

# Metabolism and Diagnostic Value of Bile Acids - A Short Review

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## Introduction

Bile salts play an important role in the absorption of fats from the intestine. They solubilize the products of lipolysis in a micellar solution.

Cholesterol is insoluble in water but is brought into aqueous solution in bile by molecular association with bile salts and phospholipid lecithin. The water-soluble bile salts are naturally occurring detergents and can break up the lecithin cholesterol crystals into small aggregates called mixed micelles. The large quantities of cholesterol present in human bile are solubilized in the water-soluble mixed micelles, allowing cholesterol to be transported harmlessly in bile via the biliary tract into the intestine. However, mixed micelles of bile salts, lecithin, and cholesterol, have a limited capacity to solubilize cholesterol. Therefore the solubility of cholesterol in bile depends on the relative proportion of these three components. When maximum quantity of cholesterol is dissolved in bile, above which its crystallization occurs, the bile is said to be at critical micellar concentration (CMC). If any change occurs in bile i.e. either increase in the quantity of cholesterol or decrease in the bile salt-phospholipid ratio to cholesterol, it may ultimately result in cholesterol stone formation in the gall bladder, under different pathophysiological conditions.

Bile acids are formed in the liver and undergo enterohepatic circulation (EHC), and since have multiple functions including (a) dispersion and transportation of their precursor-cholesterol, (b) fat absorption, (c) regulation of their own synthesis from cholesterol in hepatocyte and (d) suppression hepatic cholesterol synthesis; they are significantly involved in many hepatic, biliary, and intestinal diseases. This review places into perspective the understanding of bile acid metabolism in normal subjects and patients with various hepatic, biliary, and intestinal diseases and points out their significant diagnostic value.

## Enterohepatic Circulation

The enterohepatic circulation (EHC) involves the liver and biliary tract, the intestinal tract and the plasma compartment. However, the amount and concentration of bile acids in the various anatomical spaces varies strikingly. During fasting in man and other animals, most of the bile acid pool is present in the gall bladder. There is probably continuous basal secretion of bile acids, so that some bile acids are always entering the intestinal lumen (Norfield and Hofmann, 1975). Some of these bile acids are reabsorbed from the intestine so that measurable concentrations of bile acids are present in the portal blood, peripheral blood and, presumably, the liver cell during fasting states. When a fatty meal is eaten the gall bladder contracts and most of the bile acid pool is secreted into the small intestinal lumen. The amount of bile acids, absorbed from the intestine, increases and concentration of bile acids rises in the portal blood as bile acids return to the liver. The majority of bile acids are extracted by the liver, so that only a small fraction of the absorbed bile acids spills over into the peripheral blood. The bile acids entering the liver cell are rapidly excreted into bile and, depending on degree of spincter, concentration and bile acids excreted by liver, will either pass into the intestine or be stored in the gall bladder. In health the quantity of bile acids present in portal blood, the hepatocyte and peripheral plasma compartment is extremely low; the bulk of the bile acids in the EHC is in the gall bladder and the intestinal lumen.

## Synthesis and Normal Metabolism

The bile acids participating in EHC are primary bile acids, formed in the liver, secondary bile acids,

formed by the action of intestinal bacteria and tertiary bile acids, formed from the secondary bile acids in the liver.

In man, two primary bile acids are formed in the liver; cholic acid, a trihydroxy acid and chenodeoxycholic acid, a dihydroxy acid, sometimes termed as chenichic acid. The synthesis rate of cholic acid is about twice that of the chenichic acid. After formation, the bile acids are conjugated with glycine or taurine as N-acyl conjugates. Conjugation is believed to be complete, so that all the bile acids, leaving the hepatocyte, are conjugated.

The cholyl and chenyl conjugated bile acids are excreted in bile, and most are reabsorbed without bacterial alteration. About one fourth of primary bile acid conjugates are deconjugated by bacterial enzyme in the ileum, liberating an unconjugated steroid moiety. The majority of these bile acids, are reabsorbed and reach the liver where they are re-conjugated. Thus for primary bile acids there is synthesis and conjugation in the liver followed by partial deconjugation in the intestine, with complete re-conjugation in the liver (Hepner et al., 1972). Each day one-third to one-fourth of the primary bile acid pool is lost or converted by anaerobic bacteria to secondary bile acids. The most important change is 7 $\alpha$ -dehydroxylation, which converts cholic acid to deoxycholic acid, and chenichic acid to lithocholic acid. The fate of these two secondary bile acids differs. About one-third to one-half of deoxycholic acid, formed, is reabsorbed and undergo the same process of conjugation in the liver, excretion into the bile, deconjugation in the intestine, and reabsorption and re-conjugation in the liver, in the same way as the primary chenichic acid.

