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Serum bile acids in liver disease

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SUMMARY Serum bile acids have been measured in patients with a wide variety of liver diseases using a technique which separates the major individual conjugated and free bile acids. Total serum bile acids may be elevated up to 100 times the normal concentration in patients with liver disease and this increase consists largely of conjugated bile acids. The ratio of glycine-conjugated to taurine-conjugated bile salts is low in all types of liver disease and this is found particularly in the serum of patients with obstructive jaundice. There is a decrease in the ratio of trihydroxy:dihydroxy cholanic acid in patients with cirrhosis.

Serum bile acids have been measured by many workers in an attempt to produce an index useful in the differential diagnosis of liver pathology. The observation that total serum bile acids are increased in hepatobiliary disease (Sherlock and Walshe, 1948) has been confirmed many times (Carey, Figen, and Watson, 1955; Rudman and Kendall, 1957; Osborn, Wootton, da Silva, and Sherlock, 1959; Carey, 1961; Sandberg, Sjövall, Sjövall, and Turner, 1965; Sjövall and Sjövall, 1966). Measurements of conjugated and unconjugated serum bile acids have been the subject of more recent reports (Rauterau, Chevrel, and Caroli, 1967; Makino, Nakagawa, and Mashimo, 1969) but the detailed pattern of individual conjugated bile acids in the circulation has been described so far only in patients with the stagnant loop syndrome (Lewis, Panveliwalla, Tabaqchali, and Wootton, 1969). In this paper we report the concentration of individual bile acids in sera drawn from patients with a wide variety of liver diseases.

Patients Studied

Thirty-nine patients in hospital for the diagnosis and treatment of hepatobiliary disease were studied. Diagnoses were made on the basis of clinical findings, haematological assessment, liver function tests, liver biopsy, and, in some cases, peritoneoscopy or laparotomy. Blood samples were obtained after overnight fasting and the sera separated and stored at $-20°C$ until analysis. The same specimens of sera were used for the measurement of bilirubin, enzyme, and cholesterol concentrations.

The 39 patients were chosen to give a wide spectrum of liver disease. There were nine cases of hepatitis and one of Weil's disease. Fifteen patients had obstructive jaundice, which was due to an extrahepatic obstruction of the biliary tree in nine cases and intrahepatic cholestasis in the other six. Seven patients, of whom four were alcoholics, had cirrhosis. The aetiology of the cirrhosis in the other three patients was not determined. Four patients had ulcerative colitis or Crohn's disease with associated liver disease, the histological nature of which was assessed by examination of specimens obtained by needle biopsy of the liver. Finally, three patients with unconjugated hyperbilirubinaemia due to Gilbert's syndrome were also studied.

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Materials and Methods

Individual bile acids were isolated from 2 to 10 ml of fasting serum by ion-exchange chromatography and determined by fluoroimetry (Panveliwalla, Lewis, Wootton, and Tabaqchali, 1970). By this method, the normal range is $3{\cdot}11 \pm 0{\cdot}69$ μmol/l. In normal serum unconjugated bile acids are not detectable: the glycine/taurine ratio of serum bile acids is $4{\cdot}3 \pm 2{\cdot}4$, and their trihydroxy/dihydroxy ratio is $1{\cdot}8 \pm 0{\cdot}65$. Other biochemical measurements were carried out using standard methods (Wootton, 1964).

Results

The results are tabulated in Table I.

TOTAL SERUM BILE ACID CONCENTRATIONS

In all varieties of liver disease studied, except unconjugated hyperbilirubinaemia, serum bile acid concentrations were elevated, up to 100 times the mean normal level. There was a statistically significant correlation of total serum bile acids with serum

bilirubin, but not with serum cholesterol or serum enzymes (Fig. 1). There were no significant differences in total serum bile acids between the various groups. Thus values of more than 50 μmol/l were found in four of nine patients with hepatitis, in seven of nine patients with extrahepatic obstructive jaundice, and in three out of seven patients with cirrhosis.

The correlation between total serum bile acids and the presence or absence of pruritus was not clear-cut (Fig. 2). Patients with pruritus had values ranging from 15·4 to 285 μmol/l (mean 126 μmol/l) whilst those who did not have this symptom had values ranging from 2·7 to 225 μmol/l (mean 39·4 μmol/l). Twelve out of 15 patients with serum bile acid concentrations exceeding 50 μmol/l complained of pruritus. Only three out of 15 patients with serum bile acid concentrations of less than 50 μmol/l had this symptom.

