

Total protein output during rapid reduction of bile salt secretion rates in man

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SUMMARY An investigation was undertaken to study the effect of bile salt secretion on total biliary protein secretion in man. Bile was collected in eight patients from a tube in the bile duct. Collection was started after a meal and continued for six hours, in order to obtain bile salt secretion rates over the entire physiological range. Total protein secretion rates did not vary with change in bile salt secretion or bile flow. The protein pattern assessed by SDS-PAGE did not vary with bile salt secretion. The results indicate that bile salt secretion has little influence on biliary protein secretion under these conditions in man. Changes in bile salt secretion were associated with linear change in bile flow, but there was no relationship between bile flow and protein secretion rates. This argues against convective sieving of plasma proteins into bile.

There are two sources of hepatic bile proteins, serum proteins which pass from blood to bile and proteins synthesised in the liver and exported into bile.¹⁻⁵ Some serum derived bile proteins such as IgA are transported across the hepatocyte by specific vesicular systems^{6,7} and their bile concentration exceeds their blood concentration;^{4,8} however, most serum derived proteins have a much lower concentration in bile than serum and seem to enter bile at a rate inversely proportional to their molecular weight.^{1,4}

Little is known about factors determining the rate of protein secretion. Studies in animal models have found a relationship between bile salt secretion and secretion of membrane bound enzymes into bile.⁹⁻¹² Studies on the influence of bile salt secretion on the secretion of other biliary proteins or total protein secretion are conflicting, however, with some investigators observing an effect¹² while others have found that protein secretion is not related to biliary secretion of bile acids.^{9,10,13} In this study we have examined the effect on protein secretion of a rapid reduction in

bile salt secretion produced by interruption of the enterohepatic circulation. We found that bile salts do not appear to influence protein secretion during rapid reduction of bile salt secretion induced by interruption of the enterohepatic circulation. A second observation is that protein secretion rates are not influenced by changes in bile flow. This does not support the concept⁴ that serum proteins enter bile by convective sieving through the paracellular pathway.

Methods

PATIENTS

Bile was obtained with informed consent from patients who had the bile duct explored during an operation for gall stone disease. As part of this procedure a t-tube is placed in the bile duct and one limb is exteriorised. Four days later the bile ducts were examined by cholangiography and shown to be free of residual stones. The exterior limb of the t-tube was then clamped until the study six days later to restore the normal passage of bile into the duodenum. The tube was removed immediately after the study. Eight patients (three men and five women) were studied. The mean age was 57.1 years (20.3) (SD) and mean weight 69.1 kg (17.0) (SD).

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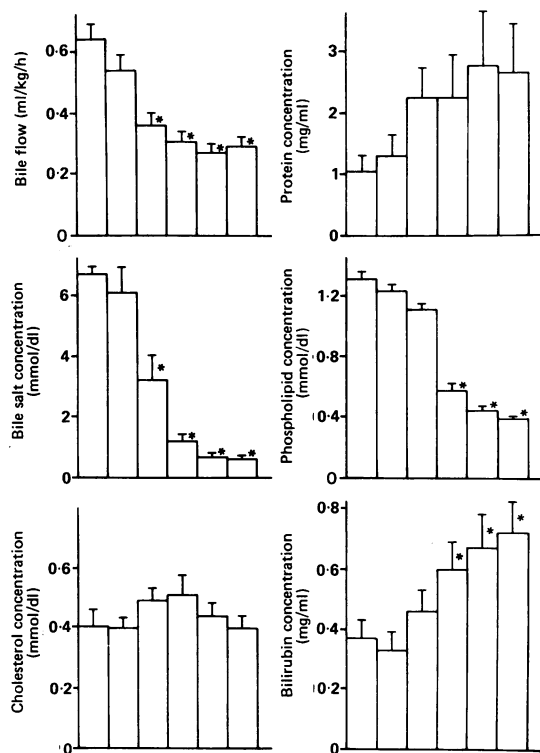


Fig. 1 Mean hourly bile flows and concentration of biliary lipids and proteins of hepatic bile collected from eight individual patients over a six hour period. Each bar represents results from bile collected over 60 minute intervals. *Significantly different from results of bile collected during first hour.