Lithocholic acid is insoluble at body temperature, and probably adsorbs strongly to colonic bacteria. The majority of the lithocholic acid conjugates are sulphated in the liver (Cowen et al., 1975). As for example, if 100 lithocholate molecules reach the liver, about 50 will be excreted as sulpholithocholyl glycine, 25 as lithocholyl glycine, 5 as sulpholitho-cholyl taurine, and 20 as lithocholyl taurine. Thus lithocholyl glycine is sulphated to greater extent than lithocholyl taurine (Allan et al., 1976). The fate of lithocholate conjugates in individual species has not yet been well defined. Probably the unsulphated lithocholyl glycine and lithocholyl taurine are reabsorbed from the small intestine and return to the liver where they are sulphated. The sulphated lithocholates are poorly absorbed from the small intestine and pass into the colon. There these are desulphated and conjugated to some extent, with the lithocholate moiety again being reabsorbed (Hofmann, 1977).

The fourth, most abundant, bile acid in man, ursodeoxycholic acid, has recently been noted repeatedly in the samples of human bile. Its occurrence was in quite high proportion in some individuals ingesting chenichic acid for the dissolution of gall-stones (Salen et al., 1974).

The bacterial dehydrogenation of chenichic acid or its conjugates or both results in the formation of 3-hydroxy-7-keto cholanoic acid (keto lithocholic acid) in the small intestine. When this reaches the liver, it is reduced to ursodeoxycholic acid as defined by Salen et al. (1974). These two bile acids are conjugated and secreted into the intestine, where they may be dehydrogenated to 7-keto derivatives. This again may be absorbed and once again reduced to ursodeoxycholic acid and chenichic acid during hepatic passage. The chenichic acid undergoes reversible oxidation and reduction at 7-position, so that chenichic acid and ursodeoxycholic acid are both precursors and products of each other.

At present the physiological significance of other secondary and tertiary bile acids, which occurs in bile only in traces, is dubious, and their presence merely indicates uncommon bacterial biotransformation.

#### **Altered Metabolism in Various Diseases**

In patients with liver disease all phases of bile acid metabolism are probably altered. The EHC is altered by shunting of bile acids away from the liver in patients with acute and chronic liver diseases. This results in appearance of significant amounts of bile acids in the systemic circulation and ascitic fluid. The patients with cirrhosis also lose a significant amount of bile acids via urine, in addition to fecal loss. By contrast, in pure cholestatic syndrome there is a significant increase in plasma due to interference with hepatic bile excretion (outflow block) and subsequent regurgitation of bile acids are no longer confined solely to EHC, but are also present in plasma. Consequently, there is a higher

urinary excretion of bile acids and their deposition in the skin and other tissues (Schoenfield and Sjøvall, 1967).

Data from studies (Vlahcevic et al., 1971; Vlahcevic et al., 1973) has demonstrated clearly that bile acid metabolism in patients with cirrhosis (with and without ascites) is markedly different from that of normal subjects. The decreased synthesis of cholic acid in comparison to chenodeoxycholic acid was found as most remarkable observation in cirrhosis, while in health the synthesis of cholic acid is double that of chenodeoxycholic acid. Recent reports have shown that patients with cholesterol gallstones have a diminished bile acid pool (Hofmann and Hofmann, 1974). The reduction in bile acid pool, coupled with increased cholesterol secretion, are probably the major factors contributing to the formation of the lithogenic bile which in turn may be the initiating factor in development of cholesterol gallstones in man. If so, then patients with cirrhosis and markedly diminished bile acid pool size should also have lithogenic bile and a higher incidence of cholesterol cholelithiasis. At least two studies have reported that the incidence of gallstones in patients with cirrhosis is three times that of the normal subject (Bouchier, 1969; Nicholas et al., 1972), but the majority of the stones were, however, pigmented (calcium bilirubinate) rather than cholesterol stones. The investigations by Vlahcevic et al. (1973) have shown that in addition to a decrease in bile acid secretion in cirrhosis there was also a marked reduction in biliary cholesterol secretion. These findings, therefore, suggest that patients with cirrhosis and diminished bile acid pool are protected from an increased incidence of cholesterol gallstones because of marked simultaneous defect of cholesterol metabolism.