SERUM BILE ACID PATTERNS

In all groups most of the circulating bile acids were

Paiient	*Disease*	*Sex and Age*	*Liver Function Tests*					*Total Bile Acids*[1] *(normal: 3·1 ± 0·7, μmol/l)*
			Bilirubin (normal: 0·1-0·8 mg/100 ml)	*Alkaline Phosphatase (normal: 3-11 K-A units/100 ml)*	*5¹-NT (normal: 2-17 IU/100 ml*	*I-C D (normal: 3-8 mg/100 ml)*	*Cholesterol (normal: 150-250 mg/100 ml)*	
Hepatitis								
1	IH	F 35	3·9	20	—	60	—	6·2
2	IH	M 23	1·5	12	25	8	210	8·3
3	IH	M 41	3·4	13	12	19	—	9·6
4	CAH	F 20	2·3	17	41	9	220	13·4
5	IH	F 40	11·2	22	—	11	—	34·0
6	IH	F 68	7·2	13	8	63	180	126·1
7	IH	F 39	14·7	14	14	150	—	151·0
8	IH	M 44	21·0	19	17	45	210	203·7
9	IH	M 23	7·3	14	—	85	140	225·0
Weil's disease								
10		M 21	42·0	9	—	16	110	58·3
Obstructive jaundice (extrahepatic)								
11	Bile duct stricture	F 42	1·1	65	—	9	260	15·4
12	Ca panc	M 48	—	—	—	—	—	24·3
13	Ca panc	F 56	—	—	—	—	—	72·9
14	Ca panc	M 65	15·0	45	46	15	370	93·0
15	Ca panc	F 43	—	—	—	—	—	118·3
16	Sclerosing cholangitis	F 34	7·9	49	—	12	445	123·8
17	Ca panc	M 53	14·5	46	100	15	355	150·3
18	Ca panc	M 54	16·0	63	33	6	255	270·2
19	Ca panc	M 52	26·0	39	—	10	—	285·3
Obstructive jaundice (intrahepatic)								
20	Lymphosarcoma	M 38	—	—	—	—	—	13·3
21	Drug jaundice	M 45	2·6	30	32	8	320	21·8
22	Drug jaundice	F 37	0·9	83	145	—	310	30·1
23	PBC	F 42	11·0	52	132	15	—	37·3
24	PBC	F 56	7·8	132	—	—	700	67·7
25	PBC	F 52	2·7	45	82	13	255	157·5
Cirrhosis								
26	Cryptogenic	F 51	0·6	14	30	6	135	2·7
27	Alcoholic	F 48	0·8	11	—	10	160	11·3
28	Cryptogenic	F 50	1·4	14	11	5	295	12·2
29	Alcoholic	M 51	0·9	15	42	13	—	19·8
30	Alcoholic	F 58	3·1	21	35	12	225	54·6
31	Cryptogenic	F 48	2·2	19	14	9	155	79·5
32	Alcoholic	M 69	1·2	14	12	10	220	100·6
Colitis and Crohn's disease								
33	Reactive hepatitis	F 28	0·4	9	—	10	125	5·6
34	Granulomata	M 44	0·5	17	33	5	190	11·6
35	Pericholangitis	M 62	6·5	70	145	18	320	28·1
36	Hepatic vein thrombosis	M 48	1·9	26	21	6	140	32·9
Unconjugated hyperbilirubinaemia								
37	Gilbert's	M 22	2·5	5	7	3	160	nd
38	Gilbert's	M 28	2·5	5	—	2	—	1·2
39	Gilbert's	M 21	3·6	6	6	3	155	4·9

Table I *Liver function tests and serum bile acids in the patients studied*[1]

IH = infective hepatitis; CAH = chronic active hepatitis; Ca panc = carcinoma of the head of the pancreas; PBC = primary biliary cirrhosis; TC = taurocholic acid; TCD = taurochenodeoxycholic acid; GC = glycocholic acid; GCD = glycochenodeoxycholic acid; C = cholic acid; CD = chenodeoxycholic acid; nd = not detectable.

[1]Normal values of serum bile acids published previously (Panveliwalla *et al*, 1970).

conjugated. At levels of total bile acids of over 50 μmol/l more than 88% were conjugated.

The ratio of glycine to taurine conjugation tended to be low in the serum bile acids of all patients with liver disease of short duration. Thus it was less than 2·0 in all nine patients with hepatitis and in all except one patient with recent obstructive jaundice. The mean ratio was lowest in patients with cholestasis and highest in those with cirrhosis (Fig. 3). This pattern, however, had no diagnostic value because of extensive overlapping of the various groups of patients. The glycine/taurine ratio showed no correlation with the total bile acid concentration. A markedly low ratio of glycine/taurine conjugation was found much more frequently in patients with pruritus than in those without (Fig. 4).

