# Bile Acid Concentrations in Systemic and Portal Serum in Presumably Normal Man and in Cholestatic and Cirrhotic Conditions

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Total bile acid concentration was determined in systemic and portal serum and in liver tissue from patients with presumably normal liver function, and from patients with extrahepatic cholestasis. Systemic and portal serum bile acids were also determined in patients with alcoholic liver cirrhosis. In 5 patients, in whom a portal catheter was inserted through the umbilical vein, the diurnal variation in systemic and portal serum bile acid concentration was studied. In patients with presumably normal liver function the fasting systemic serum bile acid concentration was $4.8 \pm 0.5$ $\mu$mol $\cdot$ $l^{-1}$, and the portal concentration was $12.9 \pm 1.5$ $\mu$mol $\cdot$ $l^{-1}$. In cholestasis and liver cirrhosis the systemic and portal bile acid concentration was substantially elevated. The bile acid concentration gradient between systemic serum, portal serum, liver tissue, and hepatic bile was 1:3:80:2600 in the patients with normal liver function. In both the cholestatic and cirrhotic condition the systemic and portal serum bile acid concentration was equilibrated. Postprandially both the systemic and portal bile acid concentration increased, but the gradient between these concentrations was unchanged. The results are compatible with the hypothesis that portal and systemic serum bile acid concentrations are determined by the intestinal absorption rate in subjects with normal liver function and by the hepatic and renal clearance capacity in cholestatic and cirrhotic onditions.

*Key-words:* Bile acids; cholestasis; cirrhosis; portal serum

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The bile acid pool in normal man is compartmentalized mainly to the enterohepatic circuit because of the high clearance capacity of the liver and the efficient intestinal absorption. In conditions with disturbances of the liver function, particularly in cholestasis, the bile acid pool is redistributed to the systemic circulation and to the body water. Sparse information is available on bile acid concentration in portal blood and liver tissue under normal as well as pathologic conditions (3, 4, 10).

The present study was aimed at examining the diurnal variation of portal bile acid concentration in man and the bile acid concentration in systemic and portal serum, and in hepatic tissue from patients with cholestasis and portal cirrhosis.

## MATERIAL AND METHODS

*Clinical material.* The clinical material consisted of 82 patients, 37 women aged 27–84 years, and 45 men aged 40–81 years. Of these patients 22 had extrahepatic cholestasis. The

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Table I. Clinical data and serum liver tests in 22 patients with extrahepatic biliary obstruction

| Diagnosis | Age | Sex | Bilirubin (mg/100 ml) | Alkaline Phosphatase (U/l) | sASAT (U/l) | sALAT (U/l) | Cholesterol (mmol/l) | TG |
|---|---|---|---|---|---|---|---|---|
| Choledocholithiasis | 30 | m | 8.0 | 500 | 98 | 52 | 6.0 | 2.5 |
| Choledocholithiasis | 49 | f | 7.8 | 1055 | 216 | 192 | 6.0 | 1.7 |
| Choledocholithiasis | 57 | f | 3.6 | 916 | 61 | 106 | 6.3 | 1.1 |
| Choledocholithiasis | 57 | m | 7.4 | 591 | 113 | 192 | – | – |
| Choledocholithiasis | 63 | m | 6.3 | 328 | 88 | 118 | 6.6 | 2.4 |
| Choledocholithiasis | 69 | m | 5.7 | 805 | 41 | 80 | – | – |
| Choledocholithiasis | 70 | m | 13.9 | 710 | 197 | 121 | 7.5 | 1.4 |
| Choledocholithiasis | 71 | f | 3.2 | 342 | 126 | 87 | 7.6 | 3.3 |
| Choledocholithiasis | 80 | f | 5.8 | 257 | 16 | 25 | – | – |
| Choledocholithiasis | 85 | m | 5.9 | 375 | 155 | 150 | 4.6 | 0.9 |
| Cholangitis | 34 | m | 11.0 | 500 | 36 | 31 | – | – |
| Pancreatitis chron. (choledochal stenosis) | 38 | m | 4.8 | 1226 | 113 | 192 | – | – |
| Pancreatic carcinoma | 58 | m | 10.4 | 1120 | 70 | 77 | 15.0 | 5.4 |
| Pancreatic carcinoma | 70 | f | 20.0 | – | – | – | – | – |
| Pancreatic carcinoma | 72 | m | 19.0 | 1250 | 27 | 17 | 5.3 | 2.3 |
| Pancreatic carcinoma | 73 | f | 26.4 | – | – | – | – | – |
| Pancreatic carcinoma | 77 | f | 5.4 | 1081 | 27 | 28 | 7.7 | 1.8 |
| Pancreatic carcinoma | 79 | f | 9.9 | 911 | 62 | 25 | 5.7 | 2.5 |
| Pancreatic carcinoma | 82 | f | 15.0 | 1250 | 175 | 130 | 14.1 | 1.7 |
| Choledochal carcinoma | 74 | f | 18.2 | 699 | 47 | 59 | – | – |
| Gallbladder carcinoma | 71 | m | 17.6 | 209 | 13 | 14 | – | – |
| Gallbladder carcinoma | 76 | m | 17.5 | – | – | – | – | – |
| Reference value | | | (<1.2) | (<260) | (<17) | (<17) | (<10.1) | (<2.5) |

