DETERMINATION OF SULFATED AND NONSULFATED BILE ACIDS IN SERUM BY MASS FRAGMENTOGRAPHY

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ABSTRACT

A Sep-Pak C_{18} cartridge was used for purification of bile acids from serum. Three kinds of deuterium labeled internal standards were required for accurate measurement of individual sulfated and nonsulfated bile acids. These internal standards were added to the serum before its application to the cartridge. Separation of sulfated and nonsulfated bile acids was performed on piperidinohydroxypropyl Sephadex LH-20 column chromatography. The nonsulfate fraction was submitted to alkaline hydrolysis, and the sulfate fraction to solvolysis followed by alkaline hydrolysis. Each fraction was converted to the hexafluoroisopropyl-trifluoroacetyl derivatives and quantitated by mass fragmentography. The recovery of each bile acid sulfate was quite satisfactory. In fasting healthy subjects the mean of total nonsulfated bile acids in serum was 1.324 µg/ml, and that of total sulfated bile acids was 0.450 µg/ml. Sulfated lithocholic acid comprised a large part of sulfated bile acids in healthy subjects.

INTRODUCTION

Several methods have been developed for the determination of sulfated bile acids in serum (1-4). However, the data for healthy controls in these reports are not consistent with each other. For example, the content of lithocholic acid sulfate (LC-S) in normal sera varies widely from 0 to 0.24 µg/ml (mean value) (1,3). Makino et al. (1), who first reported the concentrations of sulfated bile acids in human serum, failed to detect LC-S. Campbell et al. (3) showed the existence of a large amount of LC-S in serum, using an analytical method comprised of carefully selected extractive techniques to prevent loss of lithocholic acid, but they did not detect deoxycholic acid sulfate in 10 healthy subjects. Recently, Beppu et al. (5) reported a method for the determination of individual nonsulfated

bile acids and sulfated lithocholic acid in serum, using highly selective ion monitoring mass fragmentography. By reducing the number of purification steps, they established an excellent method in which loss of each bile acid was minimal. The average content of total lithocholic acid in serum of healthy controls determined by their method was 0.166 μg/ml, and that of LC-S was 0.117 μg/ml, which was obtained from the difference between the contents of total and nonsulfated lithocholic acid. Because of these conflicting data, we have studied aiming at establishing a more precise assay method for individual sulfated bile acids. Furthermore, this paper reports concentrations of individual sulfated and nonsulfated bile acids in serum of healthy subjects and patients with hepatobiliary diseases.

METERIALS AND METHODS

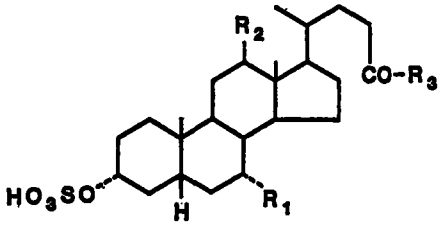
Chemicals

Lithocholic acid (LC), deoxycholic acid (DC) and cholic acid (C) were obtained from Sigma Chemicals Co. (St. Louis, MO). Taurocholic acid (T-C) was obtained from Steraloids Inc. (Wilton, NH), and glycolithocholic acid (G-LC), lithocholic acid 3-sulfate, glycolithocholic acid 3-sulfate (G-LC-S) and taurolithocholic acid 3-sulfate (T-LC-S) were obtained from P-L Biochemicals Inc. (Milwaukee, WI). Chenodeoxycholic acid (CDC) was supplied by Yamanouchi Pharmacy Co., Tokyo, and ursodeoxycolic acid (UDC) by Tokyo Tanabe Pharmacy Co., Tokyo. These commercial bile acids were tested for purity by thin layer chromatography and found to be sufficiently pure. [11,11,12β-$^2H_3$] Deoxycholic acid (D-DC) and [2,2,4,4-$^2H_4$] lithocholic acid (D-LC) were synthesized chemically. The synthetic procedure for these deuterium labeled bile acids was previously reported in detail by Takikawa et al. (6). Sep-Pak C_{18} cartridges were obtained from Waters Associates Inc. (Milford, MA). Sephadex LH-20 was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Piperidino-hydroxypropyl Sephadex LH-20 (PHP-LH-20) was prepared by the method of Goto et al. (7) and stored in the acetate form in 90 % ethanol. Trifluoroacetic anhydride was obtained from Nakarai Chemicals, Kyoto, and hexafluoroisopropanol from Tokyo Kasei Kogyo, Tokyo. All other reagents were of analytical grade.

