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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-

venous regurgitation. Bile acids are on the other hand formed in the liver and are eliminated into the intestines with the bile. Probably the main cause, why the fate of bile acids in obstructive jaundice has escaped an extensive study was the inadequacy of the chemical methods for their estimation. As a relatively simple method is now available, the passage of bile acids in blood and lymph was studied in the present investigation together with that of bilirubin. Previous studies on the regurgitation of bile constituents were limited to the examination of concentration changes in plasma and lymph. In the present experiments it was attempted to calculate the absolute amounts of these substances transported by the lymphatic and the venous routes.

Material and Methods

The investigations were made on mongrel dogs with an average body weight of 15,8 (12 to 18) kg in pentobabital general anaesthesia (initial dose 30 mg/kg). The thoracic duct was cannulated on the neck, the cystic and the common bile ducts were ligated. Lymph samples were collected for 8 hours in 2 hour periods and after that, up to the 24th hour, in two 8 hour periods. To compensate fluid losses 3% dextran in physiological saline solution was infused intravenously. The amount of the infused fluid was about the double of the volume of the collected lymph. Blood samples were obtained at the end of each collection period. Circulating plasma volume was measured at the end of the experiments with the Evans-blue dye dilution method. Total bilirubin concentration was estimated with the diazo reagent of Hyjmans van den Bergh (6). Total bile acid concentration was measured by a modification of the enzymatic fluorimetric method of *Murphy* et al. (11) using the NAD-dependent enzyme 3-α-hydroxysteroid dehydrogenase prepared from cells of Pseudomonas testosteroni (10), for the oxydation of the cholenic acids. The fluorescence of the reduced NAD-H, formed in this reaction was measured with an OPTON M4QIII. spectrophotofluorimeter. The excitation wave length was 370 nm and the emitted light was read at 470 nm.

A calibration curve was prepared by dilution, from a stock solution of cholic acid. The standard solutions were incubated simultaneously with the serum and lymph samples. The calibration curve was linear in the range between 4 and 100 μM/litre of cholic acid. The bile acids were extracted from the biological fluids after protein precipitation with hot absolute ethanol and evaporation of the alcoholic supernatant, by redissolving the residue in 50% ethanol and the elimination of the interfering substances by extraction with 1:1 ether/heptane. For each serum or lymph sample a blank was incubated simultaneously containing the extract and all reagents exept the enzyme. The reading of its individual blank and of a reagent blank, containing the enzyme, but no serum or lymph extract, was substracted from the readings of the samples. Recovery studies with added bile acid showed that there is an averaged 19% loss during the extraction procedure. Accordingly, the serum and lymph concentrations were corrected for this loss.

The values presented in the text and the figures are average with ± SEM.

Results

After the occlusion of the bile passages bilirubin and bile acid concentration in lymph rose rapidly, reaching the maximum values between the 4th and 6th hour (Fig. 1 and 2). Normal average lymphatic bilirubin concentration was 0,20 ± 0,07 mg% and in the lymph collected between 4 and 6 hours it attained 4,82 ± 0,91 mg%. Similarly, control lymphatic total bile acid concentration was 1 ± 0,5 μM/litre (range 0 to 6 μM/litre) and it attained after 4 hours 116 ± 20,5 μM/litre. After the 6th hour the lymphatic concentrations of both substances were declining. In the lymph collected between 16 and 24 hours bilirubin concentration was 1,96 ± 0,32 mg% and bile acid concentration 52 ± 20 μM/litre. The 16-24 hours lymphatic concentra-

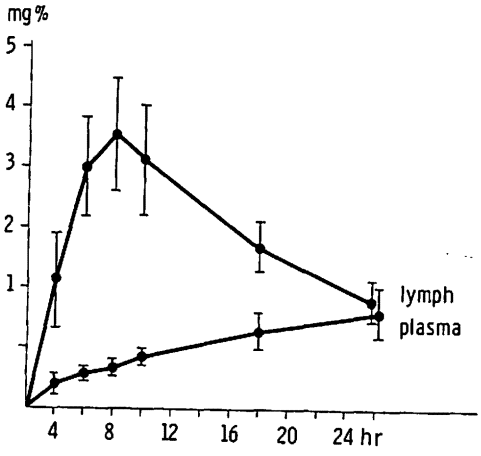

Fig. 1 Bilirubin concentrations in thoracic duct lymph and blood plasma during the obstruction of the common bile duct.

