—Editorial—

Clinical Aspects of Bile Acid Metabolism in Liver Diseases

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For a long time, it has been assumed that the liver plays an important role in the formation and excretion of bile acids and that the metabolism of bile acids, therefore, can be disturbed to some extent in liver diseases. Pathophysiological aspects of the disturbed metabolism of bile acids remained obscure at the time when procedures of qualitative and quantitative analyses, identification and estimation of bile acids began to be utilized in practice 20 years ago. Since then, the metabolism of bile acids has been clarified in individuals under normal as well as pathological circumstances. Because of a functionally and morphologically close relation between hepatic epithelial cells and the metabolism of bile acids, the pathophysiological aspects of the disturbed metabolism of bile acids in parenchymatous inflammatory diseases of the liver, fatty liver, cholestasis and primary hepatocellular carcinoma are attracting considerable attention.

First applied to the measurement of human bile acids in blood plasma in 1965, gas-liquid chromatography has been utilized as a standard method in medical practice. Recently, radioimmunoassay and enzyme linked radioimmunoassay have been utilized. This progress in the estimation of bile acids has awoken our interest in the pathophysiological significance of bile acids in liver diseases.

The author reviewed the information on bile acid metabolism in liver diseases which has been reported up to the present.

(Key Words: Bile Acid, Cholic Acid, Chenodeoxycholic Acid, Deoxycholic Acid, Hepatitis, Liver Cirrhosis, Primary Hepatocellular Carcinoma, Cholestasis, Fatty Liver)

INTRODUCTION

For a long time, it has been assumed that the liver plays an important role in the formation and excretion of bile acids and that the metabolism of bile acids, therefore, can be disturbed to some extent in liver diseases. Pathophysiological aspects of the disturbed metabolism of bile acids remained obscure at the time when procedures of qualitative and quantitative analyses, identification and estimation of bile acids began to be utilized in practice 20 years ago. Since then, the metabolism of bile acids has been clarified in individuals under normal as well as pathological circumstances. Because of a functionally and morphologically close relation between hepatic epithelial cells and the metabolism of bile acids, the pathophysiological aspects of the disturbed metabolism of bile acids in parenchymatous inflammatory diseases of the liver, fatty liver, cholestasis and primary hepatocellular carcinoma are attracting considerable attention.

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OUTLINE OF BILE ACID METABOLISM

Bile acids are formed by the hepatic epithelial cells and belong to the main constituents of the bile which is excreted into the bile capillaries and leaves the liver through the intrahepatic bile duct system to reach the duodenum via the extrahepatic bile tract. Bile acids secreted in the intestine, usually during lipid absorption. Bile acids separate from the lipids after passage through the intestinal wall and enter the portal circulation. The absorbed bile acids are virtually all taken up by the liver and reexcreted in the bile. Although about 90 per cent of the bile acids found in the bile entering the biliary tree and duodenum is retained by means of the enterohepatic circulation as a circulating pool, small amounts are constantly lost and replaced by the synthesis of new bile acid (Figure 1). This loss is the result of three possible factors: renal excretion, destruction by the liver, and fecal excretion.

![Diagram of normal and cholestatic enterohepatic circulation]

Fig. 1 The enterohepatic circulation of bile acids in normal subjects and in cholestasis. From Sherlock, S. (46).

Virtually no bile acids normally pass into the urine, but they do so after experimental removal of the liver or damage to the liver and also in various hepatobiliary diseases. In biliary obstruction, urinary excretion takes the place of part of the fecal loss. Bile acid retention in general is the result of a disturbed balance between excretion and formation of bile acids. The liver plays an important role in the formation and excretion of bile acids. The metabolism of bile acids, therefore, can be disturbed by reduced formation caused by hepatic damage, reduced biliary excretion or decreased hepatic uptake of absorbed bile acids.

The normal bile acid pool is about 2,500 mg. The number of circulations per day probably varies with the amount of food eaten. Between the pool size and circulation frequency, an inverse relationship has been demonstrated, suggesting that more frequent passages in the liver lead to
increased feedback inhibition of bile acid synthesis and shrinkage of the pool, but another possibility is that small pools are absorbed more rapidly.

**CLINICAL ASPECTS OF BILE ACID METABOLISM IN LIVER DISEASES**

From the above mentioned facts, it is easily understandable that bile acid metabolism is affected when any of the organs or tissues involved in the enterohepatic circulation i.e., the liver, biliary tract, upper small intestine, ileum, colon and portal vein are diseased. In the following sections bile acid metabolism in liver diseases will be discussed.

