Serum bile acids in hepatobiliary disease

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SUMMARY We review the estimation of total and individual serum bile acids to detect the presence and nature of hepatobiliary disease. The different methods for measuring serum bile acids are discussed.

In clinical practice, the term 'liver function test' is applied to a variety of serum measurements that are merely potential markers of hepatobiliary disease. Unfortunately, they all lack sensitivity and specificity, and alone are inefficient in detecting the presence and defining the nature of hepatobiliary disease (Winkel *et al.*, 1975). Although the importance of these tests has declined with the development of more sophisticated techniques of hepatobiliary investigation, the concept remains attractive because of the safety, rapidity, and economy of such serum measurements. Hence the search for more suitable serum tests for liver disease continues.

Bile acids are synthesised and conjugated by hepatic microsomal, mitochondrial, and lysosomal enzymes (Danielsson and Sjövall, 1975). The enterohepatic circulation normally ensures that 20 g of bile acids pass through the liver each day, during which time 4 to 5 g are newly synthesised to compensate for faecal loss (Heaton, 1972; Hofmann, 1977). Hepatobiliary disease may therefore be associated with changes in bile acid metabolism which are reflected in altered serum bile acid concentrations. It has frequently been suggested that the measurement of changes in serum bile acids might indicate the presence, and possibly the nature, of liver and biliary disease.

Early studies sought to differentiate intrahepatic from extrahepatic cholestasis on the basis of altered proportions of individual bile acids; later work investigated the value of measuring the total serum bile acids in the detection of hepatic disease. Many different methods were used in the course of these studies, and provided different information. Furthermore, until recently, accurate measurement of the low bile acid concentrations found in normal subjects could not be achieved.

Methods

The spectrophotometric methods employed in the

early studies lacked sensitivity and specificity. Some workers used thin layer chromatography and fluorimetry for measurement of individual serum bile acids, but adequate separation of dihydroxy bile acids was not possible (Panveliwalla *et al.*, 1970).

There are currently three approaches to serum bile acid estimations. First, gas liquid chromatography involves lengthy chemical modification of specimens for analysis, but provides information about all the major bile acids. Second, the enzymatic fluorimetric method is simple and rapid, but provides only an approximate value of the concentrations of total serum bile acids. Third, radioimmunoassay is a rapid method for measuring individual bile acid conjugates, but a different assay is required for each one.

The first application of gas liquid chromatography to the measurement of serum bile acids was by Sandberg *et al.* (1965). Improvements were introduced by Roovers *et al.* (1968). Enzyme hydrolysis replaced alkaline hydrolysis, which is known to destroy bile acids (Levin *et al.*, 1961), and an internal standard solved the problem of procedural losses and standardisation of the gas liquid chromatography injections. Further modifications were reported by Henegouwen *et al.* (1974), and, more recently, Ross *et al.* (1977) demonstrated the accurate measurement of serum bile acids in the normal range using only 2 ml of serum.

The enzymatic fluorimetric method was introduced by Iwata and Yamasaki (1964). It depends upon the enzymatic dehydrogenation of the 3 α hydroxyl group which is common to all the major bile acids found in man, and is based on the formation of NADH from NAD in the presence of bile acids, the enzyme 3 α dehydrogenase, and a ketone trapping agent. NADH is measured by fluorimetry as first described by Murphy *et al.* (1970). A liquid solid extraction with Amberlyte XAD2 resin improved the method (Schwartz *et al.*, 1974), but a lack of specificity was suggested by control serum bile acid values which were twice

those found by gas liquid chromatography. Some improvement, however, followed the introduction of a purified enzyme (Fausa, 1975). The use of 7α hydroxysteroid dehydrogenase has been used to measure the total primary bile acids (Macdonald, 1976; Fausa and Skålhegg, 1977). The 7α hydroxyl group is common only to the primary bile acids.

The first radioimmunoassay for conjugated cholyl bile acids was reported by Simmonds *et al.* (1973). A similar method was described by Matern *et al.* (1976), who advocated the preliminary extraction of the bile acids from serum using XAD2 resin. A method for chenodeoxycholic acid conjugates was developed by Schalm *et al.* (1977). The radioimmunoassay methods for conjugates of cholic, chenodeoxycholic, deoxycholic, and sulpholithocholic acids, described by Demers and Hepner (1976), were notable for the absence of cross-reaction between glycine and taurine conjugates. Finally, the recent report of an I^{125} radioimmunoassay for primary conjugated bile salts (Spenney *et al.*, 1977) promised a cheaper method. The Y emission avoided the tritium based assay, but control values for cholylglycine ($3.47 \pm 2.16 \mu\text{mol/l}$) appeared high.