Theodor et al. (1968) had demonstrated that cholic acid pool size and cholic acid synthesis were decreased in acute hepatitis. All those patients had a reduced cholic acid conversion to deoxycholic acid. These observations in conjunction with the high concentration of bile acids in plasma suggests that the bile salts in the enterohepatic circuit are reduced. Bile acid conjugation in hepatitis is apparently deficient, with the defect being most marked early in the course of disease (Ekdahl, 1958). A marked decrease in biliary bile acid concentrations was observed in several patients (Vlahcevic et al., 1973), suggestive of diminished bile acid secretion. It has also been reported that early in the course of hepatitis, the bile of these patients became transiently supersaturated with cholesterol (Begemann and Herget, 1974). Recently Makino et al. (1975) observed that the mean serum and urinary bile acid levels were higher in hepatitis than in patients with other forms of liver disease chronic hepatitis, cirrhosis, obstruction etc).

In chronic hepatitis the total serum and urinary bile acid levels are not significantly different from those observed in cirrhosis (Makino et al., 1975).

Decreased synthesis and biliary excretion of cholate leads to decreased formation of deoxycholate in the intestine in patients with cholestasis due to cirrhosis of the liver or biliary atresia (Vlahcevic et al., 1973). In hepatocellular injury cholate synthesis is more reduced than chenodeoxycholate synthesis (Carey et al., 1969; Vlahcevic et al., 1973), which may further diminish the already decreased deoxycholate synthesis.

In patients with extra-hepatic biliary obstruction the synthesis rate of bile acids were diminished and the pools were restricted to the liver, peripheral blood and tissue such as skin (Norman and Strandvik, 1971; Strandvik, 1973). In liver diseases with intrahepatic cholestasis, the portion of glycine conjugates may decrease (Sjøvall, 1960; Kuroda and Okuda, 1974). The normal glycine: taurine conjugated bile acid ratio is 3:1 (Sjøvall, 1960). However, no significant co-relations have been observed between the extent of liver disease and the glycine: taurine ratio (Paumgartner and Grabner, 1970).

The urinary excretion of bile acids in healthy individuals is essentially nil. A major factor contributing to the absence of urinary bile acids is the extremely low blood levels; in addition, the majority of bile acids are protein bound, the proportion depending on molecular structure. Since binding is not complete, some glomerular filtration of bile acids must occur and the urinary excretion of acids can only be explained by efficient tubular reabsorption (Hofmann, 1977).

The presence of lithocholic acid in patients with extra-hepatic biliary atresia led to the hypothesis that this bile acid was formed in liver and not, as usual, by bacteria in the intestine. This was supported by studies in a patient with extra-hepatic biliary atresia in whom lithocholic acid was excreted in the urine although no bile acids were excreted in faeces (Back, 1973).

The contaminated small bowel syndrome (GSBS) is defined as combination of diarrhoea, steatorrhea, and vitamin B12 malabsorption, which is caused by bacterial overgrowth in the small intestine. Since the fundamental defect is the development of anaerobic, colonic type of flora of the small intestine, it is better called, contaminated small bowel syndrome, as suggested by Gracey (1971).

In normal subjects all, or virtually all, bile acids in the upper small intestine are conjugated with glycine or taurine. The main bacteria isolated from the jejunum are obligate anaerobes (specially bacteroides) capable of deconjugating bile acids. There is usually a relationship between the presence of bacteroides ana, the presence of deconjugated bile acids (Gorbach and Tabaqchali, 1969). The rate of deconjugation is markedly increased (a) if bacteria have access to the circulating bile acid pool more proximally in the intestine than normal, and (b) if a larger than normal fraction of the bile acid pool descends into the bacteria-rich colon i.e., if there is dysfunction of the terminal ileum and so malabsorption of bile acids. The end product of the deconjugation of given e-conjugates, their bacterial metabolism or absorption and metabolism by body, include carbon dioxide CO<sub>2</sub>) most of which is excreted in the breath. When glycine is labelled with <sup>14</sup>C, radioactive CO is expired at a rate proportional to the rate of bile acid deconjugation. Thus the main cause of positive breath test is bacterial overgrowth in the small intestine, ileal disease of resection, or a combination of both.

The serum of patients with GSBS has been reported, to contain high concentration of unconjugated bile acids. This does not seem to be due to liver damage, but is probably best explained by the fact that unconjugated bile acids are bound to plasma albumin more tightly than conjugated bile acids, and so are cleared more slowly (Burke et al., 1971).