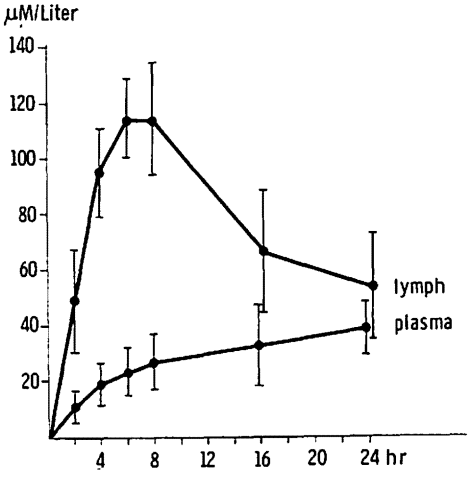

Fig. 2 Total bile acid concentrations in lymph and plasma during the obstruction of the common bile duct.

tion of bilirubin did not differ significantly from plasma concentration. Bile acid concentration was however in every animal still higher in lymph than in plasma. Plasma concentrations of both bilirubin and bile acids rose more slowly. That of bilirubin from 0,26 ± 0,07 mg% in 24 hours to 1,73 ± 0,41 mg%, and that of bile acid from 1,05 ± 0,33 μM/litre to 36 ± 9,7 μM/litre. No secondary decline of the serum concentrations was observed during the experiment.

The lymph flow was before bile duct ligation 0,37 ± 0,05 ml/min, or 0,023 ± 0,004 ml/min/kg body weight. It increased in the first 8 hours to 0,58 ± 0,08 ml/min, to decrease again in the last 8 hours to 0,33 ± 0,03 ml/min (Fig. 3). Average circulating plasma volume was 710 ml (4,5% of body weight). Multiplying lymphatic concentrations with the volume of the collected lymph in the individual periods, and the changes in plasma concentration of the same substances with plasma volume the amounts of bilirubin and bile acid transported by the lymphatics and by the veins could be calculated.

After biliary duct occlusion the lymphatic transport of bilirubin rose rapidly, from the control 0,05 μg/min/kg to 1,64 ± 0,35 μg/min/kg between 4 to 6 hours. After that, the amount of

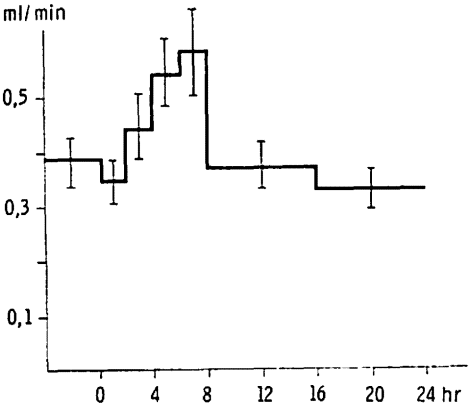

Fig. 3 Thoracic duct lymph flow during the obstruction of the common bile duct.

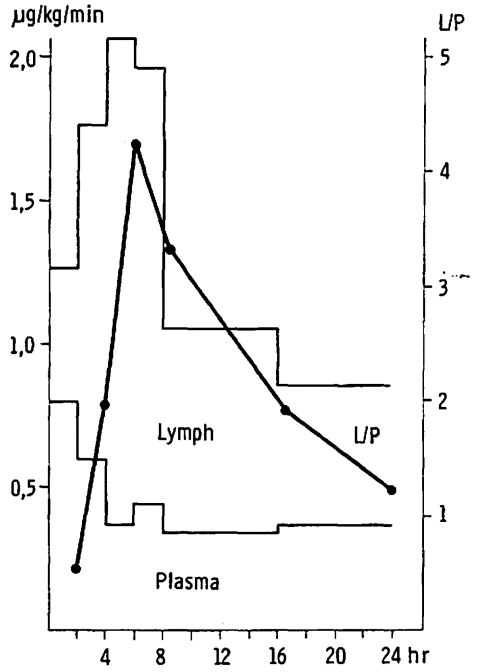

Fig. 4 Lymphatic and venous transport of bilirubin during the occlusion of the common bile duct.

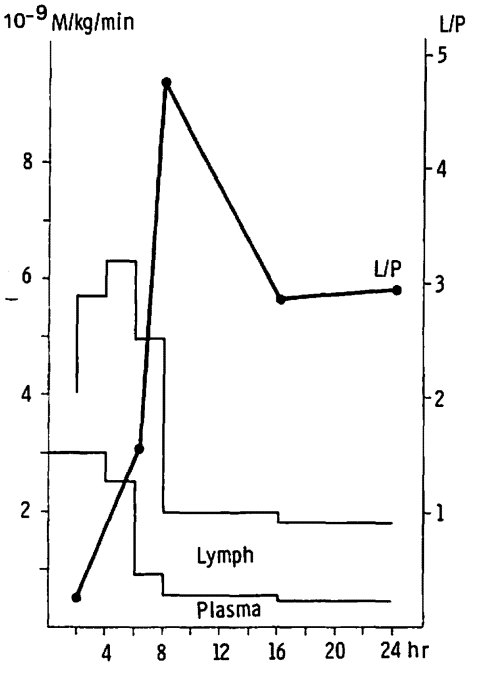

Fig. 5 Lymphatic and venous transport of bile acids during the occlusion of the common bile duct.