(1) **Acute viral hepatitis**

Table 1 shows the mean value with standard deviations of total and individual bile acid concentrations in the serum of healthy subjects as a control group and in each group of patients with various types of liver diseases.

| Table 1 Serum bile acid levels in patients with liver diseases and in healthy subjects as a control group |
|-------------------------------------------------|--------|--------|--------|--------|--------|
| CDCA    | DCA   | CA     | LCA    | Total  |
| Healthy control group (n = 20)                  | 0.38 ± 0.13 | 0.31 ± 0.15 | 0.20 ± 0.08 | 0.67 ± 0.30 |
| Acute hepatitis (n = 25)                        |        |        |        |        |
| florid   | 30.11 ± 0.54 | 26.10 ± 11.31 | 2.68 ± 0.83 | 50.12 ± 15.01 |
| convalescent | 3.11 ± 0.43 | 0.73 ± 0.43 | 1.76 ± 0.22 | 5.12 ± 0.67 |
| Chronic persistent hepatitis (n = 30)           | 1.14 ± 0.36 | 0.15 ± 0.06 | 1.43 ± 0.66 | 2.51 ± 0.83 |
| Chronic aggressive hepatitis (n = 35)           | 3.74 ± 1.63 | 0.70 ± 0.48 | 1.91 ± 0.62 | 6.01 ± 2.51 |
| Liver cirrhosis (n = 50)                        | 3.56 ± 2.03 | 0.56 ± 0.44 | 2.43 ± 1.57 | 6.14 ± 3.78 |
| Fatty liver (n = 40)                            | 1.50 ± 0.63 | 0.78 ± 0.37 | 1.56 ± 0.38 | 3.86 ± 0.83 |
| Intrahepatic cholestasis (n = 12)               | 13.75 ± 4.1 | 1.58 ± 0.91 | 15.57 ± 2.09 | 8.01 ± 0.13 | 3.14 ± 3.48 |

From Iwamura, K. et al. (21)

Data: mean ± S.D. (µg/ml)

In the florid stage of viral hepatitis, the quantity of total bile acid (TBA) increased eighty to hundred times in comparison with concentrations in serum of healthy control subjects (21, 38) in which the quantity of chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA) also increased eighty times, whereas the cholic acid (CA) level rose thirteen times (21). It is suggested that hepatic production of CA was increased (34) and CA was secreted into the bile which caused a decrease in quantity (28, 30). Despite diminished amounts secreted into the bile, CA is reabsorbed almost completely in the duodenum. Therefore, CA is not taken up by the parenchymal cells in parenchymatous liver diseases and is apt to disappear more slowly than normal from the serum of jaundiced patients with acute hepatitis (48). In the cholestatic form of acute hepatitis, CA is definitely excreted in the urine if severe cholestasis persists (3). Consequently, the quantity of CA in the serum increased thirteen times in these patients when compared with healthy subjects.

The production of DCA excreted in the bile is also marked. This
results from the lack of substrate for bacterial dehydroxylation to DCA in the colon because of decreased production of CA in the liver and of complete reabsorption of CA in the duodenum (34). Nevertheless, the elevation of DCA in the serum of patients with acute hepatitis is remarkable when compared with healthy subjects. It seems to be very difficult to determine the reason for the elevation of deoxycholic acid in the plasma in acute hepatitis.

In the convalescent stage of acute viral hepatitis, actually estimated levels of total bile acid, CA, CDCA and DCA in serum increased, but more moderately than in the florid stage. At the same time, similar patterns of elevated individual bile acids were shown, even though inflammatory changes in the liver came to a standstill both blood-chemically and morphologically. This might be caused by the acuteness and duration of hepatic inflammatory changes.

Estimation of deviations in serum transaminase activity principally reflects ferment deviations due to injured liver parenchymal cells and the extent of elevated transaminase activity in the blood serum suggests liver cell injury in general. It is important that there is a statistically significant positive correlation between total bile acid in plasma and total bilirubin (1, 21). This means that the elevation of bile acids in serum is related in part to hepatic cell injury and in part to excretory disturbances. It is more striking that elevated bile acid levels in serum result in a standstill of hepatic inflammatory changes. This finding might be used as a diagnostic procedure. In the icteric stage of acute viral hepatitis and the cholestatic form of acute hepatitis and intrahepatic cholestasis, it is important that increased synthesis following decreased bile acid levels in the portal vein as well as disturbances in permeability and secretion of the liver cells play a part in increases of bile acids in the blood serum (11, 12).

In addition to these observations, endogenous bile acid tolerance tests by intramuscularly administered caerulein, an effective cholecystokinetikum, orally given egg yolk or fatty meal were performed in turn in patients with acute viral hepatitis in the convalescent stage (Figures 2 and 3). The results suggested that serum bile acid levels are more sensitive than serum bilirubin in evaluating the stability of convalescence in the convalescent stage (19). The fluctuation tendencies of total bile acid levels in blood plasma were similar among patients with acute viral hepatitis in the florid stage, although the real values were different. The maximum values occurred 50—60 minutes after administration of stimulants and the increases were 20, 30 and 100 per cent above the values before stimulation. It appeared that the total bile acid levels before stimulation increased according to the severity of the disease (19). On the basis of these observations, it can be said that the measurement of serum bile acid levels is almost certainly more sensitive, especially if postprandial samples are analysed.