The ratio of trihydroxy bile acids to dihydroxy bile acids was reduced in the serum of patients with cirrhosis. The mean value in this group of patients was 0·7 compared with mean values of 1·2-1·7 for the other four diagnostic groups. There was, however, a considerable overlap of individual values in

Conjugated Bile Acids				*Free Bile Acids*		*Ratio*		
TC (normal: 0·38 ± 0·20, μmol/l)	*TCD (normal: 0·30 ± 0·08, μmol/l)*	*GC (normal: 1·6 ± 0·7, μmol/l)*	*GCD (normal: 0·86 ± 0·30, μmol/l)*	*C (μmol/l)*	*CD (μmol/l)*	*Free : Total*	*Glycine : Taurine (normal: 4·3 ± 2·4; range: 1·1-9·6)*	*Tri-OH : Di-OH (normal: 1·8 ± 0·6; range: 0·7-2·6)*
1·0	1·1	1·7	1·5	0·9	nd	0·15	1·5	1·3
1·7	0·9	2·5	2·1	nd	1·1	0·13	1·7	1·0
1·4	2·1	3·4	2·7	nd	nd	—	1.7	1·0
3·7	3·5	2·0	4·2	nd	nd	—	0·9	0·7
4·1	8·5	6·0	7·2	8·2	nd	0·24	1·0	1·2
50·0	19·5	34·2	20·8	1·6	nd	0·02	0·8	2·2
20·5	34·2	58·6	35·2	2·5	nd	0·01	1·7	1·2
73·5	34·4	33·2	51·5	9·3	1·8	0·11	0·8	1·3
40·2	36·3	52·6	89·2	4·7	2·0	0·03	1·8	0·8
20·5	16·5	8·8	8·9	1·9	1·7	0·06	0·5	1·2
2·5	2·8	2·8	5·8	nd	1·5	0·10	1·6	0·5
3·6	3·5	7·1	1·6	5·3	3·3	0·38	1·8	1·9
12·4	20·0	10·0	28·9	1·0	0·6	0·02	1·0	0·5
18·9	17·3	45·6	2·0	4·6	4·6	0·10	1·3	2·9
22·5	39·2	25·9	30·1	0·2	0·3	—	0·9	0·7
25·8	22·0	33·0	35·8	5·0	2·2	0·06	1·4	1·1
54·0	36·8	35·4	20·4	2·3	1·3	0·02	0·6	1·6
62·7	68·2	65·0	72·1	2·2	nd	0·02	1·0	1·0
104·0	61·2	68·1	51·8	0·2	nd	—	0·7	1·5
1·3	1·6	5·2	3·0	1·2	1·0	0·17	2·8	1·4
9·5	3·6	6·5	2·2	nd	nd	—	0·7	2·7
9·3	4·0	12·6	4·2	nd	nd	—	1·3	2·7
11·9	8·4	9·5	6·2	0·5	0·8	0,04	0·8	1·4
20·3	13·0	20·4	14·0	nd	nd	—	1·0	1·5
56·0	57·0	16·3	26·0	1·5	0·7	0·01	0·4	1·0
0·6	0·6	0·7	0·8	nd	nd	—	1·4	1·0
1·4	1·6	2·4	3·4	1·6	0·9	0·22	1·9	0·9
0·7	1·5	4·0	3·0	1·3	0·9[a]	0·30	3·2	1·1
3·4	1·9	4·1	8·5	0·8	1·1	0·09	1·9	0·8
8·1	2·8	11·9	28·8	1·6	1·4	0·05	3·7	0·6
9·7	22·2	7·1	31·4	3·9	3·8[a]	0·11	1·2	0·4
12·8	25·8	17·2	44·1	0·5	0·2	0·01	1·6	0·2
1·2	nd	1·9	1·1	nd	1·4	0·25	2·5	0·8
2·9	nd	5·5	3·2	nd	nd	—	3·0	2·6
6·9	2·7	9·2	9·3	nd	nd	—	1·9	1·3
9·0	9·6	4·6	3·0	2·5	4·2	0·20	0·4	1·0
id	nd	nd	nd	nd	nd	—	—	—
id	nd	1·2	nd	nd	nd	—	—	—
id	nd	2·7	2·2	nd	nd	—	—	—

Table I *Liver function tests and serum bile acids in the patients studied*—continued

[a] Free deoxycholic acid detected 0·8 μmol/l.
Free deoxycholic acid detected 1·4 μmol/l.