Early clinical studies of serum bile acids

An early study by Sherlock and Walshe (1948) investigated the possibility that blood cholate estimation in jaundiced patients might differentiate extrahepatic obstruction from parenchymatous liver disease. Unfortunately, cholate values were found to be similar in both groups of patients, and thus failed to distinguish between potential disorders of synthesis and excretion. Many patients, especially those with cirrhosis, had normal cholate values. This was probably due to the use of a non-specific method (Josephson, 1935) which resulted in a normal range 30-fold greater than that defined by present workers using improved techniques (Simmonds *et al.*, 1973; Matern *et al.*, 1976; Pennington *et al.*, 1978b).

The simultaneous measurement of trihydroxy and dihydroxy bile acids by spectrophotometric methods focused attention on the apparent value of the trihydroxy: dihydroxy bile acid ratio in differential diagnosis. Rudman and Kendall (1957) observed that trihydroxy bile acids could not be measured in their cirrhotic patients; the ratio was less than one in patients with chronic hepatitis, and exceeded one in a mixed group of patients with cholestasis. Similar conclusions were drawn by Carey (1958); and other workers considered that the low ratio in cirrhosis signified a poor prognosis (Osborn *et al.*, 1959).

Recent studies

TOTAL SERUM BILE ACIDS AND THE DETECTION OF HEPATOBIILIARY DISEASE

The first convincing evidence that serum bile acid measurement might be a more sensitive index for the presence of liver disease was provided by Frosch and Wagener (1967). Abnormal values of serum bile acids were observed to correspond with histological abnormalities in patients convalescing from viral hepatitis who had normal conventional liver function tests. Kaplowitz *et al.* (1973) first suggested that measuring serum bile acids two hours after a meal was the method of choice for detecting hepatobiliary disease. All 26 of their patients had raised postprandial total serum bile acids, whereas the fasting values were normal in 10 patients. In another study, total postprandial serum bile acids were increased in all 30 patients with histological evidence of liver disease. The fasting serum bile acids were normal in three patients, and normal values for bilirubin, alkaline phosphatase, and aspartate aminotransferase were found in at least seven patients (Barnes *et al.*, 1975). It is notable, however, that the former study excluded patients with non-functioning gallbladders, and the methods employed in both studies were insufficiently sensitive to discriminate between fasting and postprandial serum bile acid values in normal subjects. The latter criticism was not applicable to another study (Fausa and Gjone, 1976), in which it was claimed that estimations of fasting and postprandial serum bile acids were superior to conventional liver function tests. Unfortunately, the analysis of the data merely demonstrated that the overall increase in the values of serum bile acids exceeded that for other indices. When the data were reanalysed on the basis of frequency of abnormal response, the fasting values of serum bile acids were abnormal significantly less frequently, and the postprandial values were abnormal significantly more frequently, than the conventional liver function tests. Another report observed that the postprandial total serum bile acids remained raised after conventional liver function tests had returned to the normal range in subjects recovering from an overdose of paracetamol (Hamlyn *et al.*, 1977). In our own studies we have found no difference between the total fasting or postprandial serum bile acids, aspartate aminotransferase, and gamma glutamyltranspeptidase in the detection of anicteric patients with occult hepatic disease (Pennington *et al.*, 1977a).

The above studies measured conjugated and unconjugated individual and/or total serum bile acids. Korman *et al.* (1974) found that the measurement of cholyl conjugates by radioimmunoassay was

very useful for the early detection of relapse in patients with chronic active hepatitis. Demers and Hepner (1975) specifically measured glycine conjugates of the three major bile acids and sulpholithocholic acid, and found that the latter was the only bile acid with a raised value in all the patients they studied.

A different approach was adapted by the Mayo group, who investigated the plasma disappearance of intravenous ^{14}C cholyglycine in patients with histological evidence of hepatobiliary disease (La Russo *et al.*, 1974). Reduced plasma clearance of ^{14}C cholyglycine was a better way of identifying patients than the measurement of fasting serum cholyglycine. Subsequently, Thjodleifsson *et al.* (1977) observed that the estimation of total post-prandial serum bile acids was a better method than the intravenous bile acid tolerance test in the detection of hepatic disease.

SERUM BILE ACIDS AND NATURE OF HEPATIC DISEASE

Several studies have demonstrated a proportionate reduction in serum deoxycholic acid in patients with all forms of hepatobiliary disease (Makino *et al.*, 1969; Pennington *et al.*, 1977a). Thus the trihydroxy: dihydroxy bile acid ratio, described in earlier studies, is equivalent to the cholic:chenodeoxycholic acid ratio, which will be described as the primary bile acid ratio.