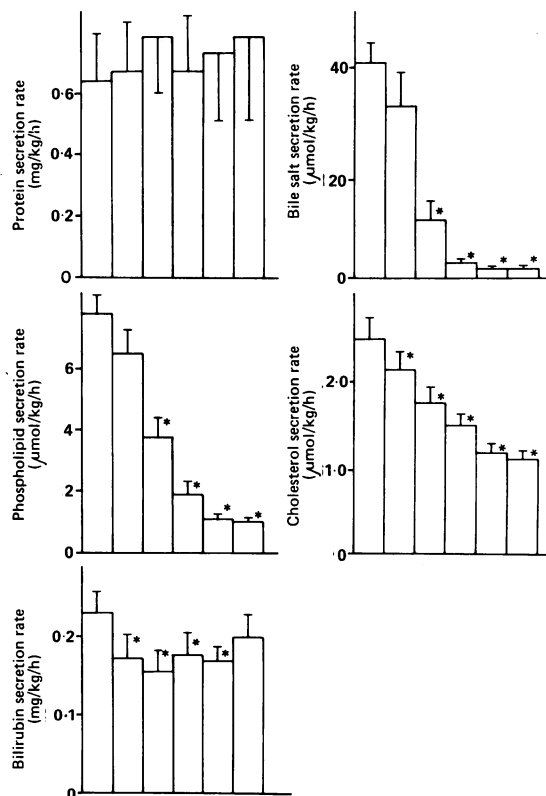


Fig. 2 Mean hourly secretion rates of biliary protein and lipids of hepatic bile collected from eight individual patients over a six hour period. Each bar represents results from bile collected over 60 minute intervals. *Significantly different from results of bile collected during first hour.

STUDY PROTOCOL

Patients were given a standard full diet breakfast at 08 30 on the study day. Thirty minutes later the t-tube was opened. The first 3 ml bile – that is, in the tubing, were discarded. The end of the drainage tube was placed in a collecting tube positioned in ice, and six, one hour collections were made. The level of the end of the drainage tube was maintained at 15 cm below the level of the common bile duct.

CHEMICAL METHODS

Bile salts were measured enzymatically using 3-OH steroid dehydrogenase,¹⁴ phospholipid by the method of Bartlett,¹⁵ and cholesterol by GLC.¹⁶ Bilirubin was determined by the diazo method.¹⁷

Biliary proteins were quantitated by a fluorometric technique¹⁸ as previously described by Gallinger *et al.*¹⁹ This method has been shown to be accurate as validated by quantitation of biliary proteins by amino acid analysis.¹⁹ Slab sodium dodecyl sulphate poly-

acrylamide gel electrophoresis was performed using a gradient of 5–20% with a 3% stacking gel.²⁰ After separation by electrophoresis, proteins were stained with silver nitrate (Bio Rad silver stain kit, Richmond California).²¹

STATISTICAL ANALYSIS

Results were compared using the Student's paired *t* test. As multiple testing was done the acceptable *p* value was lowered appropriately (acceptable level of $p=0.05/\text{number of comparisons by } t \text{ test}$). This placed the level of *p* for significance at $p<0.01$.

Results

Bile flows and concentrations of biliary lipids and proteins are given in Figure 1. Secretion rates are presented in Figure 2. Bile flow decreased progressively in the first three hours after interruption of the enterohepatic circulation, as expected. Concen-

trations of solutes followed one of three patterns. Bile salt and phospholipid concentrations fell. Cholesterol concentration was unchanged. Protein and bilirubin concentrations increased but the increase in protein concentration did not quite reach significance ($p=0.07$).

Mean protein secretion rates did not change over the six hour study period. Bilirubin secretion rate decreased slightly but significantly in the second hour, and remained steady thereafter. Secretion rates for bile salts, phospholipid and cholesterol all decreased significantly and in a stepwise manner in the first few hours. There was a close similarity in the pattern of secretion rates for bile salts and phospholipid and cholesterol, but the reductions in phospholipid secretion were less than those in bile salt secretion, and cholesterol secretion rates were least affected. The bile salt pool size calculated by the washout method^{22,23} was $6.47 \text{ mmol} \pm 0.99 \text{ (SEM)}$.