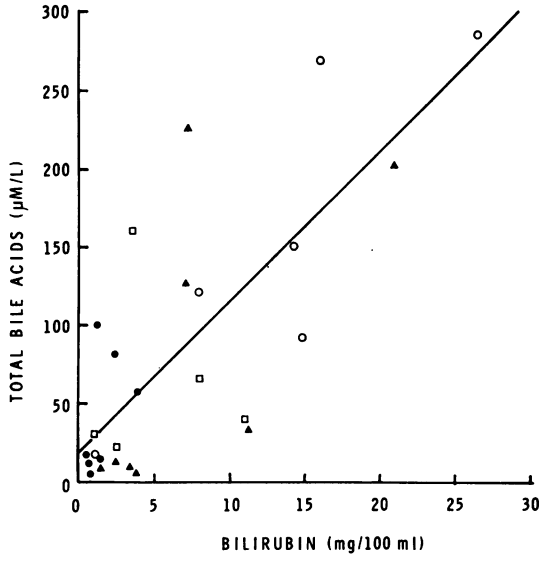

Fig. 1 *Fasting serum total bile acids (μmol/l) plotted against serum bilirubin ($y = 9{\cdot}3x + 21$; $r = 0{\cdot}76$; $P < 0{\cdot}01$). ●—Cirrhosis; ▲—hepatitis; ○—extrahepatic obstruction; □—intrahepatic obstruction.*

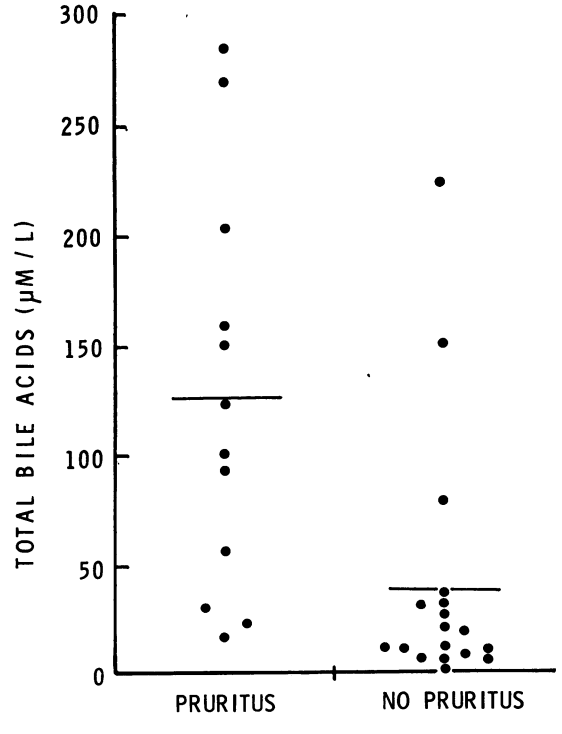

Fig. 2 *Concentrations of fasting total serum bile acids in patients with and without pruritus.*

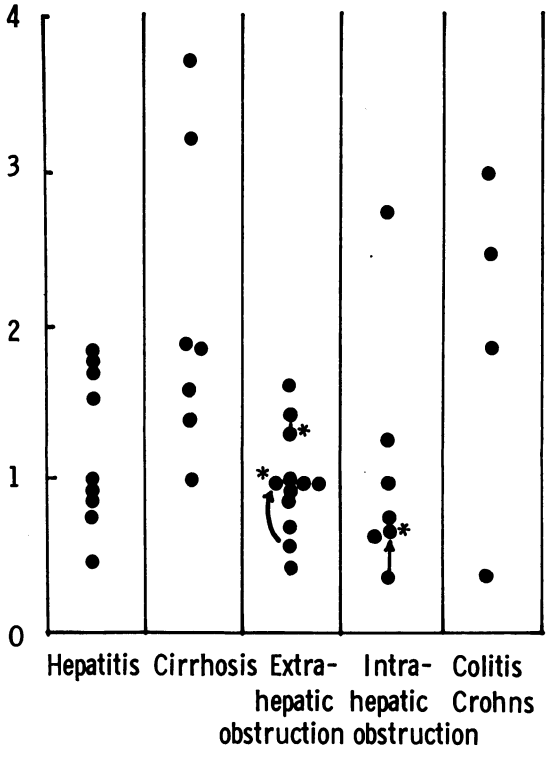

Fig. 3 *Ratio of glycine conjugated bile acids to taurine conjugated bile acids in fasting serum (* = on cholestyramine).*

