

# Serum bile acids in primary biliary cirrhosis

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**SUMMARY** Serum bile acid classes have been studied in 15 patients with primary biliary cirrhosis in five patients with cholestasis, and in five patients who had cirrhosis without cholestatic features. Conjugated monohydroxy bile acids (12-35% serum total bile acids) were found in eight of 11 sera from patients with primary biliary cirrhosis, in sera from four patients with cholestasis but not in any of the five patients with cirrhosis. The glycine conjugates/taurine conjugates (G/T) ratio in eight of 11 patients with primary biliary cirrhosis and two of four patients with cholestasis was  $<1.0$ .

Bile acid concentrations in seven patients with primary biliary cirrhosis were measured before and during cholestyramine therapy. Decreases in serum total bile acid concentrations were observed which were accompanied by small increases in the trihydroxy/dihydroxy ratio and also in the G/T ratio in six of the seven patients. No association was found between the concentration of any particular conjugated or free bile acid and the presence or absence of pruritus.

The clinical syndrome of cholestasis has long been associated with disturbances of bile acid metabolism (Sherlock, 1966). Schaffner and Popper (1969) have suggested that cholestasis is related to the production of monohydroxy bile acids within the liver cell. There has, however, been no adequate study of serum monohydroxy bile acids both conjugated and free in patients with cholestasis to test this hypothesis. Since cholestasis is one of the features of primary biliary cirrhosis, individual serum bile acids have been determined in patients suffering from this condition as well as in patients with cholestasis due to other causes, and also in patients suffering from cirrhosis without cholestatic features.

It has been known for many years that pruritus in cholestatic liver disease is usually accompanied by raised serum bile acid levels (Carey, 1958) and that relief is associated with a reduction in serum bile acids (Carey and Williams, 1961; Van Itallie, Hashim, Crampton, and Tennent, 1961; Datta and Sherlock, 1963; Neale, Lewis, Weaver, and Panveliwalla, 1971). The degree of pruritus does not, however, appear to be directly related to the absolute level of total bile acids (Ross, Murphy, and Sherlock, 1970). In the present study the relationship between individual serum bile acid levels and pruritus has been examined to see if a particular bile acid could be responsible for the condition.

## Patients

Twenty-five patients with liver disease were studied. Fifteen patients suffered from primary biliary cirrhosis; this diagnosis was made on clinical, serological, and histological grounds. Three of these patients were studied when in hepatic failure. In seven cases bile acid levels were measured both when the patient was not receiving cholestyramine and when cholestyramine had been administered for a minimum period of two weeks.

A further five patients who had biochemical features of cholestasis were grouped together: three had extrahepatic obstruction, one was recovering from chlorpromazine jaundice, and one patient had the clinical features of primary biliary cirrhosis but subsequent histological examination showed the diagnosis to be that of active chronic hepatitis. Only the patient with chlorpromazine jaundice received cholestyramine during the study.

The remaining five patients had cirrhosis without cholestatic features. The cirrhosis was of unknown aetiology in four cases and due to alcohol in one case.

None of the patients studied had features of bacterial overgrowth in the bowel. There was no evidence of intestinal diverticula and urinary indicators were normal.

## Methods

### SERUM BILE ACID ESTIMATION

Serum obtained after an overnight fast was stored at  $-15^{\circ}\text{C}$  before analysis. Protein was precipitated from 1 ml serum with ethanol and the supernatant solution evaporated to dryness. The residue was dissolved in 50% ethanol ( $\text{pH } 10.0$ ) and the resulting solution extracted with ether/heptane (1:1, v/v). The bile acid classes in the ethanolic phase were obtained by silica gel thin-layer chromatography using ethylene dichloride/acetic acid/water (1:1:0.1) (Gregg, 1966). This system separates bile acid mixtures into their free, taurine, and glycine conjugated bile acid fractions and within these groups into tri-, di-, and monohydroxy bile acids. It does not separate either the conjugated or free forms of deoxycholic acid from those of chenodeoxycholic acid. The bile acid fractions were located with iodine, eluted from the silica gel with methanolic pyrophosphate buffer, and then estimated using an enzymatic fluorimetric procedure previously described for serum total bile acids (Murphy, Billing, and Baron, 1970). The determination of serum bile acids is described in greater detail elsewhere (Murphy, 1972).

The recovery of known amounts of aqueous solutions of bile acids added to serum before protein precipitation and carried through the entire procedure ranged from 70 to 87% (mean  $77.3 \pm \text{SD } 5.0$ ). It was independent of the serum total bile acid concentration and the individual bile acid used. The standard solutions of lithocholic and tauroolithocholic acids were prepared in 50% ethanol and under these conditions the recovery in each case was 91%.

