J. clin. Path., 1975, **28**, 506-509

Diagnostic value of serum bile acid estimations in liver disease

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SYNOPSIS Bile acid concentrations were estimated enzymatically in fasting and postprandial (two-hour) serum samples from 18 normal subjects and 30 patients with histologically proven hepatobiliary disease. The serum bile acid concentration was less than 15 μmol/l in normal subjects and did not increase postprandially. The fasting serum bile acid concentration was raised in 27 of the patients with hepatobiliary disease, and following a meal was outside the normal range in all 30 patients. Other liver function tests were abnormal less frequently. These results suggest that the estimation of serum bile acids in the postprandial state is a sensitive screening test of hepatobiliary disease. They should be of particular value in patients in whom liver disease is suspected but not proven, and in those recovering from liver disease, especially following therapy.

In normal subjects the serum concentrations of bile acids are low. In most, but not all, patients with liver disease the serum concentrations of bile acids are elevated. Previous work in which serum concentrations of bile acids have been reported has been done on fasting or randomly obtained sera. Kaplowitz *et al* (1973), however, have suggested that in patients with liver disease the bile acid concentration in postprandial serum may be elevated when fasting concentrations are normal. This is because, in the fasting state, most of the bile acid pool is sequestered in the gall bladder, so that the reduced ability of the liver to clear bile acids from the portal blood may not be apparent.

In this communication fasting and postprandial serum bile acid concentrations have been measured in normal subjects and in patients with a variety of hepatobiliary diseases. In addition, the diagnostic value of the serum bile acid concentrations has been compared with other commonly used biochemical tests of 'liver function'.

Patients

Eighteen subjects aged 23-35 years (10 male) with no history of hepatic or gastrointestinal disease and with normal biochemical tests of liver function acted as controls.

Thirty patients with hepatobiliary disease, three of whom (nos. 2, 3, and 23) had had a previous cholecystectomy, were studied. Their diagnosis was established histologically by percutaneous needle biopsy of the liver performed within one week of estimating the bile acids (table I). Three patients with hepatic venous outflow block and four patients with unconjugated hyperbilirubinaemia were also studied.

Histological Diagnosis	*No. of Patients*
Cryptogenic cirrhosis	9 (2 after cholecystectomy)
Alcoholic cirrhosis	3
Primary biliary cirrhosis	6
Active chronic hepatitis	4
Cholestatic syndromes (drug jaundice)	3 (1 after cholecystectomy)
Viral hepatitis	
Acute	1 (patient no. 27)
Resolving	1 (patient no. 26)
Large bile duct obstruction	3
Other patients studied:	
Hepatovenous outflow block	3
Unconjugated hyperbilirubinaemia	4

Table I *Histological diagnosis of patients investigated in this study*

Methods

The control subjects and those with hepatobiliary disease were fasted overnight and serum was obtained at 8.00 a.m. Each subject then ate a standard fatty breakfast, and a further serum sample was obtained two hours later. The serum samples were stored at $-20°C$ and estimated within a week.

Received for publication 16 December 1974.

Serum bile acids were estimated by the fluorometric 3-hydroxy steroid dehydrogenase method (Murphy *et al*, 1970) using an Aminco SPF 125 spectrofluorometer. For bile acid concentrations within the normal range the estimate of precision was ± 1 μmol/l. The coefficient of variation within a batch for duplicate samples outside the normal range was 5·7%. The specificity of the method has been discussed previously (Murphy *et al*, 1970). In all patients serum concentrations of bilirubin, aspartate transaminase, and alkaline phosphatase were measured using routine laboratory methods, the enzyme assays being performed at 25°C.

Tests of significance between groups of data were carried out using Student's *t* test for paired or unpaired data where applicable.

Results

In normal subjects the serum bile acid concentrations ranged between 0 and 15 μmol/l. There was no significant difference in concentration between the sexes. Although five subjects had increased concentration after breakfast there was no difference between the mean of the fasting and postprandial concentrations (7·2 μmol/l and 7·7 μmol/l respectively).