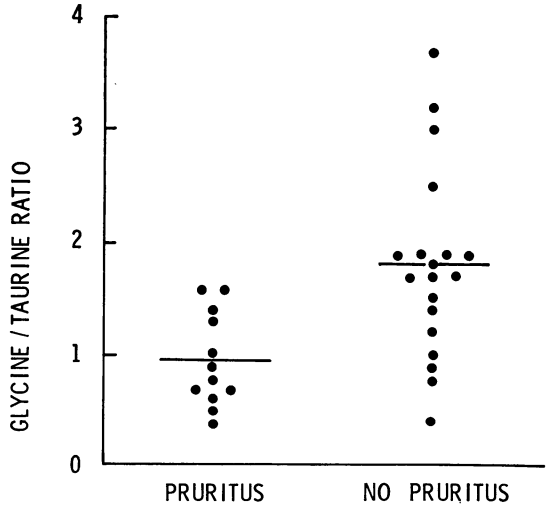

Fig. 4 *Ratios of glycine conjugated bile acids to taurine conjugated bile acids in patients with and without pruritus.*

the five groups (Fig. 5). In patients with cirrhosis, there was a negative correlation between the ratio of trihydroxy bile acids and total serum bile acids, although the numbers were too small for statistical evaluation. (Fig. 6). This correlation could not be shown in patients with other conditions. Lithocholic acid was not detected in the serum of any patient.

Serum bile acids were measured serially in a patient suffering from infective hepatitis (Fig. 7). The highest total serum bile acid concentration was found early in the course of the disease before the patient was markedly icteric and was already falling when serum bilirubin level had reached a peak. Subsequently, the serum bilirubin level and serum bile acids fell in parallel.

EFFECT OF TREATMENT OF OBSTRUCTIVE JAUNDICE

Total serum bile acids were measured in three patients before and after the administration of cholestyramine which was given in an attempt to

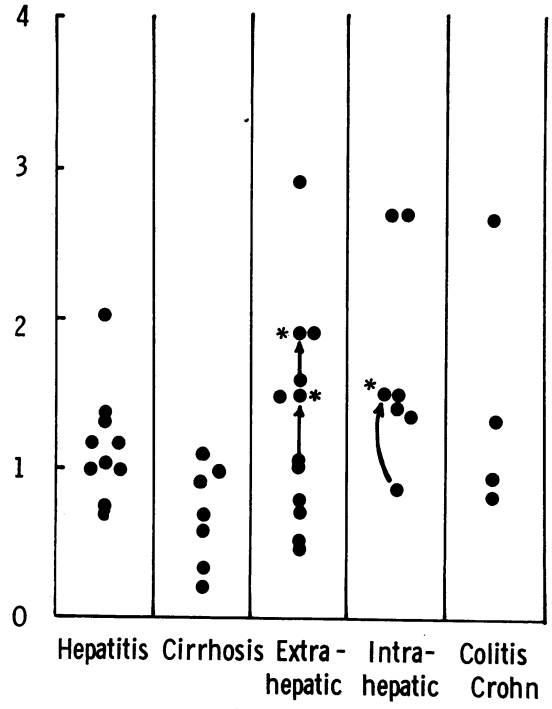

Fig. 5.

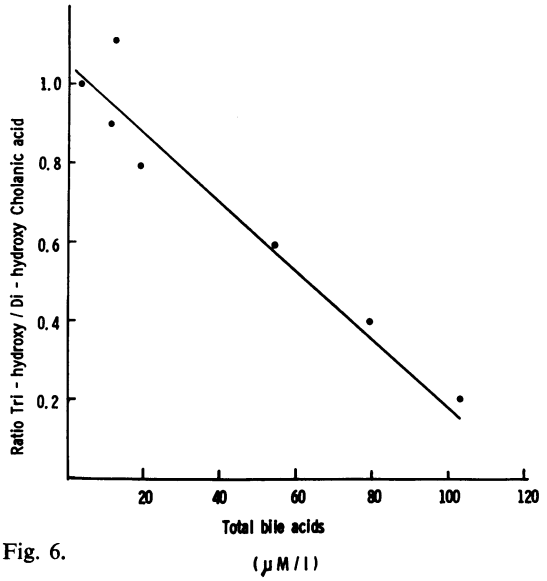

Fig. 6.

Fig. 5 *Ratio of trihydroxy to dihydroxy bile acids in fasting serum.*

Fig. 6 *Correlation of the ratio of trihydroxy to dihydroxy bile acids in fasting serum with total bile acids in patients with cirrhosis* ($y = 1{\cdot}05 - 0{\cdot}0083x$; $r = -0{\cdot}97$).

Fig. 7 *Changes in fasting total bile acid concentration in a patient with infective hepatitis.* ●—*Total bile acids* (*μmol/l*); ▲—*bilirubin* (*mg/100 ml*); ○—*ICD* (*IU*); □—*LDH* (*IU*).