The precision of duplicate estimations on each

sera was as follows: for serum concentrations of individual bile acids ranging from 2 to  $15 \mu\text{mol/litre}$  the coefficient of variation was 12% and for concentrations greater than  $15 \mu\text{mol/litre}$  it was 8%. It was not possible to measure bile acid concentrations less than  $2 \mu\text{mol/litre}$  and such concentrations have been reported as not detected.

The specificity of the determination of serum bile acids using hydroxysteroid dehydrogenase is such that  $3\alpha$ -bile acid sulphate esters (Palmer, 1967) are not estimated by this procedure.

Liver function tests performed included estimation of serum bilirubin (normal range  $0.3\text{--}1 \text{ mg/100 ml}$ ), alkaline phosphatase (normal range  $3\text{--}13 \text{ KA units/100 ml}$ ), and total cholesterol (normal range  $170\text{--}260 \text{ mg/100 ml}$ ).

## Results

The levels of serum individual bile acids found in primary biliary cirrhosis are shown in Table I and those in other liver diseases in Table II together with details of cholestyramine treatment and liver function tests.

### SERUM BILE ACIDS IN PATIENTS NOT RECEIVING CHOLESTYRAMINE THERAPY

#### Total bile acids

The total bile acids were elevated in all the sera examined (normal value  $< 8 \mu\text{mol/litre}$ ; Murphy *et al.*, 1970). The mean value ( $197.3 \pm 90 \mu\text{mol/litre}$ ) found in the sera of the group with primary biliary cirrhosis was statistically higher ( $p < 0.001$ ) than that of the cirrhotic group ( $43.5 \pm 26.4 \mu\text{mol/litre}$ ) but was not different from that found in patients

Diagnosis	Patient No.									
	1	1	1 <sup>a</sup>	2	2	3	4	5	6	6
Pruritus	+	-	-	+	-	+	-	-	+	-
Cholestyramine	Off	On	On	Off	On	On	Off	On	Off	On
Bile Acids ( $\mu\text{mol/litre}$ )										
Total	166	86	146	171	38	35	102	30	154	74
Taurotrihydroxy	12	6	33	20	9	15	44	ND	32	29
Taurodihydroxy	46	19	12	69	9	10	8	11	35	9
Tauromonohydroxy	31	19	ND	ND	ND	ND	ND	ND	23	ND
Glycotrihydroxy	36	20	55	12	ND	5	7	10	33	15
Glycodihydroxy	41	23	46	70	20	5	43	9	14	14
Glycomonohydroxy	ND	ND	ND	ND	ND	ND	ND	ND	17	7
Ratio										
Glycine/Taurine	0.87	0.98	2.24	0.92	1.11	0.40	0.96	1.73	0.71	0.95
Trihydroxy/dihydroxy	0.55	0.62	1.52	0.23	0.31	1.33	1.00	0.50	1.32	1.91
Alkaline phosphatase (KA units/100 ml)	26	36	38	47	46	48	42	57	50	64
Cholesterol (mg/100 ml)	280	340	270	240	210	220	265	320	460	420
Total bilirubin (mg/100 ml)	2.5	2.5	12.0	1.4	1.0	<0.5	<0.5	2.5	8.5	6.0

Table I Results of bile acid estimations in cases of primary biliary cirrhosis

with other cholestatic liver diseases ( $115 \pm 68$   $\mu\text{mol/litre}$ ).

*Free bile acids*

Unconjugated bile acids were not detected in any of the sera examined.

*Monohydroxy bile acids*

In eight of 11 sera from patients with primary biliary cirrhosis and in sera from four patients with cholestasis, spots were detected with  $R_f$  values corresponding to those of tauro lithocholic and glycolithocholic acids. Subsequent tests have confirmed that such spots were monohydroxy bile acids but because of the small absolute amounts present it was not possible to establish their identity. They do not, however, appear to be conjugates of lithocholic acid (Murphy, Jansen, and Billing, 1971) and consequently such compounds have been reported as taurine or glycine conjugated monohydroxy bile acids.

In the eight biliary cirrhotic patients 18 to 35% of the total bile acids were present as monohydroxy bile acids, while in the four patients with cholestasis values ranged from 12 to 28%. Monohydroxy bile acids were not detected in any of the sera from patients with non-cholestatic liver disease (Fig. 1). The amounts of monohydroxy bile acid present did not correlate with the serum total bile acid levels although the highest percentage values were found to be associated with highest total bile acid concentrations.