Bile acid sulfates

Bile acid 3-monosulfates (deoxycholic acid 3-monosulfate, DC-S;

glycodeoxycholic acid 3-monosulfates, G-DC-S; taurodeoxycholic acid 3-monosulfate, T-DC-S; chenodeoxycholic acid 3-monosulfate, CDC-S; and cholic acid 3-monosulfate, C-S) were synthesized by the method of Goto et al. (8). These synthesized 3-monosulfates were recrystallized in the eluate from methanol-ether (Fig. 1).



1) R_1=H, R_2=R_3=OH DC-S

2) R_1=R_3=OH, R_2=H CDC-S

3) R_1=R_2=R_3=OH C-S

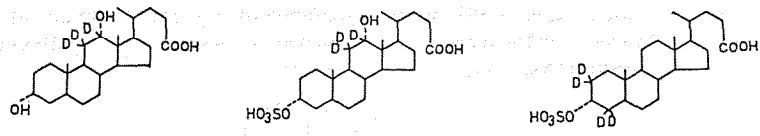
4) R_1=H, R_2=OH, R_3=NH-CH_2-COOH G-DC-S

5) R_1=H, R_2=OH, R_3=NH-CH_2-CH_2-SO_3H T-DC-S

Fig. 1. Bile acid sulfates synthesized according to the method of Goto et al.(8)

Deuterium labeled deoxycholic acid 3-monosulfate (D-DC-S) was also synthesized from D-DC by the same method, but [2,2,4,4-$^2H_4$] lithocholic acid 3-monosulfate (D-LC-S) was synthesized from D-LC as described by Palmer and Bolt (9) (Fig. 2). Melting points of bile acid sulfates were measured with a Büch micro melting point apparatus and were uncorrected. ^{1}H-NMR spectra were measured on a JEOL PS-100 spectrometer with DSS as an internal standard. IR spectra were taken with a JASCO D-701G spectrophotometer. Mass spectra were recorded with a Shimadzu LKB-9000B mass spectrometer.

Physical properties of these products were as follows: DC-S, mp 215-216°C, lit. mp 202-203°C (8), 225-239°C (10) ; NMR (D_2O, δ ppm) 0.70 (18-CH_3), 0.92 (19-CH_3), 4.01 (12β-H), 4.30 (3β-H)(8); IR (KBr) 3410, 1560, 1410, 1230, 1060, 995, 940 (cm^{-1}) (11). G-DC-S, mp 205-206°C, lit. mp 198-199°C (8), 218-220°C (11) ; NMR (D_2O, δ ppm) 0.71 (18-CH_3), 0.94 (19-CH_3), 3.73 (=N-CH_2CO-), 4.05 (12β-H), 4.34 (3β-H) (7); IR (KBr) 3440, 1590, 1225, 1060, 1035, 970, 940 (cm^{-1}) (11). T-DC-S, mp 186-188°C(dec), lit. 183-184°C (8), 189-191°C (11) ; IR (KBr) 3440, 1630, 1540, 1215, 1050, 970, 940 (cm^{-1}) (11). CDC-S, mp 204-206°C, lit. mp 201-202°C (8), 207-209°C (10), 210-212°C (11) ; NMR (D_2O, δ ppm) 0.68 (18-CH_3), 0.90 (19-CH_3), 3.84 (7β-H), 4.18 (3β-H) (8) ; IR (KBr) 3420, 1560, 1440, 1225,



[11,11,12β-$^2H_3$] DC [11,11,12β-$^2H_3$] DC-S [2,2,4,4-$^2H_4$] LC-S

Fig. 2. Deuterium labeled DC, DC-S and LC-S.