Further, it has been reported that the onset of hepatitis B could be detected in children inoculated with hepatitis B virus by the development of a raised postprandial serum bile acid level, although they remained unicteric (17).
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Fig. 2  Endogenous bile acid tolerance test in healthy control group (n = 10).
From Iwamura (19)

Fig. 3  Endogenous bile acid tolerance test in patients with acute viral hepatitis (convalescence) (n = 15).
From Iwamura, K. (19)
Based on the results of kinetics of $^{14}$C-cholic acid in fulminant hepatic failure, it has been reported that the plasma disappearance of $^{14}$C-cholic acid was more prolonged in patients who died than in those who survived. This might form the basis of a prognostic test (18).

Further, the possibility that the ratio of glycine to taurine might be of diagnostic significance has been investigated. In healthy subjects, cholic acid and chenodeoxycholic acid are conjugated in the liver with glycine or taurine through a peptide bond in a ratio of about three to one. In parenchymatous liver diseases, the ratio is decreased in general, although the extent of the decrease is more or less dominant in acute hepatitis than in other hepatic disorders. Therefore, it cannot be utilized significantly as a differential diagnostic tool. It must be also considered whether the ratio of cholic acid to chenodeoxycholic acid is of diagnostic significance. Since cholic acid possesses more hydroxyl groups than chenodeoxycholic acid, the decrease of the ratio of cholic acid to chenodeoxycholic acid can be assumed in severe liver injuries. The results estimated by gas-liquid chromatography using Sandberg's method showed an apparent decrease of this ratio in the florid stage of acute hepatitis (21). The diagnostic significance of the ratio, however, must be evaluated on the basis of total bile acid level and individual bile acid levels in serum measured by radio-immunoassay and the enzymatic method (Figure 4).

![Graph showing the ratio of cholyglycine to total bile acid level in serum](image)

**Fig. 4** The ratio of cholyglycine to total bile acid level in serum.
(From Wakushima, T. et al.: Radioisotopes 28: 437, 1979.)
The fact that the plasma disappearance of intravenously injected $^{14}$C-cholic acid was prolonged in viral hepatitis in comparison with normal subjects does not reflect the reduced formation of cholic acid, but shows the decreased hepatic uptake of cholic acid or reduced hepatic excretion of cholic acid (Figure 5). Detailed analyses of the results concerning the plasma disappearance of intravenously injected $^{14}$C-cholic acid have been carried out successively and the pathophysiological aspects involved have been clarified as follows: the metabolism of cholic acid is disturbed by decreased hepatic uptake, reduced biliary excretion or the augmented hepatic back-flow rate of cholic acid (26).

In summary, the measurement of serum bile acids is recommended for detecting minimal liver cell damage, such as in viral hepatitis, especially in the initial and convalescent stage, although increases in both hepatocellular and cholestatic jaundice limit its diagnostic effectiveness; and the estimation of serum bile acid with a definite lapse of time after a meal, i.e. the postprandial bile acid test, possesses diagnostic significance (5, 19, 23).

In patients with hepatocellular failure, the ratio of serum cholic acid to chenodexoxycholic acid is usually low, the main bile acid being chenodexoxycholic acid (39). The assessment of the plasma disappearance of intravenously injected isotopic bile acid reveals abnormal hepatocellular function (Figure 6) (49), but there is much overlapping between hepatocellular and cholestatic jaundice and the test is not available in practice. It seems unlikely, however, that this estimation will replace simpler and less costly biochemical tests of liver function, at least immediately.

Schema einer Plasmaschwundkurve nach i.v. Injektion von 24-$^{14}$C-Cholsäure und des daraus abgeleiteten Stoffwechselmodells mit den Verteilungsraumen Blut, Leber und Gallengangssystem.