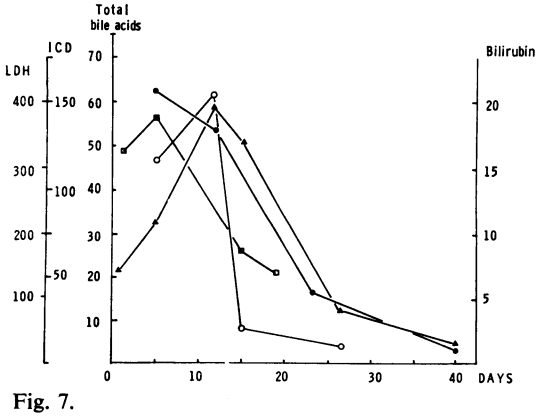

Fig. 7.

Patient		Treatment	Bilirubin (mg/100 ml)	Cholesterol	Bile Acids (μmol/l)							Bile Acid Ratios		
					Total	TC	TCD	GC	GCD	C	CD	Free : Total	Glycine : Taurine	Tri-OH : Di-OH
16	Stricture due to fibrosclerosis[1]	Nil	7·9	445	123·8	25·8	22·0	33·0	35·8	5·0	2·2	0·06	1·4	1·1
		Cholestyramine	7·5	445	82·0	22·0	11·0	24·7	19·9	2·6	1·8	0·05	1·3	1·5
		Prednisone	2·2	315	16·6	0·7	0·9	3·8	7·7	3·0	—	0·18	7·2	0·9
17	Carcinoma of the pancreas	Nil	14·5	355	150·3	54·0	36·8	35·4	20·4	2·3	1·3	0·02	0·6	1·6
		Cholestyramine	14·6	485	90·1	30·1	15·1	28·6	16·3	—	—	—	1·0	1·9
25	Primary biliary cirrhosis	Nil	2·7	255	157·5	56·0	57·0	16·3	26·0	1·5	0·7	0·01	0·4	1·0
		Cholestyramine	2·6	230	85·6	28·8	15·2	15·6	14·7	1·3	—	0·02	0·7	1·1

Table II *Effect of treatment on serum bile acids (key as in Table I)*

This patient has been reported elsewhere (Gleeson, Taylor, and Dowling, 1970).

reduce pruritus. The results are shown in Table II. In all three patients there was a marked reduction of total serum bile acids by between 30 and 40%; the serum bilirubin levels remained unchanged. This reduction was associated with some improvement in symptoms, although in no case was the pruritus relieved entirely. The reduction in total serum bile acids was associated with an increase in the ratio of trihydroxy to dihydroxy bile acids in all patients and an increase in the proportion of glycine to taurine conjugates in two of three cases.

In contrast with the clear-cut reduction in the level of circulating bile salts, there was no fall in serum cholesterol. One patient (J.W.) with multifocal fibrosclerosis which had caused a stricture of the common hepatic duct was treated successfully with prednisone. The total serum bile acids fell from 82 μmol/l to 16 μmol/l.

UNCONJUGATED HYPERBILIRUBINAEMIA

Of the three patients studied, one showed the unique finding of undetectable serum bile acids in two specimens, analysed on different occasions, and another had a subnormal concentration. Taurine conjugates were not detected in the serum of any of these patients.

Discussion

In this paper we describe work undertaken to try to characterize the pattern of bile acids in the sera of patients with hepatic and biliary disease more clearly than has been possible hitherto. We have used the method described by Panveliwalla *et al* (1970) by which it is possible to measure the major conjugated and free bile acids. The technique does not separate conjugates of deoxycholic acid from conjugates of chenodeoxycholic acid; this is probably not of great importance as other workers have shown that the increase of circulating conjugated chenodeoxycholic acid is relatively small in hepatobiliary disease (Makino *et al*, 1969).

TOTAL BILE ACID CONCENTRATIONS IN SERUM

Serum bile acid levels were found to be elevated in all forms of liver disease except Gilbert's syndrome. The defect of bilirubin excretion in Gilbert's syndrome clearly does not affect the excretion of bile salts. Indeed in two of three cases studied, fasting serum bile acids appeared to be below the normal range, and in one patient they could not be detected in the serum on two separate occasions.

In all the other conditions studied the total serum bile acids were elevated and results were similar to those reported by Sherlock and Walshe (1948), Carey *et al* (1955), Rudman and Kendall (1957), and Makino *et al* (1969). There is a significant correlation between total serum bile acids and serum bilirubin but the range of total serum bile acids for the various diagnostic groups overlaps markedly and thus this estimation does not appear to be of value in the differential diagnosis of liver disease. No correlation could be demonstrated between serum bile acids and the other indices of liver disease measured (serum alkaline phosphatase, 5[1]-nucleotidase, isocitric dehydrogenase, and cholesterol).