clinical data on these patients are given in Table I. None of the cancer patients in this group had macroscopic liver metastasis. The liver tests and plasma lipids in the cancer patients did not differ from those in the other cholestatic patients.

Thirteen patients, 12 men aged 40–72 years, and one woman aged 59 years, had portal liver cirrhosis according to microscopy of liver biopsies. All of them had a long history of alcohol consumption, and they were electively or emergency portacaval shunted (end to side) because of bleeding oesophageal varices. All of them had a slight increase of serum bilirubin concentration, mean 2.8 ± (S.E.M.) 0.7 mg/100 ml serum. The serum albumin was decreased, mean 28 ± 2 g/l.

In five patients with various diagnoses (see Table IV) the diurnal variation of bile acids in systemic and portal serum was studied.

Forty-three patients with uncomplicated gallstone disease, 23 women aged 56 ± 19 (S.D.) years, and 20 men aged 62 ± 11 (S.D.) years, served as controls. These control patients had normal liver function, as judged from routine liver tests, and serum lipids were within normal values.

*Experimental procedures.* The cholestatic, cirrhotic, and control patients were studied at the time of operation. They were fasted for routine practice. Anaesthesia was given as hexobarbital (Evipan®), nitrous oxide, and pancuronium bromide (Pavulon®). Liver biopsies (13) and blood samples were taken from the portal vein by direct puncture or via an umbilical vein catheter as the first procedure during the operation.

Hepatic bile was sampled as described previously (14).

In the five patients, in whom diurnal variation or bile acids in portal serum was studied, an umbilical vein catheter was inserted into the portal vein at the time of operation and left in place. The study was performed when the pa-

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Table II. Bile acid concentration in serum, liver, and bile (mean±S.E.M.)

| | Systemic serum | | | Portal serum | | | Liver tissue | | Bile |
|---|---|---|---|---|---|---|---|---|---|
| | Controls (n, 26) | Extrahepatic Cholestasis (n, 11) | Cirrhosis (n, 13) | Controls (n, 23) | Extrahepatic Cholestasis (n, 11) | Cirrhosis (n, 13) | Controls (n, 25) | Extrahepatic Cholestasis (n, 11) | Controls (n, 20) |
| | | $\mu mol \cdot l^{-1}$ | | | $\mu mol \cdot l^{-1}$ | | | $\mu mol \cdot kg^{-1}$ | $\mu mol \cdot l^{-1}$ |
| Mean | 4.8 | 42.9*** | 21.0*** | 12.9 | 47.8*** | 20.1* | 400 | 430 | $27 \cdot 10^3$ |
| S.E.M. | 0.5 | 9.1 | 4.4 | 1.5 | 9.2 | 3.6 | 30 | 40 | $1.9 \cdot 10^3$ |

Level of significance within compartment; *$P<0.05$; ***$P<0.001$.
Level of significance between systemic and portal serum: Controls, $P<0.001$; Cholestasis, n.s.; Cirrhosis, n.s.

tient was mobilized and had eaten an ordinary diet for at least 4 days (8–28 days after the operation). Before the study was started, the catheter position in the portal vein was checked by contrast examination. Two of these patients had normally functioning gallbladder according to peroral cholegraphy at the time of the study (cases 1 and 2, Table IV).

*Analytical procedures.* The liver biopsies were sliced, and the slices were then washed in ice-chilled Krebs Ringer bicarbonate solution, pH 7.4, for 15 minutes to diminish bile acid contamination from bile. The bile acids were extracted from liver homogenates by alkaline ethanol extraction, as described by Okishio & Nair (15).