Fig. 5  Diagram of plasma disappearance of intravenously injected 24-$^{14}$C-cholic acid and its metabolic pathway (26).
From Klapdor, R. (26).
(2) Chronic hepatitis

Total and individual bile acid levels in serum in patients with chronic aggressive hepatitis and liver cirrhosis showed fluctuation tendencies similar to those of concentrations in the serum of patients in the convalescent stage of acute viral hepatitis (Table 1). In patients with chronic persistent hepatitis and fatty liver (Figure 7), the quantity of TBA increased four times compared with the concentrations in the serum of healthy subjects in the control group, although the concentrations of individual bile acids in serum did not always show the same fluctuations in the two groups. In particular, DCA levels in the serum of patients with chronic persistent hepatitis were apparently less than those in patients with fatty liver. DCA levels in the serum of patients with chronic persistent hepatitis were less than those in healthy subjects in the control group (21). In any case, attention has been focussed on deoxycholic acid levels in the serum of patients with chronic persistent hepatitis to which several factors seem to contribute as stated above, while total and individual bile acid levels in the serum increased in patients with chronic aggressive hepatitis and liver cirrhosis (6, 20, 39, 40). In patients with chronic aggressive hepatitis, total bile acid levels in serum increased. In chronic hepatitis, a positive correlation between total bile acid levels in serum and the bromsulphalein (BSP) retention rate 45 minutes after intra-
venous injection was confirmed (Figure 8) and it was statistically significant \( r=0.742, \ p<0.001 \) (21). BSP is based mainly on the effective circulating blood volume and the amount of binding protein for transport in blood serum. In chronic liver diseases, the effective circulating blood volume and the amount of binding protein for transportation tend to diminish, so that the correlation between the elevation of bile acid levels in serum in chronic inflammatory liver disease and excessive synthesis due to diminished bile acid concentration in the portal vein blood must be considered.

Icterus appears if the bilirubin elimination from the serum is disturbed. However, there were no patients with chronic hepatitis in which total bilirubin levels in serum increased in quantity during clinical observations. Nevertheless, the correlation between TBA and total bilirubin levels in serum was investigated but no statistically significant correlation could be found (19). At the present time, the two-hour postprandial serum bile acid concentration seems to be the most sensitive liver function test for chronic hepatitis (Figure 9) (5, 10, 25, 49).

\begin{center}
\begin{tabular}{|c|c|c|c|}
\hline
Tissue & Cells \\
\hline
florid convalescence & chronic persistent hepatitis & chronic aggressive hepatitis & compensated decompensated \\
\hline
acute hepatitis & n=25 & n=30 & n=35 \\
\hline
liver cirrhosis & n=42 & n=50 \\
\hline
fatty liver & n=40 \\
\hline
intrahepatic cholestasis & n=12 \\
\hline
\end{tabular}
\end{center}

Fig. 7 Total bile acid levels in plasma in patients with liver diseases.
Form Iwamura, K. et al. (21)
Fig. 8  Correlation between total bile acid levels in plasma and BSP (45') retention rate in patients with chronic hepatitis (n = 60). From Iwamura, K. et al. (21)

Fig. 9  Two hour postprandial total plasma bile acid concentrations. From Thjodleifsson, B. et al. (49)
Further, in a series of serum bile acid levels after administration of fatty meal, egg yolk or caerulein respectively to patients with chronic persistent hepatitis, total bile acid levels began to increase 10 minutes after administration of the stimulants, reached maximum values after 50—60 minutes, and returned to approximately the same values as those before stimulation of the gallbladder after 240 minutes (Figure 10). The maximum value was double or triple that before stimulation of the gallbladder. There were no differences among total serum bile acid levels due to the three different stimulants.

![Graph showing bile acid tolerance test](image)

**Fig. 10** Endogenous bile acid tolerance test in patients with chronic persistent hepatitis (n = 10)
From Iwamura, K. (19)

In patients with chronic aggressive hepatitis, the values of total serum bile acid began to increase 10 minutes after administration of the stimulants, reached a maximum after 60 minutes and returned to approximately the same value as that before administration of the stimulants 240 minutes or longer after stimulation of the gallbladder (Figure 11). The maximum values were doubled. There were no differences among the fluctuation tendencies of total bile acid levels due to the three different stimulants. In patients with chronic aggressive hepatitis and liver cirrhosis, total bile acid levels in serum showed fluctuation tendencies similar to those of serum concentrations in patients in the convalescent stage of acute viral hepatitis in which the patients no longer had any complaints and the inflammatory changes of the liver came to a standstill with respect to both blood chemistry and histology. In patients with chronic persistent hepatitis and
fatty liver, the quantity of TBA increased four-fold compared with the concentrations in the serum of healthy subjects in the control group. The range of fluctuations of total bile acid concentrations in each type of liver disease showed no relation to age and sex of the patients, but they might be related to the condition of the liver diseases.