Patients with GSBS are occasionally found to have stones within the involved part of small bowel. Such enteroliths consist mainly of three bile acids especially deoxycholic acid, with fatty acids as minor constituents (Haslewood, 1967).

Malabsorption in GSBS has a dual mechanism. Unconjugated dihydroxy bile acids cause toxic injury to the small bowel provided there is a moderate reduction in the concentration of conjugates; and when the concentration of conjugated bile acids falls very low there is, in addition, a reduced micellar solubilization of fatty acids and monoglycerides. In cases involving terminal ileal resection and bypass (TIRB), the return of bile acids in liver increases (negative feed-back mechanism) 2 to 10 fold over the normal 300 to 500 mg per day, depending on amount of ileum removed (Woodbury and Kern, 1971; Hofmann, 1972; Findlay et al, 1973).

The increased concentration of bile acids in ileal effluent, entering colon, causes diarrhoea. The best evidence of this is the relief of diarrhoea by oral administration of cholestyramine, an insoluble bile acid binding resin. Cholegenic diarrhoea is usually ascribed to the inhibitory effect of bile acids on colonic absorption. The main features of this phenomenon were established in a classic study by Forth et al. (1966).

An increased frequency of gall-stones has been described but this seems to apply only when appreciable amount of ileum has been resected or the patient has Crohn's diseases, presumably with latent ileal involvement (Hill et al., 1975).

### **Diagnostic Value of Serum Bile Acids**

In time, as routine methods become available for the measurement of serum bile acids, it is likely that the same type of information can be obtained by the use of the BSP (Sulphobromoph-thalein) and the ICG (Indocyaninegreen) tests with less risk and more convenience to the patients. In addition because the bile acids are synthesized in the liver and undergo EHC, it is possible to gain information relating to other aspects of liver disease (Javitt, 1977).

It is essential to estimate bile acids in serum before deciding whether the problem should be classified

as hyperbilirubinemia or cholestasis. A normal serum bilirubin with elevated bile acids is classified as cholestasis and elevation of both as cholestatic jaundice (cholestasis and hyperbilirubinemia). This classification appears to be very useful clinically but is relatively new and needs further evaluation. As cholestasis develops the liver progressively loses its capacity to transport bile acids. Because the bile acids undergo enterohepatic recycling related to meals and the great load of hepatic excretion, postprandial bile acid determination has been found to be more sensitive than measurements of fasting bile acid levels for "detecting liver disease". A normal level in the morning after and overnight fast with elevated two hour postprandial level is considered "minimal cholestasis". An elevated fasting level with a postprandial rise is classified as moderate cholestasis" and marked elevation in both the levels with no statistically significant difference between the two values are classified as "severe cholestasis" (Javitt, 1977).

## References

1. Allan, R.N., Thistle, J.L. and Hofmann, A.F. (1976) Lithocholate metabolism during chemotherapy: for gallstone dissolution. Absorption and sulphation. *Gut*, 17:413.
2. Back, P. (1973) Die Primäre Hepatose Synthese Von. Monohydroxy-Gallensäuren bei extrahepatischer Gallen-gagctresie. *Klin. Wochens.*, 51:926-932.
3. Begemann, F. and Herget, H. J. Lithogenic bile in acute hepatitis, in advance in bile acid research Ed. sted by Matern, S., Hackenschmidt, J., Back. P. and Gerok, W.P. New York; Verlag, 1974, p. 335.
4. Bouchier, I.A.D. (1969) Postmortem study of the frequency of gallstones in patients with cirrhosis of the liver. *Gut*, 10:705.
5. Burke, C.W., Lewis, B., Panveliwalla, D. and Tabaqchali, S. (1971) The binding of cholic acid and its taurine conjugate to serum proteins. *Clin. Chim. Acta*, 32:207.
6. Carey, J.B. Jr., Hanson, R.M. and Monson, B.K. (1969) Primary bile salt synthesis in human liver injury. *Gastroenterology*, 56:1142.
7. Cowen, A.E., Korman, M.G., Hofmann, A.F. and Cass, O.W. (1975) Metabolism of lithocholate in health man. I. Biotransformation and biliary excretion of intravenously administrated lithocholate, lithocholyglycine and their sulphates. *Gastroenterology*, 69:59.
8. Ekdahl, P.H. (1958) On the conjugation and formation of bile acids in human liver. V. On the conjugation of cholic acid-2414C in homogenates of human liver specimens stored in deep-freeze unit; bile acids and steroids. *Acta Chir. Scand.*, 115:208.
9. Findlay, J.M., Eastwood, M.A. and Mitchell, W.D. (1973) The physical states of bile acids in diarrhoeal stool of ileal dysfunction. *Gut*, 14:319.
10. Forth, W., Rummel, W. and Glasner, H. (1966) Zur resorptions, hammelden wirkung von gallensäuren. *Naunyn-schmiedebergs. Arch. Exp. Path.*, 254:364.
11. Gorbach, S.L. and Tabaqchali, S. (1969) Bacteria, bile, and the small bowel. *Gut*, 10:963.
12. Gracey, M. (1971) Intestinal absorption in CSBS. *Gut*, 12:403.
13. Haslewood, G.A.D. Bile salts. London, Methuen, 1967.
14. Hepner, G.W., Hofmann, A.F. and Thomas, P.J. (1972) Metabolism of steroids and amino acid moieties of Conjugated bile acids in man. I. Cholyglycine. *J. Clin. Invest.*, 51:1889.
15. Hill, G.L., Mair, W.S.J, and Goligher, J.C. (1975) Gallstones after ileostomy and ileal resection. *Gut*, 16:932.
16. Hofmann, A.F. (1972) Bile acid malabsorption caused by ileal resection. *Arch. Intern. Med.*, 130:597-605.
17. Hofmann, A.F. and Hofmann, W.E. (1974) Measurement of bile acid kinetics by isotop dilution in man. *Gastroenterology*, 67:314.
18. Hofmann, A.F. (1977) The EHC of bile acids in man. *Clin. Gastroenterol.*, 6:3.