*Hydroxylation patterns*

The raised concentrations of total bile acids were the result of increases in both total trihydroxy and total

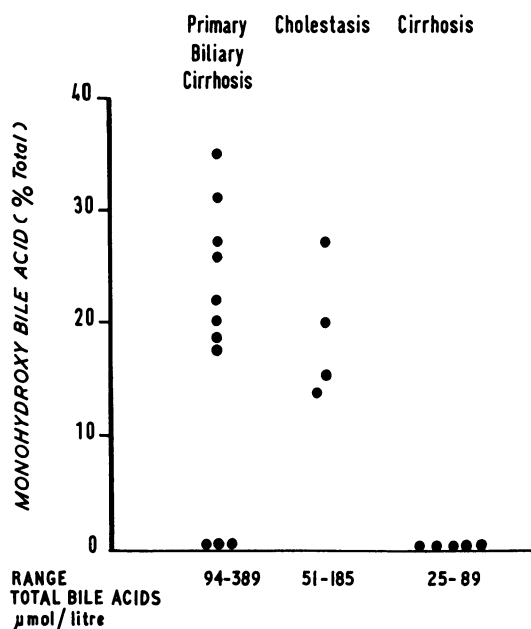


Fig. 1 Serum monohydroxy bile acids (expressed as a percentage of serum total bile acids) in patients with primary biliary cirrhosis, cholestasis, and cirrhosis.

dihydroxy fractions of all sera examined. The lowest values of the ratio of the total trihydroxy: total dihydroxy bile acids (TRI/DI ratio) were found in the primary biliary cirrhotic group. There was, however, considerable overlap in the value of this ratio in the three groups of patients. No correlation was found

7	7	7 <sup>1</sup>	8	8	9	10	11	12	13	13	14	14	15 <sup>1</sup>
+ Off	- On	- On	+ Off	- On	- On	+ On	- Off	+ Off	+ Off	+ On	+ Off	- On	- Off
389	367	301	322	179	128	110	224	91	190	96	141	75	220
120	31	19	34	7	54	11	23	10	26	23	6	4	37
76	88	52	68	49	5	35	88	27	46	16	39	23	29
32	44	19	57	ND	5	6	ND	16	26	12	31	9	17
61	119	107	34	49	24	29	93	26	13	17	33	21	40
57	85	96	74	49	29	18	20	12	46	14	25	12	65
43	ND	8	55	25	11	11	ND	ND	33	14	7	6	32
0.71	1.25	2.34	1.03	2.19	1.00	1.12	1.02	0.72	0.94	0.88	0.86	1.08	1.65
1.36	0.90	0.85	0.48	0.57	2.29	0.75	1.07	0.93	0.43	1.33	0.61	0.72	0.82
52	76	80	50	51	49	7	184	62	35	31	84	52	45
400	240	140	360	340	270	615	740	140	370	345	390	300	465
16.0	16.0	27.0	4.5	7.5	13.6	8.0	15.0	6.1	8.8	9.6	8.8	8.0	9.0

Table I—Continued

<sup>1</sup>Indicates terminal specimen. ND indicates not detected.

Diagnosis	Patient No.									
	Extrahepatic Obstruction			Drug Jaundice	Active Chronic Hepatitis	Cirrhosis				
	16	17	18	19	20	21	22	23	24	25
Pruritus	+	+	-	+	-	-	-	-	-	-
Cholestyramine	Off	Off	Off	On	Off	Off	Off	Off	Off	Off
Bile Acids ( $\mu\text{mol/litre}$ )										
Total	161	51	63	153	185	24	89	35	26	44
Taurotrihydroxy	27	7	3	37	10	7	14	9	ND	4
Taurodihydroxy	33	12	18	63	58	3	18	8	9	16
Tauromonohydroxy	32	9	9	24	18	ND	ND	ND	ND	ND
Glycotrihydroxy	56	6	19	9	56	8	13	15	8	12
Glycodihydroxy	13	12	14	9	35	6	44	3	9	12
Glycomonohydroxy	ND	5	ND	11	8	ND	ND	ND	ND	ND
Ratio:										
Glycine/Taurine	0.75	0.82	1.10	0.23	1.15	1.40	1.71	1.06	1.88	1.20
Trihydroxy/dihydroxy	1.80	0.54	0.69	0.64	0.71	1.07	0.44	2.18	0.44	0.57
Alkaline phosphatase (KA units/100 ml)	140	104	142	45	55	7	9	9	22	24
Cholesterol (mg/100 ml)	630	260	345	200	950	210	180	200	230	210
Total bilirubin (mg/100 ml)	12.2	2.8	18.4	0.8	6.0	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

Table II Results of bile acid estimations in extrahepatic obstruction, drug jaundice, active chronic hepatitis, and cirrhosis

between the TRI/DI ratio and the corresponding total bile acid concentration in any of the groups.