In 27 of the 30 subjects with hepatobiliary disease

Patient No.	*Sex*	*Age*	*Serum Bile Acid (μmol/l)*		*Total Bilirubin (mg/100 ml)*	*Alkaline Phosphatase (KAu/100 ml)*	*Aspartate Transaminase (iu/l)*
			Fasting	*Postprandial*			
Cryptogenic cirrhosis							
1	F	56	135	180	2·4	19	11
2[1]	M	54	70	192	18·6	64	52
3[1]	M	47	62	47	0·5	14	66
4	M	63	97	122	1·1	11	9
5	M	55	22	63	0·4	11	25
6	M	62	191	174	8·0	96	38
7	M	45	245	190	2·5	8	96
8	F	30	12	30	0·4	10	13
9	M	60	150	232	1·5	15	20
Active chronic hepatitis							
10	M	47	77	115	1·5	29	35
11	M	59	41	53	1·9	30	34
12	M	25	30	37	1·5	19	9
13	M	62	65	104	1·3	21	26
Alcoholic cirrhosis							
14	M	36	242	176	16·0	45	32
15	M	32	66	82	1·3	14	31
16	M	48	31	40	0·7	ND	9
Primary biliary cirrhosis							
17	F	62	268	245	10·3	85	61
18	F	54	237	329	11·3	64	113
19	F	67	11	92	1·3	86	40
20	F	60	17	21	0·7	77	29
21	M	57	34	30	0·6	45	25
22	F	50	600	565	12·2	96	62
Cholestatic syndromes							
23[1]	F	63	42	78	2·1	27	24
24	F	26	24	33	0·9	161	57
25	F	31	115	108	29·0	54	14
Viral hepatitis							
26	M	26	9	42	0·7	7	9
27	M	31	487	532	5·0	26	59
Large bile duct obstruction							
28	F	30	436	422	9·7	93	23
29	M	60	34	32	3·0	138	33
30	F	62	275	256	20·4	62	21
Hepatic venous outflow block							
31	F	29	12	40	0·9	28	17
32	M	32	10	21	1·4	10	13
33	F	75	26	46	1·0	21	14
Unconjugated hyperbilirubinaemia							
34	M	22	6	0	4·4	6	17
35	M	18	12	15	1·4	11	10
36	M	51	15	14	22·5	9	15
37	M	51	8	8	22·0	14	12
Normal range values			0-15	0-15	0·1-0·8	3-13	4-15

Table II *Total serum bile acid concentrations and other liver function tests in individual patients in this study*

[1]These patients had undergone cholecystectomy.
ND = not determined.

the fasting concentrations were above the normal range (22-600 μmol/l). In three subjects with liver disease the fasting serum bile acid concentrations were normal (less than 15 μmol/l), but two hours after the fatty breakfast they became markedly elevated. In two of these three subjects (nos. 8 and 26) the other biochemical estimations were normal (table II). Compared with the other biochemical estimations the postprandial serum bile acid concentrations were the only estimations which were consistently abnormal (table II). For the other liver function tests clearly abnormal results were less frequent. For total bilirubin 22/30 were abnormal, for alkaline phosphatase 20/30, and for aspartate transaminase 23/30.

In the four patients with unconjugated hyperbilirubinaemia both the fasting and two-hour postprandial bile acid concentrations were normal as were the liver function tests. Of the three patients with hepatic venous outflow block, all had elevated postprandial serum bile acid concentrations although two had normal fasting concentrations.

Discussion

This study has shown that the two-hour postprandial serum bile acid concentrations are elevated in patients with histological hepatobiliary disease, even when fasting concentrations and other liver function tests are normal. This is in contrast to normal subjects whose bile acid concentrations did not rise outside the normal fasting range. Similar findings were reported by Kaplowitz *et al* (1973) in a series of 26 patients with hepatobiliary disorders. These workers also showed that the postprandial serum bile acid concentration was elevated when bromsulphthalein excretion was normal. In the present study, using an enzymatic assay with a fluorometric endpoint, three patients had normal fasting serum bile acid concentrations, while in the study of Kaplowitz *et al* (1973), using a gas-liquid chromatographic method, 10 of 26 patients had normal fasting serum concentrations. In addition to the differences in methodology, the type of patient studied in each of these investigations was quite different, and this probably accounts for the differences in the incidence of normal fasting serum bile acid concentration. Franz and Bode (1973), using an enzymatic assay, found normal concentrations of fasting serum bile acids in six of 42 patients with liver cirrhosis, one of 15 with chronic active liver disease, and 19 of 25 patients with chronic persistent hepatitis. Unfortunately, the serum bile acid concentrations after a meal were not reported.