Examination of the protein secretion rates in individual patients (Fig. 3) reveals that five of eight

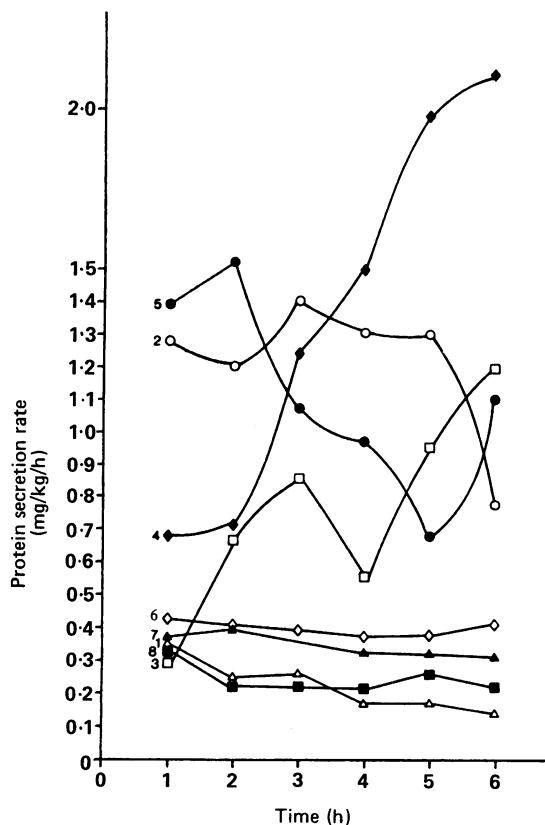


Fig. 3 Hourly protein secretion rates into hepatic bile collected in eight individual patients over a six hour period.

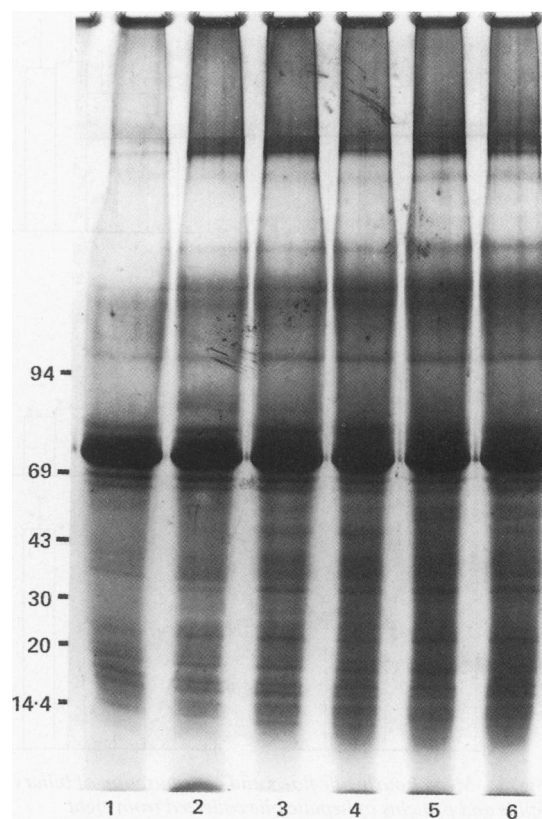


Fig. 4 SDS-polyacrylamide gel electrophoresis (5–20% gradient) of biliary proteins. Hepatic bile was collected over 60 minute intervals for six hours. Proteins were precipitated in 7% trichloroacetic acid followed by an ether-ethanol (1:1) wash. A constant mass of protein (50 μg) as determined by fluorometric assay was applied to each lane. The gel was stained for protein with Bio Rad's silver staining kit. The number beneath each lane indicates the hourly interval over which the bile sample was collected. Migration of molecular weight markers are indicated (kDa).

patients (patients 1, 2, 6, 7, and 8) had fairly steady secretion rates. Large increases in protein secretion were recorded in patients 3 and 4 and protein secretion decreased in the other patient. The range of secretion rates was large.

SDS-PAGE electrophoresis of samples from one patient are shown in Figure 4. There were no obvious changes in the bands over the six hour period. Results shown are from a patient who had a constant protein secretion rate (patient 8). SDS-PAGE of proteins from patients with increasing or decreasing protein secretion rates also showed no obvious changes in protein bands.