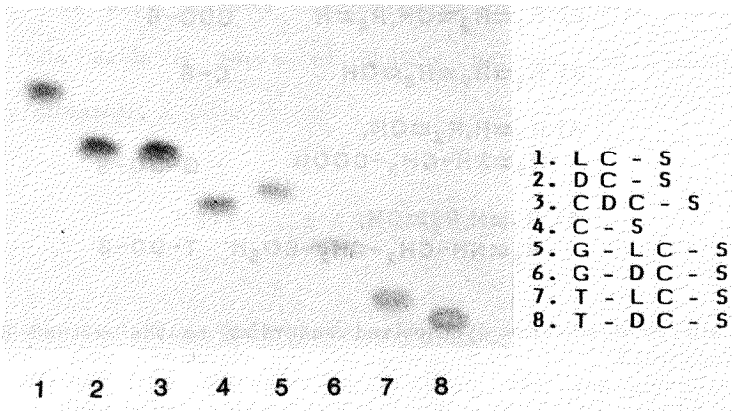


Fig. 3. Thin layer chromatogram of bile acid sulfates.

1060, 960 (cm^{-1}) (11). C-S, mp 205-207°C, lit. mp 198.5-199.5°C (8), 220-233°C (10) ; NMR (D_2O, δ ppm) 0.70 (18-CH_3), 0.93 (19-CH_3), 0.99 (21-CH_3), 3.89 (7ß-H), 4.06 (12ß-H), 4.12 (3ß-H) (8); IR (KBr) 3410, 1560, 1225, 1060, 965 (cm^{-1}) (11). D-DC-S, IR (KBr) 3410, 1560, 1405, 1225, 1060, 995, 940 (cm^{-1}); Mass spectrometry : d_4 6%, d_3 91%, d_2 2%, d_1 0%, d_0 0%. D-LC-S, mp 196-198°C; IR (KBr) 1560, 1405, 1215, 1060, 960, 940 (cm^{-1}); Mass spectrometry : d_5 5%, d_4 81%, d_3 13%, d_2 0%, d_1 0%, d_0 0%.

Thin layer chromatography was carried out with silica gel precoated plates (E. Merck, Darmstadt), and separation of individual bile acid sulfates was achieved with a solvent mixture of chloroform, methanol, acetic acid and water (13:4:2:1, v/v) (Fig. 3). Bile acids were detected by spraying 50% sulfuric acid and heating the plate to about 100°C.

Conditions for mass fragmentography

Mass fragmentography was performed by the method reported previously (5,6). A combined GC-MS instrument, GC-MS 9000B (Shimadzu-LKB, Kyoto, Japan), equipped with a data system (GC-Mass-Pack 90) was employed. Gas chromatographic separation was carried out on a 1-m glass column (3 mm, ID) packed with 3% DC-QF-1 on Gas Chrom Q (80-100 mesh). Helium was used as the carrier gas at a flow rate of 40 ml/min. The temperatures of the column oven, injection port, separator and ion source were maintained at 215°C, 225°C, 230°C and 230°C, respectively. The ionization energy was set at 70 eV, ionization trap current at 60 μA and ion acceleration voltage at 3.5 KeV.
The bile acids were analyzed as hexafluoroisopropyl-trifluoroacetyl (HFIP-TFA) derivatives (11). The following ions were used: LC, m/z 622 (M^+); DC, CDC and UDC, m/z 620 ($M^+ - CF_3COOH$); C, m/z 618 ($M^+ - 2xCF_3COOH$); D-LC, m/z 626 (M^+); D-DC, m/z 623 ($M^+ - CF_3COOH$).

General procedures

The method described by Makino et al. (1) was extensively modified for the qualitative and quantitative analysis of sulfated and nonsulfated bile acids in serum. Briefly, our procedure consists of the following successive steps: Purification through Sep-Pak C_{18} cartridges, PHP-LH-20 column chromatography for the separation of sulfated and nonsulfated bile acids, solvolysis for the sulfate fraction, alkaline hydrolysis, preparation of HFIP-TFA derivatives, and mass fragmentography.

Solid-liquid extraction of bile acids with Sep-Pak C_{18} cartridges

A Sep-Pak C_{18} cartridge was prewashed in the following sequence : 5 ml of methanol, 10 ml of distilled water, 5 ml of ethanol, 10 ml of distilled water, 10 ml of methanol and finally 10 ml of distilled water. 0.5 ml of a serum sample was diluted with 5 ml of 0.5 M phosphate buffer (pH 7.0) and introduced onto the Sep-Pak C_{18} cartridge. The adsorbed bile acids were eluted with 5 ml of methanol after washing with 5 ml of distilled water. For sample analysis, the internal standards (D-DC, D-LC-S and D-DC-S) were added to serum and sonicated for several minutes with buffer before passing through the cartridge.