![Graph showing endogenous bile acid tolerance test](image)

**Fig. 11** Endogenous bile acid tolerance test in patients with chronic aggressive hepatitis (n = 15).
From Iwamura, K. (19)

A series of total bile acid levels in serum can be determined under such liver disease conditions, especially with respect to liver cell function (20). If stimulants are given to healthy subjects and patients with several types of liver disease in whom the normal contractibility can be confirmed prior to contraction of the gallbladder, bile mainly in the gallbladder and also some intrahepatic bile can be excreted via the choledochus. The bile acid in the excreted bile passes through the intestinal wall and enters the portal circulation. The absorbed bile acids are virtually all taken up by the liver and re-excreted in the bile. Contraction of the gallbladder loads the liver cells with endogenous bile acids. Therefore, this procedure is regarded as a tool for evaluation of the liver and serves as an endogenous tolerance test of liver disease (1, 9, 31). In each group of liver diseases, series of TBA in blood serum at definite time intervals before and after administration of stimulants showed similar fluctuation tendencies of TBA in blood serum, but the extent and duration were different in accordance with the condition of the liver disease.

Several authors pointed out that endogenous bile acid tolerance tests
can be utilized as a differential diagnostic tool (1, 9, 20) between chronic hepatitis and liver cirrhosis. However, series of TBA levels in serum at definite time intervals before and after administration of the stimulants in patients with chronic aggressive hepatitis and liver cirrhosis are rather difficult because of overlapping in the differentiation of both groups of liver disease.

(3) Liver cirrhosis

Not only in acute and chronic hepatitis but also in liver cirrhosis, synthesis, secretion and uptake of the bile acids are all altered (53). In patients with liver cirrhosis, total and individual bile acid levels in serum increased (6, 7, 21, 39, 40). It is also well known that substantial amounts of bile acids undergo portal systemic shunting after reabsorption and enter the systemic circulation without passing through the liver cirrhosis. The decreased bile acid pool in patients with liver cirrhosis seems to be due mostly to reduced cholic acid synthesis (34, 51). Blocking of cholic acid synthesis may therefore be related to the lack of deoxycholic acid in the bile (50) and in the blood serum (52), which may be further aggravated by decreased enterohepatic circulation of cholic acid due to cholestasis. According to the author's own observation, however, the deoxycholic acid level in serum does not always decrease (21), although recent observations in cirrhotic patients support this concept of multifactorial decrease of deoxycholic acid levels (35). The mechanism of low deoxycholic acid levels has been obscure up to the present although dehydroxylating activity due to fecal bacteria had also been discussed (27). In a large group of cirrhotics, cholic acid synthesis, measured isotopically, was reduced to less than a third of normal levels (53). In any case, there was a statistically significant correlation between cholic acid synthesis and the severity of the disease in patients with liver cirrhosis, which could be assessed by clinical and laboratory parameters including liver biopsy. Cholic acid synthesis was markedly reduced even in patients with mild liver cirrhosis (54). These findings suggest that cholic acid synthesis is selectively impaired in cirrhosis, which may be due to a specific failure of 12 alpha-hydroxylation. An alternative explanation is that cholic acid synthesis is easily suppressed because there is only one major pathway from cholesterol to cholic acid, whereas chenodeoxycholic acid can be formed by at least two pathways. Chenodeoxycholic acid synthesis is normal or virtually normal even in severe liver disease (55). Although the synthesis rate is further reduced as the disease advanced, the greatest decrease is seen during the earlier phase of the disease (13). This means that significant alterations in cholic acid synthesis must occur before the onset of clinical symptoms. Subsequently, the bile acid pool is reduced to about half its normal size because of shrinkage of the cholic acid pool in patients with liver cirrhosis. In addition to reduction of the cholic acid pool, the pools of the secondary bile acids, deoxycholic and lithocholic, are dramatically reduced. The total bile acid pool is 3—5g and the average daily production of cholic acid about 330mg and chenodeoxycholic acid 162mg.

In the decompensated and compensated stages of liver cirrhosis,
positive correlations between total bile acid levels in serum and BSP in patients with liver cirrhosis were confirmed in both cases and they were statistically significant (Figure 12). The correlation in the decompensated stage of liver cirrhosis was even more statistically significant \((r = 0.674, p < 0.01)\) than that in the compensated stage \((r = 0.565, p < 0.01)\). In the decompensated and compensated stages of the cirrhosis, positive correlations between total bile acid levels in serum and total bilirubin levels in serum were recognized and they were statistically significant at 0.67 and \(P < 0.001\) in the former group and at \(r = 0.64\) and \(p < 0.001\) in the latter group (Figure 13) (21).

The values of series of total bile acid levels in blood serum at definite time intervals before and after administration of three different stimulants in patients with liver cirrhosis began to increase 10 minutes after administration of stimulants, reached a maximum after 40–50 minutes and returned to approximately the same value as that before stimulation of the gallbladder after about 240 minutes (Figure 14). The maximum value was triple the value before stimulation of the gallbladder. There were no differences among series of serum total bile acid levels at definite time intervals before and after administration of the stimulants (19). As mentioned above, several authors pointed out that endogenous bile acid tolerance tests can be utilized as a differential diagnostic tool (1, 9, 20) between chronic hepatitis and liver cirrhosis. However, it is said that individual bile acids in serum have been studied many times in the hope of discovering a pattern diagnostic of cirrhosis but there is no consistent picture.