Discussion

Bile total protein secretion rate was not related to the bile salt secretion rate in this study. The range of bile salt secretion rates obtained was large so that the failure to detect a relationship was not the result of examination of protein secretion rates over a narrow range of bile salt secretion rates. In order to obtain a high bile salt secretion rate in the first hour, the study was started 30 minutes after eating at a time when the bile salt pool would be cycling rapidly. The initial secretion rate of 40 $\mu\text{mol/kg/h}$ is at the upper range of bile salt secretion rates previously reported in man.²⁴⁻²⁷ Northfield and Hofmann found that the highest bile salt secretion rates were observed in the first hour after a meal.²⁴ In seven control subjects the rate was 35.0 $\mu\text{mol/kg/h}$ and in seven patients with stones 37.0 $\mu\text{mol/kg/h}$. This is very similar to our own results in the first hour (about 40 $\mu\text{mol/kg/h}$). The mean daily output in their study was about 60% of these levels. The highest bile salt secretion rates recorded by Shaffer and Small were about 30 $\mu\text{mol/kg/h}$.²⁶ On the other hand, low bile salt secretion rates (less than 2 $\mu\text{mol/kg/h}$) were obtained as expected in the fifth and sixth hours of our study due to the washout design of the study. We therefore conclude that over the physiological range of bile salt secretion rates, total biliary protein secretion rates are not related to bile salt secretion rates. Furthermore, no evidence was found to suggest that individual biliary proteins are substantially influenced by the bile salt secretion rate as the protein pattern evaluated by SDS-PAGE was similar throughout the study. This does not exclude the possibility that proteins present in very low concentrations such as membrane enzymes in bile are influenced by bile salt secretion rates.

This study is in agreement with that of Kakis and Yousef who used the washout method in rats.¹³ Total protein secretion rates remained constant over 48 hours despite large variations in the bile salt secretion rates.¹³ Total protein secretion rates were increased by infusion of bile salts in one study¹² but the infusion rates used were high and associated in many cases with bile flow rates which fell during the course of the study, suggesting that liver cell injury may have occurred. In two other studies intravenous bile salt infusions produced raised biliary secretion of alkaline phosphatase, 5' nucleotidase or phosphodiesterase.^{9,10} The increases in these proteins did not result in a detectable increase in total biliary protein secretion rates. We did not measure these enzymes and it is unlikely that subtle changes in their concentration would be detectable by SDS-PAGE.

This study was carried out in patients with gall stones and the conclusions might not apply to normal individuals. This is an unlikely possibility as we have

shown that protein concentrations in hepatic bile of patients with a variety of gall bladder problems other than gall stones are similar to those of patients with pigment or cholesterol gall stones.¹⁹ Furthermore, the patients in this study were able to rapidly restore the bile salt pool size to a normal level after the depletion associated with surgery and the maximal bile salt secretion rates were similar to that previously reported in controls.²⁴⁻²⁷ It would be of interest to study normal subjects but duodenal sampling could not be used because of mixing with protein of intestinal origin, and placement of t-tube is very uncommon in patients without stones. Therefore, it is unlikely that such a study can be conducted.

A number of investigators have noted that the bile-to-plasma ratio of proteins is inversely proportional to their molecular weights. It has been suggested that paracellular transport with sieving of protein may be responsible for this.⁴ Under these circumstances, however, one would predict that changes in bile flow would affect protein secretion substantially. This was not observed in the present study even though bile flow rates fell to less than one half of initial values. Perhaps the relative retardation of larger molecular weight protein is caused by another mechanism. It is possible for instance that larger molecular weight proteins could be hindered from approaching the plasma membrane of the hepatocyte where they are internalised in the process of fluid phase endocytosis.

It has been previously reported that bilirubin secretion rates are steady throughout the day.²⁸ It appears, however, that at the highest bile salt secretion rates, such as those induced at the start of this study, bilirubin secretion may be augmented. It may be that this would only be seen in the immediate postprandial period when a high bile salt secretion rate follows a low bile salt secretion rate. Perhaps there is some facilitation of bilirubin transport under these circumstances or a washout of 'stored' hepatic bilirubin.²⁹ Bile salt micelles are known to increase bilirubin solubility in bile.³⁰

In summary, bile salts do not affect total protein secretion rates or secretion rates of proteins detectable by SDS-PAGE. The molecular size selection favouring entry of low molecular weight protein does not seem to be the result of convective sieving.

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