#### Conjugation patterns

The values for the ratio of total glycine/total taurine conjugates (G/T) are shown in Tables I and II. In eight of 11 patients with primary biliary cirrhosis and two of four patients with cholestasis the G/T ratio was < 1.0 whereas the values of this ratio found in the sera of patients without cholestasis ranged from 1.06 to 1.88.

No significant correlation was found between the value of the G/T ratio and the corresponding total bile acid concentration in any of the groups. Nor was there any significant correlation between the TRI/DI ratio and the G/T ratio. Only one patient (case 15) had a G/T ratio > 1.2 and also had serum monohydroxy bile acids. There was, however, no significant correlation between G/T ratio and the absolute or relative amounts of monohydroxy bile acids present.

#### Bile acid concentration and other liver function tests

No correlation was found between the concentration of serum total bile acids or any specific bile acid fraction and either serum alkaline phosphatase, serum cholesterol concentration, or serum bilirubin.

#### SERUM BILE ACIDS AND PRURITUS

The results of bile acid estimations made before and during cholestyramine therapy are given in Table I. Serum total bile acids fell in each of the seven patients (cases 1, 2, 6, 7, 8, 13, and 14). This fall was not

always associated with relief from pruritus (case 13) and a high level of serum total bile acids was not necessarily accompanied by pruritus (cases 4, 15, 20, and 22).

No association was found between the concentration of any particular bile acid and the presence or absence of pruritus.

The fall in serum total bile acids as a result of cholestyramine therapy was accompanied by small increases in the TRI/DI ratio and also in the G/T ratio in six of the seven patients studied.

There was a significant difference ( $P < 0.001$ ) between the G/T ratios of sera taken from patients with pruritus (mean  $0.78 \pm 0.05$ ) and sera taken when pruritus was absent (mean  $1.39 \pm 0.2$ ) (Fig. 2). Pruritus was found to be associated with low G/T ratios even when the patients were receiving cholestyramine therapy (cases 3, 13, and 19). Relief from pruritus was not experienced in patient no. 13 who was the only patient who did not show an increase in G/T ratio when on treatment.

#### Terminal cases

The three patients studied when in liver cell failure did not complain of pruritus although their serum bile acid levels remained high (cases 1<sup>1</sup>, 7<sup>1</sup>, and 15<sup>1</sup> in Table I). All three had relatively high G/T ratios including case 15 who was not receiving cholestyramine.

#### Discussion

As indicated in Fig. 1 levels of conjugated monohydroxy bile acids as high as 35% (15-112  $\mu\text{mol/l}$ )

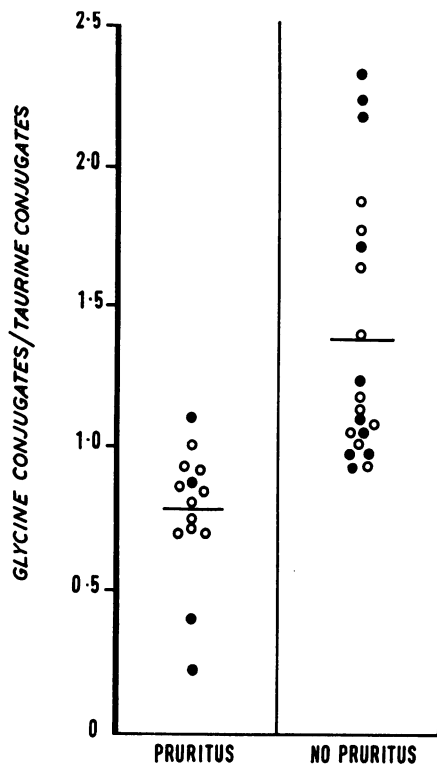


Fig. 2 Ratio of glycine conjugated bile acids to taurine conjugated bile acids from patients with and without pruritus. Bar indicates mean value. ● Patients receiving cholestyramine, ○ Patients not receiving cholestyramine

litre) of the total serum bile acids have been found in the serum of patients with primary biliary cirrhosis.