Bile acids were extracted from portal and systemic serum in principle as described by Murphy et al. (12). During the study the extraction technique was changed to the Amberlite-XAD-2 method as described by Makino & Sjövall (11). Parallel analyses with the two extraction methods showed no significant differences, i.e. the difference between the methods did not exceed the standard error of the bile acid determination (4.8 per cent). The bile acids were quantitated with the fluorescence enzymatic method (14) by the use of fluorescence spectrophotometry (Hitachi MPF-2A). Biliary bile acids were extracted and quantitated as described previously (14).

*Statistical methods.* Linear regression relationships were calculated by the least square fit method, and standard statistical methods were used to calculate correlation coefficients. The t-test was applied to determine statistically significant differences between groups.

## RESULTS

The bile acid concentration in systemic and portal serum was statistically significantly higher in patients with extrahepatic cholestasis and in patients with liver cirrhosis than in controls (Table II). In extrahepatic cholestasis the bile acid concentration was significantly higher in both systemic and portal serum than corresponding values in cirrhosis ($P<0.025$ and $P<0.01$). The bile acid concentration in portal and systemic serum differed statistically sig-

Table III. Bile acid concentration gradients between systemic serum, portal serum, liver tissue, and bile (bile acid concentration in systemic serum is designated as 1)

| | Systemic serum | Portal serum | Liver tissue | Bile |
|---|---|---|---|---|
| Controls (n, 23) | 1 | 3 | 80 | 2600 |
| Extrahepatic cholestasis (n, 11) | 1 | 1 | 10 | – |
| Cirrhosis (n, 13) | 1 | 1 | – | – |

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Table IV. Diurnal variation of bile acid concentration in systemic and portal serum in 5 patients

| Diagnosis | Age | Sex | Bile acid concentration 8 a.m. Fasting | | 9 a.m. After breakfast | | noon Before lunch | | 1 p.m. After lunch | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Syst. | Portal | Syst. | Portal | Syst. | Portal | Syst. | Portal |
| Pancreatic insuloma* | 47 | m | 6.2 | 17.2 | – | – | 5.5 | 16.6 | 8.4 | 26.1 |
| Pancreatic insuloma* | 66 | f | 3.5 | 5.1 | – | – | – | – | – | – |
| Intestinal carcinoid (liver metastases) | 74 | f | 3.6 | 13.5 | 10.1 | 34.5 | 6.1 | 47.8 | 8.7 | 50.4 |
| Chron appendicitis +cholecystitis chron. (dist. ileum resected) | 64 | f | 4.8 | 16.4 | 8.7 | 19.2 | 3.8 | 12.5 | 6.7 | 23.7 |
| Colon carcinoma (liver metastases) | 67 | m | 14.2 | 45.8 | 33.6 | 75.1 | 25.8 | 50.6 | 38.0 | 70.1 |

*Functioning gallbladder.

nificantly only in the controls ($P<0.001$). The patients with extrahepatic cholestasis had a bile acid concentration in the liver tissue of the same magnitude as in controls.

The bile acid concentration gradient between portal and systemic serum was extinguished in extrahepatic cholestasis and cirrhosis (Table III). The gradient between liver tissue and serum was considerably decreased in cholestasis.

The diurnal variation of bile acid concentration in systemic and portal serum in five patients is reported in Table IV. These patients showed almost the same variation pattern, but the interindividual variation in bile acid concentrations in both systemic and portal serum was high. The bile acid concentration increased postprandially in systemic and portal serum.

Linear regression analysis revealed a statistically significant correlation between the bile acid concentration in systemic and portal serum in controls as well as in the patients with liver disease (Table V). In the controls a significant correlation was also found between the concentration of bile acids in systemic serum and in liver tissue. A strong correlation ($r^2=0.76$) was found between liver tissue bile acids and the bile acid concentration in hepatic bile in these subjects.

## DISCUSSION

The bile acid concentration in portal serum was about three times that of systemic serum

Table V. Correlation coefficients of relationships between bile acid concentrations in systemic and portal serum, liver tissue, and biliary bile

| | Portal serum | | | Liver tissue | Bile |
|---|---|---|---|---|---|
| | Controls | Cholestasis | Cirrhosis | Controls | Controls |
| *Systemic serum* | | | | | |
| Controls | 0.56 | | | 0.72 | n.s. |
| Cholestasis | | 0.95 | | | |
| Cirrhosis | | | 0.93 | | |
| *Portal serum* | | | | | |
| Controls | | | | n.s. | 0.42 |
| *Liver tissue* | | | | | |
| Controls | n.s. | | | – | 0.87 |