Separation of sulfated and nonsulfated bile acids on PHP-LH-20 column chromatography

The eluate from the Sep-Pak C_{18} cartridge was evaporated to dryness and the residue was dissolved in 2 ml of 90% ethanol. This solution was then added to a column (6.5 x 20 mm), containing 140 mg of PHP-LH-20, which had been packed and equilibrated overnight with the same solvent. After washing with 2 ml of 90% ethanol, the nonsulfated bile acids were eluted with 7 ml of 0.3 M acetic acid-potassium acetate in 90% ethanol (pH 6.5). The elution was carried out at a flow rate of 4 ml/hr. The eluate was evaporated to dryness and subjected to alkaline hydrolysis. The sulfates were then eluted with 8 ml of 1% sodium carbonate in 70% ethanol at a flow rate of 8 ml/hr. After evaporation in a round-bottom flask, the sulfate fraction was reapplied onto a Sep-Pak C_{18} cartridge for desalting. The eluate from the cartridge was submitted to solvolysis.

Solvolytic procedure

The method for solvolysis which was employed in the sample analysis was essentially the same as that of Parmentier and Eyssen (13). The sulfate fraction was dissolved in 0.5 ml of methanol, 4.5 ml of acetone and 50 µl of 4N HCl. The solution was incubated at 37°C for 12 hr. After neutralization with ammonium hydroxide, it was evaporated to dryness under a stream of air, and the residue was subjected to alkaline hydrolysis. The other solvolytic procedure which was reported previously by Beppu et al. (5) was also tested.

Alkaline hydrolysis and derivatization

The sulfate and nonsulfate fractions were separately subjected to alkaline hydrolysis for 4 hr with 2 ml of 4 N aqueous NaOH at 120°C in an autoclave (5). The hydrolysate was acidified to pH 1 with 2 N HCl and then extracted three times with ether. Ether extracts were evaporated to dryness and derivatized to the HFIP-TFA conjugates.

Patients studied

Fifteen control subjects (7 males and 8 females) were studied. These subjects showed no clinical evidence and normal values for serum albumin, glutamic oxaloacetic transaminase and bilirubin. Eight patients (4 males and 4 females) with extrahepatic obstructive jaundice and 8 patients (7 males and one female) suffering from compensated liver cirrhosis were studied. The diagnosis of extrahepatic obstructive jaundice was confirmed by several clinical investigations and from operative findings, and that of liver cirrhosis by liver biopsy. Peripheral venous blood was collected from all subjects following a 12 hour overnight fast. Serum samples were stored at -20°C until analyzed.

Results

Mass fragmentography of individual bile acids

Figure 4 shows a mass fragmentogram of a mixture of five authentic bile acids together with D-LC and D-DC obtained by tracing the respective ions mentioned under "Materials and Methods". Amounts of individual bile acids were calculated automatically under computer control. For the nonsulfate fraction, the five bile acids were calculated from the peak area ratios to D-DC. For the sulfate fraction, LC was calculated from the peak area ratio to D-LC and the others from the ratios to D-DC. All determinations were based on standard calibration curves.

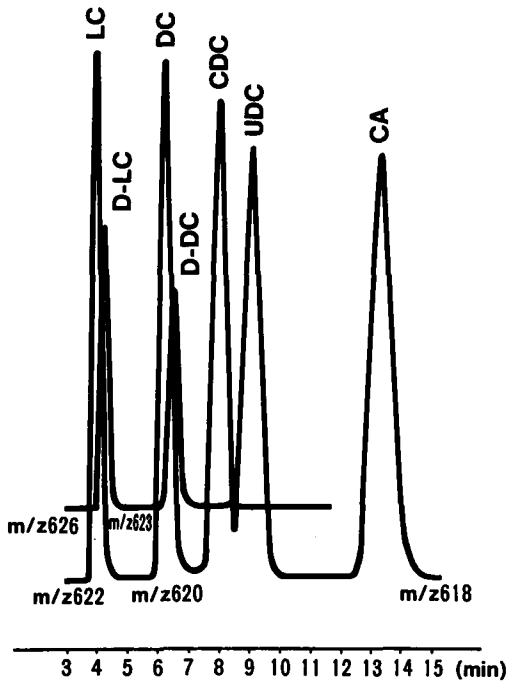


Fig. 4. Mass fragmentogram of five authentic bile acids and deuterium labeled bile acids on monitoring the respective ions.