One patient with infective hepatitis was followed for six weeks and repeated estimations showed that total serum bile acids were markedly elevated early in the disease and fell slowly to normal values. It seems that serum bile acids are a sensitive parameter of hepatic function in infective hepatitis (Frosch and Wagener, 1969).

FREE BILE ACIDS

It has not been possible to demonstrate free bile acids in the serum of *fasting* normal subjects by the method used in this study (Panveliwalla *et al*, 1970) although small concentrations have been detected by gas chromatography of unhydrolysed serum extracts (Sandberg *et al*, 1965; Makino *et al*, 1969). The maximum concentration of free bile acids found in the serum of patients with liver disease was only 11·1 μmol/l (a patient with infective hepatitis in

whom the total serum bile acid concentration was 204 μmol/l). There was no correlation between the concentration of free bile acids and the amount of total bile acids in the circulation. The highest ratio of free to total bile acids was found in patients with cirrhosis of the liver (1 to 30%). Makino *et al* (1969) suggested that the conjugation of bile acids with taurine or glycine might be defective in cirrhosis of the liver and that the finding of free bile acids could be a useful diagnostic index in the differential diagnosis of portal cirrhosis, chronic hepatitis, and obstructive jaundice. Our findings do not support this thesis.

It has been shown previously (Lewis *et al*, 1969) that free bile acids are found in the circulation of patients with the stagnant loop syndrome in whom there is significant deconjugation of bile acids by bacteria in the small intestine. Further work is necessary to determine whether a similar mechanism may operate in patients with chronic liver disease, some of whom may have significant colonization of the small intestine with colonic bacteria (Martini, Phear, Ruebner, and Sherlock, 1957; Gorbach, Lal, and Levitan, 1970).

Free bile acids were not found in high concentration in the sera of four patients with inflammatory bowel disease in whom needle biopsy specimens of liver had shown histological abnormalities. Patient 33 had non-specific reactive hepatitis, patient 34 had granulomata in the liver, patient 35 had pericholangitis, and patient 36 had hepatic venous occlusion. Free bile acids were not detected in the circulation of two of these three patients and in the third (patient 36) the concentration of cholic acid was 2·5 μmol/l and of chenodeoxycholic acid 4·2 μmol/l. Deoxycholic acid and lithocholic acid were not detected. Thus these studies on the bile acids of peripheral blood do not support the suggestion that lithocholic or other free bile acids absorbed from inflamed intestine may cause liver disease (Carey, 1964).

DEGREE OF HYDROXYLATION

In series previously quoted it has been shown that the ratio of trihydroxy to dihydroxy cholanic acids is low in patients with cirrhosis, and Carey (1958) suggested that this index has a prognostic value. We have confirmed these findings and have shown a correlation between total serum bile acids and the ratio of trihydroxy to dihydroxy cholanic acid in seven patients with histologically proven cirrhosis (Fig. 5). The mean trihydroxy: dihydroxy cholanic acid ratio of patients with obstructive jaundice was twice as high as that obtained in patients with cirrhosis. The distinction was not as clear-cut as described by Carey (1958) and was more in accord with the results of Makino *et al* (1969). The lowest ratio recorded in this group was 0·5. This result was found in a patient with terminal chronic non-suppurative destructive cholangitis (primary biliary cirrhosis) which had reached stage IV of the disease (fully developed cirrhosis). The alteration in metabolic pathways causing a reversal of the bile acid ratio in severe liver disease has not yet been elucidated.

CONJUGATION OF CIRCULATING BILE ACIDS TO GLYCINE AND TAURINE

The ratio of glycine to taurine conjugation was less than the mean normal value of 4·3 in all patients with hepatitis or biliary tract disease except Gilbert's syndrome. There was no correlation between total serum bile acids and the ratio of glycine to taurine conjugation. The lowest ratios were found in patients with obstructive jaundice (both intra- and extrahepatic) but with some overlapping of the diagnostic groups. These findings are consistent with studies *in vitro* on the conjugation of bile acids by homogenates of liver tissue obtained from patients with hepatic disease (Ekdahl, 1958).