Failure to detect monohydroxy bile acids by previous investigators may be related to the type of assay used. Many modern methods of bile acid estimation employ gas liquid chromatography procedures for which it is essential that conjugates are hydrolysed and derivatives prepared. With respect to monohydroxy bile acids such methods have been criticized on two counts. First, conjugates of lithocholic acid are particularly resistant to hydrolysis and hence quantitative recoveries are difficult to obtain (Carey and Williams, 1965). Secondly, lithocholic acids and other relatively non-polar bile acids, if present, may be lost if the customary alumina column procedure is not modified (Okishio and Nair, 1966; Ali and Javitt, 1970). The method used for the present study employed a simple extraction procedure followed by enzymatic analysis and thus allowed bile acids of widely divergent polarities to be quantitatively assayed (Murphy, 1972).

The origin of the high levels of serum monohydroxy bile acids found in the present study remains obscure. If such bile acids were the result of  $7\alpha$ -dehydroxylation of primary bile acids in the intestinal tract then one might expect to find high serum levels associated with increased amounts of lithocholic acid in intestinal contents. A study by Portella, Ross, and Sherlock (1972) has reported relatively large amounts of conjugated monohydroxy bile acids present in duodenal fluid from patients with primary biliary cirrhosis. Mass spectral analysis of derivatives of the monohydroxy bile acid fraction in bile from one such patient demonstrated that the only monohydroxy bile acid present was lithocholic acid. However, a similar examination of the monohydroxy bile acid fraction present in serum pooled from five patients with primary biliary cirrhosis indicated that the compound under investigation was not lithocholic acid but an unsaturated monohydroxy bile acid (Murphy *et al*, 1971). This preliminary evidence suggests that the serum monohydroxy bile acids found in the present study may be the result of primary biosynthesis in the liver, and that they are not readily excreted in the bile.

In a study of bile acids in urine from infants with biliary atresia, Makino, Sjøvall, Norman, and Strandvik (1971) have reported the presence of significant amounts of a similar unsaturated monohydroxy bile acid ( $3\beta$ -hydroxy-5-cholenoic acid). The occurrence of such unsaturated compounds may be evidence of a biosynthetic pathway for bile acids such as that described in an *in vitro* study by Mitropoulos and Myant (1967) and in an *in vivo* study by Anderson, Ali, Kok, and Javitt (1971). The fact that only one of the five patients with serum bilirubin levels less than 3 mg/100 ml had monohydroxy bile acids present in serum (case 1) suggests that these acids are not a cause of cholestasis but a consequence of it.

The values of the TRI/DI ratio found in this study are similar to those of Neale *et al* (1971). The taurotrihydroxy/taurodihydroxy ratio was often quite different from the corresponding glycotrihydroxy/glycodihydroxy ratio even in patients not receiving cholestyramine therapy. Similar differences have been reported by Panveliwalla, Tabaqchali, Lewis, and Wootton (1970) in the serum of normal subjects and by Neale *et al* (1971) in the serum of patients with liver disease.

The finding of low G/T ratios in cholestatic liver disease is in accord with the results of Neale *et al* (1971). The levels found in the patients with non-cholestatic cirrhosis are somewhat higher (range 1.06-1.88) but there is considerable overlap between the groups.

Since the relief from pruritus which accompanies

cholestyramine therapy is generally associated with a fall in serum total bile acids, it might be anticipated that a particular bile acid is responsible for this association (Ross *et al*, 1970). However, no positive association between any particular individual bile acid fraction and the presence or absence of pruritus could be demonstrated. The G/T ratio does appear to have some significance in that the mean level ( $0.78 \pm 0.23$ ) found when pruritus was present was significantly different ( $P < 0.001$ ) from that found when pruritus was absent ( $1.39 \pm 0.45$ ) (Fig. 2). This is also in accord with the results of Neale *et al* (1971), who found an increase in the proportion of glycine to taurine conjugates in two of their three cases after treatment. Kenny and Garbutt (1970) have demonstrated in normal subjects that the major alteration of bile acid metabolism by cholestyramine administration was enhanced hepatic synthesis of glycocholic acid. However, studies *in vitro* (Hagerman, Cook, and Schneider, 1971) have demonstrated that cholestyramine has a greater affinity for taurine-conjugated than glycine-conjugated bile acids. Three of the highest values for the G/T ratio in the present study were found in serum from patients in hepatic failure (cases 1<sup>1</sup>, 7<sup>1</sup>, 15<sup>1</sup> in Table I). Two of these patients had been taking cholestyramine for longer than two years. This suggests that the increase in G/T ratio observed in the present study is a consequence of cholestyramine selectivity rather than an indication of improved liver function. It may be that the association observed between pruritus and bile acids is a reflection of amino acid distribution rather than a direct consequence of bile acid metabolism.

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