Purification of bile acids with Sep-Pak C_{18} cartridges

Recoveries of bile acid sulfates added to pooled serum (0.5 ml) are shown in Table I. Although the recovery of LC-S, G-LC-S and T-LC-S was about 50% , it was improved by adding D-LC as an internal standard to the serum samples before introdution onto the cartridge. In contrast with serum, recovery experiments of bile acid sulfates on water solution gave good results of 98% for LC-S, 95% for G-LC-S, 103% for T-LC-S, 98% for DC-S, 105% for CDC-S and 94% for C-S.

Separation of sulfated and nonsulfated bile acids on PHP-LH-20 column chromatography

The exchange capacity of PHP-LH-20 was 0.8 mEq/g, which was

Table I. Recovery rate of sulfated bile acids in serum on Sep-Pak C_{18} cartridges.

Bile acid added	Amount added (nmol)	Amount recovered (nmol)	Recovery rate (%)
LC-S	0.684	0.342	50.0
DC-S	0.618	0.633	102.4
CDC-S	0.709	0.733	103.2
C-S	0.766	0.750	97.9
G-LC-S	0.560	0.291	52.0
G-DC-S	0.553	0.539	97.5
T-LC-S	0.504	0.223	44.2
T-DC-S	0.524	0.431	82.3

(Means of triplicate determinations)

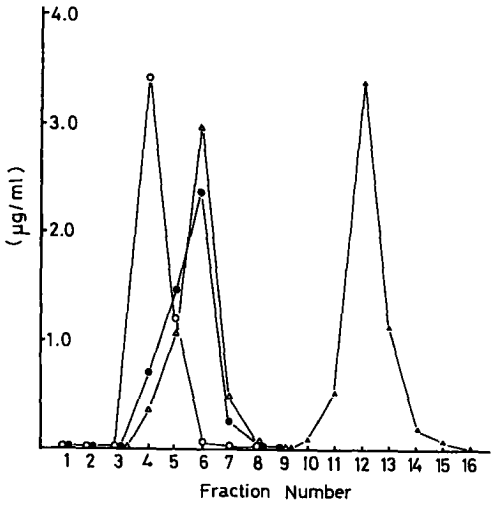


Fig. 5. Separation of sulfated and nonsulfated bile acids on PHP-LH-20 column chromatography.(○,CDC; ●,G-DC; △,T-C; ▲ LC-S).

obtained by adding T-C to the column. As shown in Fig. 5, the sulfates and nonsulfates were completely separated on PHP-LH-20 column chromatography. The nonsulfates were eluted in fractions 3-8 with 0.3 M acetic acid-potassium acetate in 90% ethanol, while the sulfates were eluted in fractions 10-15 with 1% sodium carbonate in 70% ethanol. The flow rate of elution critically influenced the separation of bile acids. When the elution was carried out at a flow rate of 8 ml/hr instead of 4 ml/hr, an increased elution volume was needed in order to elute all nonsulfates in the nonsulfate fraction and a small amount of the sulfates (LC-S) contaminated the nonsulfate fraction.

Conditions for solvolysis

Sulfated bile acids were dissolved in 0.5 ml of methanol and 4.5 ml of acetone with 50 µl of 4 N HCl. The solution was incubated at 4°C and 37°C. At 37°C, bile acid 3-monosulfates were completely solvolysed regardless of the number of hydroxyl groups in the steroid ring structure. Figure 6 shows the time course of the solvolysis at different temperatures. Solid lines represent the time courses at 37°C, and dashed lines those at 4°C. Solvolysis of bile acid 3-monosulfates was completely achieved within 6 hr at 37°C. Although the difference between LC-S and C-S was not distinct at 37°C, it was shown in the experiment at 4°C that the liberation of the sulfate group was faster in LC-S than in C-S.