![Graph](image-url)
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Fig. 13 Correlation between total bile acid levels in plasma and total bilirubin levels in serum in liver cirrhosis (n = 50).
From Iwamura, K. et al. (21)

Fig. 14 Endogenous bile acid tolerance test in patients with liver cirrhosis (n = 20).
From Iwamura, K. (19)
In patients with liver cirrhosis, shunting of blood round the liver and hepatocellular dysfunction cause impaired uptake of bile acids from the blood, which appears as an augmented serum bile acid concentration. This is observed in the fasting state as well as two hours after a meal if there is histologically significant liver disease (19, 25, 49). As stated above, at the present time, the two-hour postprandial serum bile acid concentration seems to be the most sensitive liver function test. Although the findings, using radioimmunoassay to follow the clearance of cholyglycine, raised hopes that a simple, sensitive and specific test of liver function would be available (29), subsequent studies have shown no correlation between clearance rates and the severity of liver disease (49). In patients with liver cirrhosis, the small bile acid pool and the abundant flow of dilute bile are characteristics which suggest a hepatic block in enterohepatic circulation. In addition, in patients with liver cirrhosis, urinary excretion of bile acids is increased and to a great extent preceeded by sulphation, which results in sulphated bile acids in a proportion of 100 per cent for lithocholate, 85 per cent for deoxycholate, 76 per cent for chenodeoxycholate and 24 per cent for cholate. Sulphated bile acids have a far greater renal clearance because of their polar nature than non-sulphated ones.

(4) Cholestasis

In patients with intrahepatic cholestasis, increased synthesis following decreased bile acid levels in the portal vein as well as disturbances in permeability and secretion of the liver cells play a part in increases of bile acid in serum (11, 12, 39). As mentioned above, bile acids are produced by the hepatic epithelial cells and it can be easily speculated that the formation of bile acids can be disturbed in cases of hepatic damage and that total bile acid levels in serum are consequently reduced. The blood bile acid levels is, however, usually higher than normal and it does not fall as the disease progresses. Certainly, the activity of rate-limiting enzyme for synthesis, 7 alpha-hydroxylase, is remarkably inhibited (41), which is caused presumably by intense feedback inhibition from the high serum levels of bile acids. In any case, it can be assumed in practice that reduced formation of bile acids is probably outweighed by their reduced excretion and decreased hepatic uptake. There are some clinical and experimental observations to show that bile acids are not taken up by parenchymal cells in hepatic cell degeneration (24). In obstructive jaundice, in contrast to inflammatory or toxic liver disease, permeability disturbances and secretion disturbances of the parenchymal cells as well as excessive synthesis due to diminished bile acid concentration in the portal vein blood might be a reason for the elevation of bile acid in the serum. In complete biliary obstruction, excretion is virtually limited to the urine, and urinary excretion increases markedly. Fecal excretion decreased to 33 μmol per 24 hours in six obstructed patients studied by Makino et al. (33), in contrast to the normal turnover of bile acids of 600 to 1,000 μmol per 24 hours.

Cholestasis is bile flow stagnation due to a failure of biliary secretion of the liver cells with a concomitant accumulation in the blood of constituents normally excreted in the bile (46). In extrahepatic cholestasis,
blocking of the bile flow into the duodenum is primarily caused by common bile duct stones and cancer of the biliary tract or of the pancreas. In both cases, total and basic bile acid levels increase in the serum. In one series they ranged from 15 to 285 μ mol per liter, which is four to 60 times the normal level (38). For a long time, it has been discussed whether analyses of the relative amounts of the individual bile acids in the blood of patients with hepatobiliary disease would be clinically helpful in distinguishing parenchymal liver disease from biliary obstruction (32, 38), but these analyses have been of little clinical value. In these studies, it is of interest that a marked reduction of deoxycholic acid concentrations in serum is observed in cases of cholestasis, which is caused presumably by lack of contact between the cholic acid pool and intestinal bacteria. When hepatic cells are minimally injured, the ratio of cholic acid to chenodeoxycholic acid is elevated, whereas this ratio tends to fall if cholestasis is prolonged. In general, depending on the severity of the disease, patients with intrahepatic cholestasis show the same bile acid pattern as patients with extrahepatic biliary obstruction and no information for differential diagnosis can be obtained even though there is a difference in the quantity of lithocholic acid in the serum. In intrahepatic cholestasis, lithocholic acid appeared in the serum at a concentration of 0.8 μg per milliliter, while in extrahepatic cholestasis, it appeared at 0.4 μg per milliliter in the serum. This also indicates that changes in the bile acid pattern in cholestasis are a consequence, rather than a cause, of the obstruction. In any case, a close correlation can be recognized concerning the extent of cholestasis, and the cholic acid to chenodeoxycholic acid ratio indicates a bad prognosis (7, 22).