In patients with unconjugated hyperbilirubinaemia the serum bile acid concentrations were normal, which was to be expected as they had no histological liver disease. Patients with hepatic venous outflow block, however, did have raised postprandial serum bile acid concentrations, probably indicating a degree of hepatocellular dysfunction.

The serum bile acid concentration is not raised exclusively in hepatobiliary diseases, and elevations have been reported in patients with intestinal resection and the contaminated bowel syndrome (Lewis *et al*, 1969). In contrast to patients with liver disease (Neale *et al*, 1971), the elevations were characterized by an increase in the concentrations of unconjugated bile acids.

The elevation in serum bile acid concentration observed postprandially in the majority of patients is dependent on many factors. Gall-bladder contraction in response to a meal increases the amount of bile acid entering the small intestine and hence returning to the liver. However, two of the three patients who had undergone a cholecystectomy (nos. 2 and 23) also showed a postprandial rise. Postprandial elevations of serum bile acids in these patients probably result from the passage of bile acids down the intestine to the absorption site in the terminal ileum. In liver disease the elevation of postprandial bile acids results from the inability of the liver cell to remove bile acids from portal blood or from portosystemic shunting by which the bile acids bypass the hepatocyte (Kaye *et al*, 1973).

In addition to their value as a screening test for hepatobiliary disease, serum bile acid determinations are useful in following the progress of viral hepatitis (Cronholm *et al*, 1970), particularly in the resolving phase where they remained abnormal although other liver function tests had returned to normal. This observation was confirmed in this study in patient 26. Korman *et al* (1974) have shown that serum bile acid determinations are useful in judging the

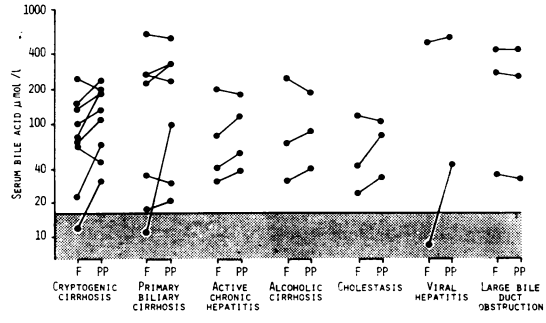

Figure *Serum bile acid concentrations in the fasting state and two hours after a standard fatty breakfast. Values from the same patient are joined up by the black line.*

response to therapy of patients with chronic active liver disease, and have demonstrated that they are superior to liver biopsy in predicting those patients who subsequently relapsed following biochemical and histological resolution.

Despite the obvious value of serum bile acids in indicating the presence of histological liver damage the present study shows that such measurements are of no value in distinguishing between particular liver diseases, particularly in deciding whether cholestasis is of intra or extrahepatic origin (figure). This confirms the findings of many other workers (Rudman and Kendall, 1957; Carey, 1958; Osborn *et al*, 1959; Carey, 1961; Sandberg *et al*, 1965; Makino *et al*, 1969).

The introduction of serum bile acid estimation as a routine laboratory test of liver function depends on the ease with which the estimation can be performed. The isolation of bile acids from serum and the high background fluorescence in the enzyme assay have, in the past, been factors reducing both accuracy and sensitivity. However, the recently introduced liquid solid extraction procedure for the isolation of bile acids from serum using the resin XAD-2 (Schwarz *et al*, 1974), which eliminates the background fluorescence, is a significant advance and permits measurements accurate to ± 0·1 μmol/l. This may well prompt some laboratories to introduce this test, particularly if the procedure can be automated. Alternatively, analysis by radioimmunoassay, as described for 'cholyl conjugates' (Simmonds *et al*, 1973), may be more useful since it is very sensitive and requires only small amounts of serum. It has yet to be established whether estimation of total bile acids or of a specific bile acid will provide the more sensitive indicator of liver disease.

G.G. was supported by The British Council and J.M. was in receipt of a grant from the Medical Research Council.

We are grateful to Professor Sheila Sherlock for allowing us to study her patients and to the Chemical Pathology Department for the results of the liver function tests.

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