Liberation of the sulfate group during ether extraction

Sulfated bile acids were dissolved in 2 ml of 1 N HCl and an equal volume of ether was added. The samples were incubated at 25°C and then vigorously shaken for about one minute every one hour. At various times, the ether layer was separated, evaporated to dryness, derivatized and subjected to mass fragmentography. D-DC as the internal standard was

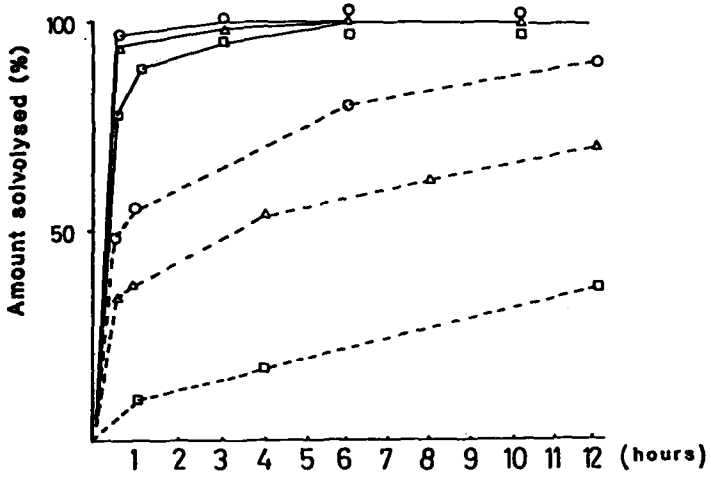


Fig. 6. Time course of solvolysis at 4°C (---) and 37°C (——). (○ ,LC-S; △ ,DC-S; □ ,C-S).

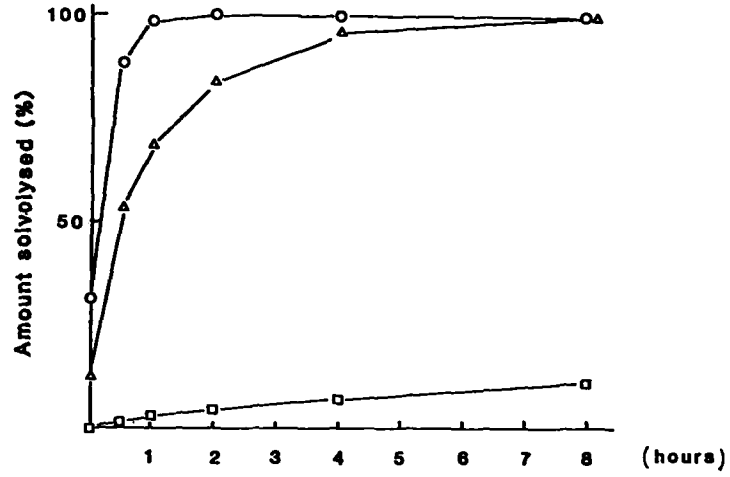


Fig.7. Liberation of the sulfate group during ether extraction.(○ ,LC-S; △ ,DC-S; □ ,C-S).

added to the sample prior to the derivatization. The results of solvolysis during ether extraction from acidic aqueous solution are given in Fig. 7. LC-S and DC-S were solvolysed quantitatively in 2 hr and 8 hr, respectively. However, only 10% of C-S was solvolysed even after 8 hr incubation.

Analysis of serum samples

Figure 8 shows a mass fragmentogram of the sulfate fraction from serum of a healthy subject. The separation of each peak was satisfactory and no interfering compounds appeared. With the present method, 0.5 ml of serum was enough to determine the amount of sulfated as well as nonsulfated bile acids in healthy subjects.

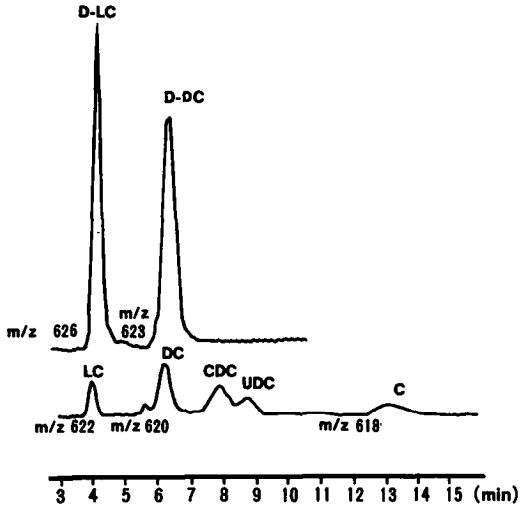


Fig. 8. Mass fragmentogram of the sulfated fraction from the serum of a healthy subject with internal standards.