In cholestasis, aberrant bile acids, such as sulphates, glucuronides, an unsaturated monohydroxy bile acid, ursodeoxycholic acid, etc., are produced. In these bile acids, many substances remain to be identified. Sulphates, glucuronides and ursodeoxycholic acid are not only less hepatotoxic but more efficiently excreted in the cholestatic urine (4, 8).

For a long time, it has been assumed that pruritus in cholestasis is caused by the deposition of bile acids in the skin. Of course, it is certain that serum bile acid levels are elevated in the serum but the correlation between pruritus and elevated serum bile acid levels is poor. It has been reported, however, that bile acid concentrations on the skin are consistently increased, and that skin bile acids return to normal with recovery of the disease and pruritus also disappears (45). The most effective treatment for pruritus is cholestyramine, a non-absorbable resin which sequestrates bile acids in the intestine and promotes their excretion. It presumably acts by lowering first serum then skin bile acid concentrations. Phenobarbital is also effective in intrahepatic cholestasis, relieving pruritus and reducing serum bile acids (47). Concerning the mode of action of this medicine, two possibilities are generally assumed: stimulation of the bile salt-independent fraction of bile flow and induction of microsomal glucuronidating enzymes.

In patients with intrahepatic cholestasis, a positive correlation between total bile acid in plasma and total bilirubin levels was observed and it was statistically significant (r = 0.646, p < 0.01) (Figure 15). Disturbances in
bilirubin elimination from the blood serum bring about jaundice, and increased bile salts in plasma are accompanied by obstructive jaundice. Bilirubin elimination is disturbed during the course of glucuronization, transportation to bile capillaries and secretion into bile capillaries. Increased synthesis following decreased bile acid levels in the portal vein as well as disturbances in the permeability and secretion of the liver cells play a part in bile acid increases in the plasma of jaundice patients.

In patients with primary biliary cirrhosis, monohydroxy bile acid in a form of peptide are mostly detectable in the serum and monohydroxy bile acid concentrations in the serum account for more than 30 per cent of total bile acid levels in the serum if they have an elevated total bile acid concentration in the serum (37).

![Graph](image)

**Fig. 15** Correlation between total bile acid levels in plasma and total bilirubin levels in serum of patients with intrahepatic cholestasis (n = 12)
From Iwamura, K. et al. (21)

(5) **Fatty liver**

In patients with fatty liver and chronic persistent hepatitis, the total bile acid quantity increases four times compared with the concentrations in the serum of healthy subjects in the control group, although individual bile acid concentrations in serum did not always show the same fluctuations in both groups. In particular, deoxycholic acid levels in serum of patients with chronic persistent hepatitis were apparently less than those of patients with fatty liver. Deoxycholic acid levels only in serum of patients with chronic persistent hepatitis were not less than those of healthy subjects in the control group. The reduction of deoxycholic acid excreted in bile is marked. This results from the lack of substrate for bacterial dehydroxylation to deoxycholic acid in the colon because of
Decreased production of cholic acid in the liver and of complete reabsorption of cholic acid in the duodenum (34). Deoxycholic acid in serum actually decreased in quantity in patients with chronic persistent hepatitis and this finding seems to be theoretically reasonable. This is the pertinent finding for the differential diagnosis of fatty liver from chronic persistent hepatitis. In addition, correlations between the extent of fatty metamorphosis and serum bile acid level were statistically significant in comparison with moderate and severe fatty metamorphosis (p < 0.001).

Figure 16 shows graphs of the series of total serum bile acid levels for three different stimulants. The values of total serum bile acid began to increase 10 minutes after administration of the stimulants, reached a maximum after 60 minutes and returned to approximately the same value as that before administration of the stimulants 240 minutes or longer after stimulation of the gallbladder. The maximum value was double. There were no differences among the fluctuation tendencies of total bile acid levels due to the three different stimulants (19).

![Figure 16](image)

**Fig. 16** Endogenous bile acid tolerance test in patients with fatty liver (n = 15)
From Iwamura, K. (19)

(6) **Primary hepatocellular carcinoma**

In patients with primary hepatocellular carcinoma, raised cholesterol level in serum can be detected. Actually, hypercholesterolemia without jaundice has sometimes been reported in patients with primary hepat-
cellular carcinoma in Uganda (2). In such cases, the increased cholesterol level in serum is caused by a disturbance of bile flow. In fact, a statistically significant correlation between cholesterol level and alkaline phosphatase activity in serum was recognized (15). In general, cholesterol concentrations in the hepatic epithelial cells is increased so that cholesterol synthesis is decreased because of inhibition of beta-hydroxy-beta-methylglutary (HMG)-CoA reductase activity in cholestasis. Therefore, it can be considered that hypercholesterolemia in cholestasis is caused by back flow of bile into the circulating blood.