Some workers have advocated the use of this ratio in differential diagnosis. Makino *et al.* (1969) observed primary bile acid ratio values of less than 1.5 in 26 out of 30 patients with portal cirrhosis. Values were greater than 1.5 in 17 out of 18 patients with obstructive jaundice. Similar findings were reported by Neale *et al.* (1971). In both studies the normal range of the primary bile acid ratio was large, and overlapped the values for the different diagnostic groups. Clearcut separation of cirrhotics, from controls, from patients with large duct obstruction, on the basis of this ratio, has recently been reported (Pennington *et al.*, 1977a). Unfortunately, there is no evidence that this ratio, or any other bile acid measurement, can separate patients with intrahepatic cholestasis from those with extrahepatic cholestasis in adult practice. Javitt *et al.* (1973) considered that the ratio was useful in distinguishing biliary atresia (low values) from intrahepatic cholestatic syndromes (high values) in infants. This observation, however, has been disputed (Mowat, 1976).

The primary bile acid ratio has also been applied to the staging of disease. Patients with primary biliary cirrhosis were noted to have falling values as their disease progressed, and ratios of less than one

were associated with terminal illness (Bloomer *et al.*, 1976). In a study of alcoholic liver disease, the ratio was low in active cirrhosis, and high in patients with inactive cirrhosis (Milstein *et al.*, 1976). Similarly, the ratio was noted to increase from abnormally low values as patients with chronic active hepatitis entered remission (Pennington, 1977). In the course of viral hepatitis it has been reported to increase (Pennington *et al.*, 1978c) or decrease (Erb *et al.*, 1972).

Makino *et al.* (1969) reported that half the serum bile acids in normal subjects were unconjugated. The conjugated fraction increased in patients with extrahepatic obstruction, and an increase in the unconjugated fraction was observed in patients with cirrhosis. The study by Neale *et al.* (1971), however, demonstrated that most serum bile acids were conjugated, and the state of conjugation with glycine or taurine was variable. Several studies have reported that a small proportion of serum bile acids are sulphated (Makino *et al.*, 1972; Stiehl, 1974; Makino *et al.*, 1975; Henegouwen *et al.*, 1976). No evidence has been presented to suggest that the measurement of sulphates is of diagnostic value.

The ability of the liver to conjugate intravenous ^{14}C cholic acid has been used to assess the potential recovery of patients in grade IV coma from acute hepatic failure (Horak *et al.*, 1976).

Pathological mechanisms

Many factors influence serum bile acid values, of which intestinal absorption plays an important part (La Russo *et al.*, 1974). In health the primary bile acid pool sizes are similar and the predominance of chenodeoxycholic acid in the serum of healthy subjects may reflect more rapid absorption of chenodeoxycholic acid (Angelin *et al.*, 1976), and the preferential hepatic clearance of cholic acid (Cowen *et al.*, 1975).

An increase in serum bile acid values will be expected in the presence of portasystemic shunting, or damaged hepatocytes which are unable to extract the bile acids from the portal blood. It is also apparent that bile acids 'leak' directly from the liver to the systemic circulation, as very high serum bile acid concentrations are found in patients with extrahepatic obstruction (Makino *et al.*, 1969; Pennington *et al.*, 1977a).

It is known that the cholic acid pool is decreased in cirrhotic patients (McCormick *et al.*, 1973). This may be due to a failure of 12α hydroxylation in the major synthetic pathway, or a possible increase in the relative role of an alternative pathway (Mitropoulos and Myant, 1967) from which chenodeoxycholic

acid may be derived (Anderson *et al.*, 1972). Proportionately more cholic acid has been described in livers following large duct obstruction (Greim *et al.*, 1972). Unfortunately, many aspects of bile acid metabolism which influence serum bile acid values are poorly understood.

Conclusions

During the past 30 years papers have appeared with increasing frequency as the technical difficulties posed by serum bile acid measurements have been partially resolved. Early reports suggested that the measurement of total serum bile acids was unhelpful, but this reflected a lack of specificity in the methods used. Subsequently, the measurement of total and single serum bile acids has been acclaimed as the most sensitive means of detecting occult liver disease. More recently, the measurement of total values two hours after a meal has been advocated, and has been found to be superior to an intravenous bile acid tolerance test.

Initial reports that changes in the bile acid ratios reflected the nature of hepatobiliary disease aroused interest in the application of these indices in differential diagnosis. Subsequent work confirmed these trends, the clinical value of which remains to be established.

The assumption has been made that serum bile acids have the advantage of specificity compared to other liver function tests. This assumption requires examination. High serum bile acid values have been reported in patients with small bowel colonisation (Lewis *et al.*, 1969); abnormal serum bile acids have been described in hyperlipidaemia (Pennington *et al.*, 1977a), and changing serum bile acids have been noted with alteration of the dietary vitamin C (Kallner, 1977). The influence of drugs which alter hepatic metabolism or bind bile acids in the bowel lumen merit further study. The possibility that measurement of the serum bile acids may be useful in monitoring gallbladder and intestinal function requires investigation.

After 30 years, and many clinical and laboratory studies, further work is required to establish the place of serum bile acid measurement in clinical practice.

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