Recoveries of both sulfated and nonsulfated bile acids are between 82 and 109 % for the entire procedure (Table II). Replicate analysis (n=6) of pooled serum showed the following coefficient variation : LC-S, 6.6% ; DC-S, 6.1% ; CDC-S, 5.9% ; sulfated ursodeoxycholic acid, 6.7% ; C-S, 10.2%.

Serum levels of sulfated and nonsulfated bile acids

Table III shows the concentrations of sulfated and nonsulfated bile acids in serum from fasting healthy subjects, from patients with extrahepatic obstructive jaundice and from patients with compensated liver cirrhosis. In healthy subjects, sum of the sulfated bile acids comprised on about 25% of total serum bile acids and LC-S was the major sulfated bile acid.

Table II. Recovery of nonsulfated and sulfated bile acids added to 500µl of pooled serum and carried through the entire procedure.

Bile acid added	Amount added (nmol)	Amount recovered (nmol)	Recovery rate (%)
LC	1.89	1.94	103
DC	1.51	1.48	98
CDC	1.43	1.38	97
UDC	1.43	1.35	94
C	1.79	1.87	104
G-LC	1.53	1.31	86
T-C	1.48	1.21	82
LC-S	0.684	0.687	100
DC-S	0.618	0.604	98
CDC-S	0.709	0.727	103
C-S	0.766	0.836	109
G-LC-S	0.560	0.546	98
G-DC-S	0.553	0.540	98
T-LC-S	0.504	0.451	89
T-DC-S	0.524	0.461	88

The mean concentration of total sulfates in patients with extrahepatic obstructive jaundice was 7.759 μg/ml, which comprised about 16 % of serum bile acids, and that in patients with compensated liver cirrhosis was 0.803 μg/ml, about 10% of serum bile acids. In these pathological conditions, CDC-S was the major sulfated bile acid. The percentages of

Table III. Amounts of sulfated and nonsulfated bile acids in serum of healthy subjects and patients with hepatobiliary diseases (μg/ml, mean ± SD) and the sulfated ratio to total.

Materials		LC	DC	CDC	UDC	C	Total
Healthy subjects (n=15)	NS[a]	0.014 ±0.013	0.381 ±0.226	0.533 ±0.278	0.045 ±0.048	0.326 ±0.289	1.324 ±0.722
	S[b]	0.205 ±0.075	0.110 ±0.063	0.093 ±0.049	0.029 ±0.048	0.013 ±0.012	0.450 ±0.160
	%sulfates[c]	94%	22%	15%	39%	4%	25%
Obstructive jaundice (n=8)	NS	0.049 ±0.044	0.519 ±0.223	15.884 ±9.854	0 0	23.931 ±13.203	40.384 ±19.963
	S	0.545 ±0.313	1.237 ±1.641	5.551 ±2.815	0	0.427 ±0.485	7.759 ±3.390
	%sulfates	92%	70%	26%		2%	16%
Liver cirrhosis (n=8)	NS	0.023 ±0.016	0.352 ±0.432	3.877 ±1.824	0.116 ±0.102	2.478 ±1.621	6.845 ±3.484
	S	0.113 ±0.070	0.145 ±0.127	0.431 ±0.186	0.066 ±0.066	0.047 ±0.034	0.803 ±0.336
	%sulfates	83%	29%	10%	36%	2%	10%

[a] NS : nonsulfates

[b] S : sulfates

[c] %sulfates = (sulfates / nonsulfates + sulfates) x 100

total sulfates in serum total bile acids were significantly decreased ($p < 0.001$) in patients with liver cirrhosis when compared to healthy controls, but not significantly different between patients with obstructive jaundice and healthy controls, or between patients with obstructive jaundice and those with liver cirrhosis. However, the difference of percentages of sulfates in CDC was significant ($p<0.02$) between patients with obstructive jaundice and those with liver cirrhosis.