PRURITUS

The cause of pruritus in patients with liver disease is still not completely clear although there is a good deal of evidence to implicate bile salts. As in other studies (Carey, 1958; Rauterau *et al*, 1967) the correlation between total serum bile acid concentrations and the presence or absence of pruritus was not distinct (Fig. 2). Nevertheless, patients with a total serum bile acid concentration of more than 50 μmol/l are very likely to complain of pruritus. There was no relationship of pruritus to the concentration of circulating free bile acids nor to the ratio of trihydroxy to dihydroxy cholanic acid. Patients with pruritus tended to have a low ratio of glycine to taurine conjugated bile acids. This probably reflects the reduction in glycine conjugation which occurs with severe obstructive jaundice, although it does raise the question of whether bile acids conjugated with taurine are deposited more readily in the skin or free nerve endings than those conjugated with glycine. The problem is complicated by the finding that 85% of bile acids extractable from skin are free (Schoenfield, Sjövall, and Perman, 1967), and yet in patients with the stagnant loop syndrome very high levels of circulating free bile acids (up to 50 μmol/l) may occur without pruritus (Lewis *et al*, 1969). Further studies will be necessary to elucidate this problem.

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References

Carey, J. B., Jr. (1958). The serum trihydroxy-dihydroxy bile acid ratio in liver and biliary tract disease. *J. clin. Invest.*, **37**, 1494-1503.

Carey, J. B., Jr. (1961). Bile acids in the serum of jaundiced patients. *Gastroenterology*, **41**, 285-287.

Carey, J. B., Jr. (1964). Bile acids, cirrhosis and human evolution. *Gastroenterology*, **46**, 490-493.

Carey, J. B., Jr., Figen, J., and Watson, C. J. (1955). The bile acids in normal human serum with comparative observations in patients with jaundice. *J. Lab. clin. Med.*, **46**, 802-803.

Ekdahl, P. H. (1958). On the conjugation and formation of bile acids in the human liver. VI. On the conjugation of cholic acid-24-^{14}C in human liver homogenates in various diseases with special reference to patients with jaundice. *Acta chir. scand.*, **115**, 208-226.

Frosch, B., and Wagener, H. (1969). Conjugated bile acids in the serum of patients with acute hepatitis. *Germ. med. Mth.*, **14**, 121-126.

Gleeson, M., Taylor, S., and Dowling, R. H. (1970). Multifocal fibrosclerosis. *Proc. roy. Soc. Med.*, **63**, 1309-1311.

Gorbach, S. L., Lal, D., and Levitan, R. (1970). Intestinal microflora in Laennec's cirrhosis. *J. clin. Invest.*, **49**, 36a.

Lewis, B., Panveliwalla, D., Tabaqchali, S., and Wootton, I. D. P. (1969). Serum-bile-acids in the stagnant-loop syndrome. *Lancet*, **1**, 219-220.

Makino, I., Nakagawa, S., and Mashimo, K. (1969). Conjugated and unconjugated serum bile acid levels in patients with hepatobiliary diseases. *Gastroenterology*, **56**, 1033-1039.

Martini, G. A., Phear, E. A., Ruebner, B., and Sherlock, S. (1957). The bacterial content of the small intestine in normal and cirrhotic subjects: relation to methionine toxicity. *Clin. Sci.*, **16**, 35-51.

Osborn, E. C., Wootton, I. D. P., Silva, L. C. da, and Sherlock, S. (1959). Serum-bile-acid levels in liver disease. *Lancet*, **2**, 1049-1053.

Panveliwalla, D., Lewis, B., Wootton, I. D. P., and Tabaqchali, S. (1970). Determination of individual bile acids in biological fluids by thin-layer chromatography and fluorimetry. *J. clin. Path.*, **23**, 309-314.

Rauterau, M., Chevrel, B., and Caroli, J. (1967). Les acides biliaires sanguins en hépatologie. *Rev. med.-chir. Mal. Foie*, **42**, 167-236.

Rudman, D., and Kendall, F. E. (1967). Bile acid content of human serum. I. Serum bile acids in patients with hepatic disease. *J. clin. Invest.*, **36**, 530-537.

Sandberg, D. H., Sjövall, J., Sjövall, K., and Turner, D. A. (1965). Measurement of human serum bile acids by gas-liquid chromatography. *J. Lipid Res.*, **6**, 182-192.

Schoenfield, L. J., Sjövall, J., and Perman, E. (1967). Bile acids on the skin of patients with pruritic hepatobiliary disease. *Nature (Lond.)*, **213**, 93-94.

Sherlock, S., and Walshe, V. (1948). Blood cholates in normal subjects and in liver disease. *Clin. Sci.*, **6**, 223-234.

Sjövall, K., and Sjövall, J. (1966). Serum bile acid levels in pregnancy with pruritus. (Bile acids and steroids 158.) *Clin. chim. Acta*, **13**, 207-211.

Wootton, I. D. P. (1964). *Macro-analysis in Medical Biochemistry*, 4th ed., p. 145. Churchill, London.