Lymphatic transport: concentrations (µg/ml or 10^{-9}M/ml) lymph multiplied by the amount of the excreted lymph (ml/min/kg body weight).
Venous transport: changes in plasma concentration (µg/ml/min or 10^{-9}M/ml/min)multiplied by circulating plasma volume (ml/kg body weight).

bilirubin transported by the lymph decreased again, to 0,47 ± 0,09 µg in the samples collected between 16 and 24 hours. The amount of bilirubin present in the blood increased in the first 2 hours at a rate of 0,81 ± 0,12 µg/min/kg. In the consecutive period the rise of plasma bilirubin content was slower again, and in the last 8 hours period attained only 0,36 ± 0,06 µg (Fig. 4).

Lymphatic bile acid transport was negligible before bile duct occlusion (0,067 ± 0,042.10^{-9} M/min/kg), it increased however rapidly, and the maximum was observed between 6 to 8 hours (4,06 ± 0,081.10^{-9}M/min/kg). Later the amount of the bile acid transported in lymph decreased again. The amount present in blood plasma increased in the first 4 hours by 3,0.10^{-9} M/min/kg body weight, and in the last 8 hours by only 0.45.10^{-9}M/min/kg (Fig. 5). After 2 to 4 hours of biliary obstruction the amount of bilirubin and bile acid transported by the lymphatics exceeded the amount gaining access directly to blood plasma. The peak average L/P transport ratios (over 4,0) were observed for bilirubin between 4 and 6 hours and for bile acid between the 6th and 8th hour of biliary stasis. The lymphatic transport remained higher than venous transport until the end of the observation (24 hrs).

Discussion

In acute biliary obstruction regurgitating bilirubin and bile acids are transported mainly by the lymphatics. As it was shown (9,18) this is to be expected for colloidal molecules. Both substances have however a molecular weight around 500. In plasma and lymph they are bound to serum albumin, but not so in the bile. Accordingly, in biliary obstruction bilirubin and bile

acid escaping accross the wall of the bile canaliculi and of the small bile ducts is not bound to protein. In consequence of the high permeability of the sinusoids, protein content of the interstitial fluid in the liver is high, nearly equal to plasma protein concentration (15). Therefore, the escaped bile constituents will be bound in the liver by serum protein and transported from the interstitium by the same mechanisms as the colloidal substances.

According to different in vitro investigation 2 to 3 molecules of bilirubin are bound by 1 molecule of albumin. The results of clinical and toxicity studies suggest that 1 molecule of albumin binds only 1 molecule of bilirubin. The in vitro determinations of the binding between bilirubin and albumin may detect bounds that are of no importance in the body. Actually, in clinical studies it has been shown that one molecule of bilirubin is bound fast and reversibly to albumin and two additional molecules are bound more losely (1, 5, 12, 16, 19). In the present experiments peak average thoracic duct lymph bilirubin concentration was 5 mg%. About 1/3 to 1/2 of thoracic duct lymph is of hepatic origin, accordingly, in the interstitial fluid of the liver an average bilirubin concentration of about 15 mg% (0,26 μm/litre) can be assumed.

The binding of various cholenic acids (mol/mol albumin) varies between 0,6 and 3,0 (14). The maximal average total bile acid content in the thoracic duct lymph was in the present experiments 0,11 mM/litre. This corresponds to a 0,33 mM/litre total bile acid concentration in hepatic lymph and interstitial fluid. Albumin concentration is in dog serum and liver interstitial fluid about 0,3 mM/litre. Accordingly, the molar ratio of bilirubin respectively bile acid and albumin in liver interstitial fluid should be around 1.