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$\mu mol \cdot l^{-1}$

| 4 p.m. Before dinner | | 5 p.m. After dinner | | 8 p.m. After supper | | 12 p.m. | | 5 a.m. | |
|---|---|---|---|---|---|---|---|---|---|
| Syst. | Portal | Syst. | Portal | Syst. | Portal | Syst. | Portal | Syst. | Portal |
| 7.0 | 32.9 | 12.4 | 30.1 | 9.8 | 30.4 | 8.6 | 35.6 | 6.7 | 30.2 |
| – | – | – | – | 4.0 | 29.2 | 3.8 | 16.3 | 3.5 | 17.0 |
| 3.6 | 19.9 | – | – | 4.6 | 23.9 | 6.8 | 29.9 | – | – |
| 4.8 | 13.5 | – | – | 6.7 | 24.1 | 2.9 | 16.0 | – | – |
| 21.0 | 33.7 | 24.8 | 45.8 | – | – | 22.9 | 51.5 | 19.0 | 18.6 |

in presumably normal fasting subjects. In the postprandial state this ratio was almost the same, namely $3.5 \pm 0.5$ (S.E.M.) ($n=13$). The finding that the fasting and postprandial ratio did not differ is consistent with the hypothesis of a constant fractional hepatic clearance of bile acids (6). If this hypothesis is true, it is reasonable to assume that in normal man not only the portal bile acid concentration but also the systemic serum bile acid concentration should be a direct function of the intestinal absorption rate as previously suggested by LaRusso et al. (9). The efficiency of hepatic bile acid clearance has been studied in animals and found to be very high, between 70–95 per cent during one hepatic passage (16, 17). The hepatic extraction capacity in normal man is probably also very high, as judged from the plasma disappearance rate of injected labelled bile acids (2). With such a high extraction capacity in the liver it is surprising to find that the ratio of the bile acid concentration in portal serum and systemic serum was not higher than three, and also that the correlation coefficient in the relationship between the bile acid concentration in these compartments was not higher than 0.56 (Table V). It thus seems possible that there might be other determinants for the systemic serum bile acid concentration than the degree of spillover during the hepatic passage. Bile acids are more or less lipophilic. The least polar and thus most lipophilic bile acids are chenodeoxycholic and deoxycholic acid. The passive intestinal absorption of bile acids occurs in the proximal small intestine and most rapidly for these least polar bile acids (5, 8). The intestinal lymph is a lipid phase and a fractional uptake of bile acids to the lymph would be possible, and such an uptake should imply a more direct passage to the systemic blood. The postprandial elevation in systemic serum bile acid concentration could thus be a function of the lymphatic transport. This hypothesis offers an explanation of the otherwise confusing phenomenon that chenodeoxycholic acid, the least polar bile acid, rises in serum shortly after eating and continues to be elevated for a couple of hours, but cholic acid does not rise until after an hour and falls within the next hour (19). It also explains the low ratio (3/1) between portal and systemic serum.

In patients with extrahepatic cholestasis, independently of the underlying cause, as well as in patients with portal cirrhosis, the bile acid concentration in systemic serum was substantially elevated, which is in accordance with many other reports (1, 10, 18, 20). In both these conditions the portal and the systemic serum bile acid concentrations were equilibrated (Table III). This indicates that the determining role of the intestinal absorption rate of bile acids for systemic and portal serum bile

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acid concentration is probably taken over by the hepatic and the renal clearance capacity. This means that the systemic serum bile acid concentration in patients with liver disease may reflect the hepatic function as suggested previously (7).

Very few determinations of bile acid concentrations in human liver are available. Greim et al. (3) found in six noncholestatic patients a mean value of 70 $\mu$mol · $kg^{-1}$ and in five patients with severe cholestasis 475 $\mu$mol · $kg^{-1}$. Our cholestatic patients showed a mean hepatic tissue bile acid concentration of 430 $\pm 40$ $\mu$mol · $kg^{-1}$, which is in good agreement with Greim et al. (3). However, our noncholestatic patients had a much higher bile acid concentration in liver tissue than those reported by Greim and coworkers. It has to be emphasized that determinations of bile acids in liver tissue, especially in normals, are inevitably associated with uncertainties because of the risk of contamination with bile acids in bile canaliculi and ductuli. The highly significant correlation between hepatic and biliary bile acid concentrations ($r=0.87$) may thus be an expression of this possible contamination.

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