DISCUSSION

The Sep-Pak C_{18} cartridge has been used for the extraction and purification of bile acids from biological fluids with good results (14, 15). These authors determined the recovery rate of bile acids on this cartridge to be good enough. However, purification of sulfated bile acids from serum resulted in lower recoveries (16). In our study it was shown that while the recovery rate was about 50% for LC-S and its conjugates, that of other sulfates, DC-S, CDC-S and C-S, was in an agreeable range. The possible reason for the low recovery of LC-S is that it may be bound most firmly to the plasma protein (possibly albumin) among these sulfates (17, 18). To remove this drawback of the cartridge, two deuterium labeled bile acid sulfates (D-LC-S and D-DC-S) should be added as internal standards to the sulfate fraction. With the method of mass fragmentography using deuterium labeled internal standards, any possible loss is favourably compensated.

Sephadex LH-20 column chromatography has widely been used for the separation of bile acid sulfates from nonsulfates since the report of Makino *et al.* (19). Recently, some weak points of the use of Sephadex LH-20

have been reported by several workers (20, 21). Eyssen et al. (20) reported that the presence of a large amount of nonsulfated bile acids in the sample resulted in the elution of trace amounts of nonsulfates in the sulfate fraction. Pageaux et al.(21) reported incomplete separation of conjugated nonsulfates and unconjugated sulfates. Takikawa et al. (6) used PHP-LH-20 column for the group separation of nonglucuronide-nonsulfates, glucuronides and sulfates, after enzymatic hydrolysis of bile acid conjugates. Furthermore, sulfates can be completely separated from conjugated nonsulfates on this column (22), which has also been confirmed in the present study. As shown in Fig. 5, the separation of T-C and S-LC was complete on PHP-LH-20. Since T-C is the most polar of nonsulfates and LC-S the least polar of sulfates, other less polar nonsulfates and more polar sulfates are easily separated.

Trimethylsilyl or trifluoroacetyl derivatives of bile acid methyl esters have frequently been used for gas chromatographic analysis. However, Edenharder and Slemr (23) indicated the superiority of HFIP-TFA derivatives because of the simplicity of preparation, absence of artifacts and good resolution on a QF-1 column. Beppu et al. (5) reported that the HFIP-TFA derivatives were superior to other conventional derivatives since each bile acid specific fragment ion in the high mass region could be used for monitoring C_{24} bile acids without interference of C_{27} sterols in mass fragmentography.

The present results indicate that a large proportion of LC in serum was sulfated in healthy controls, which means that the sulfation of monohydroxy bile acids is very important pathway even in healthy people. The contents of LC-S shown in this study are somewhat higher than that reported by Beppu et al. (5). The reason for this discrepancy is

unclear, but it is considered that the liberation of the sulfate group from LC-S does occur to some extent during ether extraction from acidic medium even with particular care. Van Berge-Henegouwen et al. (24) showed that the solvolysis of bile acid sulfates was easily achieved with a brief incubation in ether containing HCl after deconjugation by alkaline hydrolysis. Our study also showed that LC-S is easily solvolysed in HCl-ether solution, whereas C-S is hardly solvolysed. Two-phase solvolysis can be achieved with great ease (5), but considering the serious disadvantage described above, this procedure was abandoned for the routine assaying of the five major bile acids.

The percentage of sulfates in total bile acids was lower in serum of patients with liver cirrhosis than healthy subjects. The present results appear to imply that healthy subjects have a greater sulfotransferase activity than patients with liver cirrhosis. There was no significant difference in the percentage of total sulfates between patients with liver cirrhosis and obstructive jaundice. In our study, sulfate esters of CDC accounted for 26% in patients with obstructive jaundice, which was significantly greater than 10% in patients with liver cirrhosis. Although Stiehl (2) did not mention the difference in the percentage of CDC-S, patients with cholestatic liver cirrhosis whom Stiehl studied should differ from those with compensated liver cirrhosis in our study.

Our data are only an initial step in examination of the possible relationships between bile acid sulfates in serum and liver diseases. The results in this study should encourage further studies of serum bile acid assays in various hepatobiliary diseases and examination of the physiological significance of the activity of bile acid sulfotransferase in humans.

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