the branches of hepatic artery are forming a dense peribiliary plexus. In the wall of these vessels there are no large intercellular gaps and the capillary hydrostatic pressure is relatively high. Accordingly, a bulk flow of fluid into the peribiliary capillaries is rather unlikely and the diffusion of the large protein molecules is strongly restricted. On the other hand, water can be absorbed in consequence of the gradient maintaned by the differences between extra- and intravascular hydrostatic and colloid osmotic pressures and the diffusion of the small electrolyte ions accross the vessel walls may be but little restricted. Consequently, during their leakage from the biliary tract, the concentration of colloid molecules in the fluid accumulating in the Mall's spaces may rise considerably.

It was observed in the present experiments that at lower biliary tract pressure less labelled albumin regurgitates into the circulating plasma than at high pressure, but the total amount of protein carried away by the veins is still higher than the amount transported by the lymphatic system. The lymphatic transport of the colloid was not influenced by changes in infusion pressure, i.e. the same fraction of the infused albumin load is carried away by the lymph vessels irrespective of the actual volume of the infused fluid and of the pressure inside the biliary tract. Finally, in another series of experiments, reported elswhere (15), it was observed that in the first hours after the ligation of the common bile duct more bilirubin and bile acid (both substances are bound to plasma proteins) regurgitates into the lymphatic system than into the blood stream. In these experiments no external pressure was applied and the pressure in the biliary tract presumably just reached the treshold value at which bile leaks from the biliary passages. Accordingly, the sequence of events occuring after biliary obstruction may be reconstructed as follows:

When bile pressure rises, at a certain level (about 20 mmHg), the bile first leaks accross the wall of the small bile ducts into the spaces of Mall. From this confined part of the hepatic interstitial fluid water is absorbed by the peribiliary blood capillaries, and electrolyte ions and small crystalloid molecules diffuse freely into the same blood vessels. In consequence of the restricted permeability of the peribiliary capillaries to colloids, protein molecules and substances bound to plasma proteins will be carried away mainly by the lymphatics. In this situation a significantly lower fraction of the colloid molecules escaping from the biliary passages than of the electrolyte ions of the same origin gains access directly to the blood stream. If bile pressure further rises, or if it is raised artificially by fluid infusion, bile is also forced from the small bile canaliculi embedded in the grooves on the contact surfaces of liver cells, and it leaks into the spaces of Disse, i.e. into the connective tissue separating the liver cells from the sinusoids. As the structure of these vessels and the pressure gradient allow a bulk flow of solvent and solutes, the rates of regurgitation into the blood stream of large and small molecules will be similar or nearly identical. At the level of the sinusoids this process is not limited, i.e. the transfer rates are directly proportional to the solvent and solute loads.

The portal tracts are surounded by a firm limiting plate of liver cells. When fluid leaks into this restricted space its compliance rapidly decreases. This limits the leakage into the periportal spaces. As it was shown, the composition of hepatic lymph reflects the composition of the interstitial fluid of this territory. During biliary obstruction the relative concentration of protein or protein bound molecules of biliary origin increases in the lymph to a very high level in consequence of water absorption and diffusion of small molecules from the interstitial fluid of the Mall's spaces into the peribiliary blood capillaries. The above mentioned limitation of the leakage into the peribiliary spaces limits however the accumulation of the large molecules in this part of the hepatic interstitial fluid. This explains the "restricted transporting capacity of the lymphatic system" reported previously (9).

Finally, as it was shown, at higher rates of leakage the amount of solutes escaping into the Disse's spaces increases and that escaping into the periportal spaces remains unchanged. This leads to an increase of the relative importance of the bulk flow of solvent and solute into the sinusoids and a to a decrease of the effect of the selective absorption of water and electrolytes. As a consequence, the difference between the rates of regurgitation into the blood stream of ions and colloid molecules practically disappears. This was observed in the present experiments at high infusion pressure and flow rate.

The above mechanisms of biliary-lymphatic and biliary-venous reflux are fully operative only in the early stages of biliary obstruction, probably only in the first 48 hrs. With the cessation of bile secretion the regurgitation also stops. The retention of bile constituents in chronic obstructive jaundice is a consequence of the disorder of liver cell function.

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Bile Constituents in Blood and Lymph During Biliary Obstruction

II. The absorption and transport of bile acids and bilirubin

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Summary

The lymphatic and venous transport of bilirubin and total bile acid was examined in dogs after the occlusion of the common bile duct. Lymphatic concentrations of both substances attained maximum levels between the 4 th and 6 th hours, but remained during the entire time of observation (24 hours) above plasma concentrations. The concentrations in blood plasma rose more slowly, but continuously. The amounts of both substances transported by the lymphatics rose steadly for 6 or 8 hours respectively and exceeded after 2 hours of occlusion the amounts transported by the veins. The results are explained by the changes in bilirubin and bile acid formation and secretion during biliary obstruction and on the basis of observations made in experiments with electrolyte and colloid infusions into the biliary passages.

In complete biliary obstruction much attention was paid to the problem of the biliary-lymphatic regurgitation (see 18). In these studies, however, only the passage of bilirubin was examined. Among the other major bile constituents, cholesterol is not an end product excreted exclusively into the bile. It is present in appreciable concentrations normally the body fluids and participitates in rather complicated metabolic processes. The changes of cholesterol content in blood and lymph after biliary obstruction cannot be explained by biliary-lymphatic or biliary-