19. Javitt, N.B. (1977) Diagnostic value of serum bile acids. *Clin. Gastroenterol.*, 6:219.
20. Kuroda, T. and Okuda, K. (1974) Bile acids in hepatic bile in liver disease. *Acta Hepatogastroenterol.*, 21:120.
21. Makino, I., Hashimoto, H., Shinozaki, K., Yashino, K. and Nakagawa, S. (1975) Sulphated and nonsulphated bile acids in urine, serum, and bile of patients with hepatobiliary diseases. *Gastroenterology*, 68:545.
22. Nicholas, P., Rinaudo, P.A. and Conn, H.O. (1972) Increased incidence of cholelithiasis in Laennec's cirrhosis. A postmortem evaluation of pathogenesis. *Gastroenterology*, 63:112.
23. Norfield, T.C. and Hofmann, A.F. (1975) Biliary lipid output during three meals and overnight fast. I. Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects. *Gut*, 16:1.
24. Norman, A. and Strandvik, B. (1971) Formation and metabolism of bile acid in hepatic biliary atresia. *J. Clin. Lab. Med.*, 78:181.
25. Paumgartner, G. and Grabner, G. (1970) Das Verhalten Kojugierter Gallensauren des Serums bei Erkrankungen der Leber. *Acta Hepatogastroenterol.*, 5:344.
26. Salen, G., Tint, G.S., Deering, N. and Mosbach, E.H. (1974) Increased formation acid. *J. Clin. Invest.*, 53:612.
27. Schoenfield, L.J, and Sjoval, J. (1967) Bile acids on the skin of patients with pruritic hepatobiliary disease. *Nature*, 213:93.
28. Sjoval, J. (1960) Bile acids in man under normal and pathological conditions. *Clin. Chim. Acta*, 5:33.
29. Strandvik, B. (1973) Bile acid metabolism in colestasis on infancy. *Opuscula Medica*, (Supplementum) 1:24.
30. Theodar, E., Spritz, N. and Slesinger, M.H. (1968) Metabolism of intravenously injected isotopic cholic acid in viral hepatitis. *Gastroenterology*, 53:183.
31. Vlahcevic, Z.R., Buhac, I., Farra, J.T., Sell, CC.Jr. and Swell, L. (1971) Bile acid metabolism in patients with cirrhosis. I. Kinetic aspects of cholic acid metabolism. *Gastroenterology*, 60:49.
32. Vlahcevic, Z.R., Yoshida, T., Jutti judata, P., Bell, C.C.Jr. and Swell, L. (1973) Bile acid metabolism in cirrhosis. III. Biliary lipid secretion in patients with cirrhosis and its relevance to gallstone formation. *Gastroenterology*, 64:298.
33. Woodbury, J.F. and Kern, F.Jr. (1971) Fecal excretion of bile acids, a new technique for studying bile acid kinetics in patients with ileal resection. *J. Clin. Invest.*, 50:253.