From the above data it can be concluded, that in the first hours after biliary occlusion the concentration of bilirubin and especially that of bile acid in the liver interstitial fluid is near to the maximal binding capacity of the proteins present in the same fluid. Consequently, it can be assumed that at least in some cases a part of the above substances is not bound to plasma protein. It was shown, that electrolyte ions /and obviously also the small bilirubin and bile acid molecules) are, at the site of lymph formation, in the Mall's spaces, freely diffusing into the blood capillaries, which have on the other hand a restricted permeability for proteins. This conclusion was made from the observation, that the L/P concentration ratio of electrolytes introduced at a moderate pressure into the biliary tract is significantly lower than the same ratio for colloids. Accordingly, if there is free bilirubin or bile acid present, it diffuses into the capillaries and will be consequently transported by the venous route. The limited availability of protein for the binding of these substances my be one of the causes for the observed "limitation of the bilirubin transporting capacity" of the lymphatic system (9). In the experiments where labelled protein was infused into the biliary tract the lymphatic/venous transport ratios were pressure dependent: the venous transport increased with the rise of the pressure in the biliary tract. It can be assumed therefore, that if the biliary pressure is low, i.e. equal to, or just exceeds the secretory pressure of the bile, and that is what usually happens after the occlusion of the common bile duct, then the L/P transport ratio for colloids will be high, i.e. lymphatic transport will be significantly greater than venous transport. It was also shown (18), that if albumin leaks from the bile ducts at a constant rate, the fraction transported by the lymphatics increases progressively. but the fraction gaining access to the plasma (carried away by the hepatic vein) remains constant. The rising lymphatic transport was explained by the accumulation of the albumin in the interstitial fluid of the periportal spaces. This accumulation is due to the low protein permeability of the peribiliary capillaries.

In the present experiments the greatest venous transports of bilirubin and bile acid were observed in the first 2 and 4 hours, respectively after the occlusion. On the other hand, the lymphatic transport attained its maximum between the 4th and 6th (bilirubin) and 6th and 8th (bile acid) hours. From this, it can be concluded that the leakage of the bile constituents is maximal in the first 2 to 4 hours of the occlusion but up to 8 hours after the ligation of

the common bile duct its rate remains high enough, to produce further concentration increases in the interstitial fluid of the liver. After this time the transport of bilirubin and bile acid from the interstitium is greater than their leakage from the biliary ducts. Consequently, their concentration in the interstitial fluid and their lymphatic transport decrease progressively.

The leakage of the bile constituents diminishes with time because their secretion by the liver cells decreases. Bilirubin is mainly formed extrahepatically and it is conjugated and excreted into the bile by the liver cells. Elevated biliary tract pressure, even if it does not reach the secretory pressure of the bile, reduces significantly bilirubin secretion (7). In complete biliary obstruction bilirubin secretions stops completely after 2 to 3 days (4). This is due to some damage to the hepatic excretory mechanism because in chronic biliary tract obstruction the hepatic excretion of intravenously injected sulfobromophthalein, a dye taken up, metabolized and released into the bile by the same mechanisms as bilirubin, is similarly impaired (5, 20). Finally, in patients with chronic obstructive jaundice, the lymphatic concentration of bilirubin lies below plasma concentration. Actually, its L/P concentration ratio corresponds approximately of that of albumin (3, 13).

Bile acids are, on the other hand, formed in the liver and secreted into the bile. There is also an important entero-hepatic circulation. At high biliary pressure bile salt secretion rate is consistently reduced and synthesis completely inhibited (17). After the oclusion of the common bile duct the interruption of entero-hepatic circulation influences profoundly the total amount of bile acid secreted by the liver cells. If the enterohepatic circulation is interrupted, the entire pool is "washed out" in about 24 hours (2).

Accordingly, if biliary obstruction persists, as a result of the cessation of the synthesis and of the elimination of the enteral pool, after an appropriate time there will be no bile acid secretion into the bile and no regurgitation into the venous and lymphatic system. In the present experiments this stage was not reached, but only closely approached.

Finally, as it was shown (18), at moderately increased biliary tract pressure bile leaks mainly from the small bile ducts into the periportal connective tissue. Water and electrolytes, but not colloids are freely absorbed into the "true" blood capillaries of this area. This leads to a progressive increase of the concentration of the colloid molecules in the interstitial fluid of the periportal spaces. As hepatic lymph is formed from the interstitial fluid of the periportal spaces, the lymphatic concentration of colloidal or colloid-bound substances leaking from the bile ducts will attain very high levels.

With higher supply of bile, i.e. if biliary pressure rises still further, bile begins to leak also from the canaliculi lying between the hepatic cell. In the Disse's spaces the bile comes in direct contant with the sinusoids. Sinusoidal hydrostatic pressure is low, and the walls of the sinusoids are practically freely permeable to proteins. If bile leaks into the interstitial space surronding the sinusoids a bulk flow of water, electrolytes and colloids into these capillaries is to be expected. In obstructive jaundice, when biliary tract pressure rises above the threshold value allowing a leakage from the bile canaliculi into the Disse's spaces the venous transport of bilirubin and bile acid increases. Bile leakage into the Mall's spaces is at the same time limited by the decreased compliance of this part of the liver interstitium. This limits the lymphatic transport of the bile constituents. Actually, that might be the principal factor leading to the limited transporting capacity of the lymphatic system for bilirubin and bile acids.

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