In experimental hepatocellular carcinoma in animals, cholesterol synthesis is not inhibited because of lack of an inhibitory process in hepatic cancer cells (43), which cause hypercholesterolemia due to augmented cholesterol synthesis in the hepatic cancer cells. These observations showed that there may be a possibility of hypercholesterolemia due to augmented cholesterol synthesis in human hepatocellular carcinoma cells.

In experimental hepatic cancer, cholesterol-7-alpha-hydroxylase activity persists in the liver and it is not regulated by cholesterol and bile acid concentration in hepatic cells. Synthesis of cholesterol and bile acid in hepatic cancer cells are not subject to feedback control. Therefore, the cholesterol pool and bile acid pool are increased in experimental hepatic cancer (36), whereas the metabolic pool is decreased in cholestasis.

In patients with hepatocellular carcinoma, serum bile acid concentrations are increased and they predominate over those in liver cirrhosis (49, 52). This observation suggests a disturbance of bile flow in the liver because of compression of tumors on intrahepatic biliary tracts. In practice, cholesterol and bile acid concentrations in serum are increased in cholestasis, whereas cholesterol and bile acid concentrations are increased even though there is no increase in the bilirubin level in serum of patients with hepatoma. In addition, there is no definite correlation between serum bile acid level and serum cholesterol concentration in chronic liver diseases, especially in liver cirrhosis (14). Therefore, a different, indefinite metabolic pathway of cholesterol synthesis may be assumed in primary hepatocellular carcinoma but it has not been detected at present. There are also some observations indicating an indefinite metabolic pathway of cholesterol synthesis in patients with primary hepatocellular carcinoma which causes an increase in bile acid concentration in serum. In hepatocellular carcinoma cells, increased cholesterol synthesis caused an elevation of serum lipoprotein, especially high density lipoprotein (HDL) cholesterol, which is presumably a substrate of bile acid synthesis (44), and then HDL cholesterol concentration in serum brought about bile acid synthesis. Decreases in HDL as well as increases in low density lipoprotein (LDL) are recognized in cholestasis, while the normal pattern of HDL and LDL are observed in primary hepatocellular carcinoma (42).

Serum squalene level reflects cholesterol synthesis in the liver and it is decreased in cholestasis, while it is increased in primary hepatocellular carcinoma (15, 16). Alpha-fetoprotein is produced in hepatic epithelial cells and in most patients with primary hepatocellular carcinoma, it is increased. It has been observed that there is a correlation between elevation
of alpha-fetoprotein concentration in serum and augmentation of squalene, cholesterol and bile acid (Table 2). On the basis of this information, it can be assumed that in primary hepatocellular carcinoma cells, there is an indefinite cholesterol synthesizing process which causes raised cholesterol synthesis, increases in the cholesterol metabolic pool and bile acid synthesis in succession.

Table 2  Relationship of serum alpha-fetoprotein to serum cholesterol, squalene and bile acids in primary hepatocellular carcinoma

<table>
<thead>
<tr>
<th>AFP (mg/dl)</th>
<th>squalene (ug/dl)</th>
<th>cholesterol (mg/dl)</th>
<th>bile acids (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.9</td>
<td>109 ± 36</td>
<td>161 ± 24</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>1.0-9.9</td>
<td>120 ± 28</td>
<td>174 ± 19</td>
<td>53 ± 18</td>
</tr>
<tr>
<td>10.0</td>
<td>140 ± 38</td>
<td>242 ± 53</td>
<td>76 ± 22</td>
</tr>
</tbody>
</table>

Each AFP range was significantly correlated with corresponding means of cholesterol (p < 0.01), squalene (p < 0.05) and bile acids (p < 0.05)

CONCLUSION

In hepatobiliary diseases, the quantity of total and individual bile acids increases in the serum. This elevation, however, has complex causes such as excessive synthesis due to diminished bile acid concentration in the portal vein blood, permeability disturbances and secretion disturbances in the hepatic parenchymal cells. An indefinite cholesterol synthesizing process must also be assumed in primary hepatocellular carcinoma. In any case, elevated total and individual bile acid levels in the serum remain, resulting in a blood chemical and morphological standstill in hepatic inflammatory changes. This means that the metabolism of bile acids may reflect the pathophysiological condition of liver diseases more sensitively than the routinely applied diagnostic parameters. In this way, bile acid levels in serum can be utilized as a diagnostic procedure in liver diseases.

REFERENCES


33) Makino I, Hashimoto H, Shinozaki K, Yoshino K and Nakagawa S: Sulfated and non-