Analysis of the Total Bile Acid Distribution in Human Intestinal Aspirates by Gas Liquid Chromatography

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ABSTRACT

A method for the quantitative determination of the total bile acid content of human intestinal aspirates is presented. This method was applied to duodenal samples from ten normal subjects and distal jejunal samples from seven of these subjects. Enzymatic and radioactive monitoring of the procedures utilized yielded recoveries of 89 to 100 percent of bile acids.

Introduction

A limited number of studies on the analysis of total bile acid distribution in intestinal samples of normal human subjects is available. A wide variety of techniques of bile acid analysis have been applied to samples from normal subjects including: paper chromatography, paper chromatography with radioisotope studies, thin layer chromatography (TLC) and radioisotope studies, column chromatography and enzyme analysis utilizing 3-hydroxysteroid dehydrogenase (STDH).

A single study using gas liquid chromatography (GLC) to determine intestinal bile acid distribution in normal subjects has been reported. These investigators obtained intestinal aspirates from levels ranging from 100 to 250 cm rather than duodenal samples alone as reported in previous studies. This study is in general agreement with previous publications except that lithocholic acid (mean 1 to 3 percent) not previously noted in normal subjects was present at all levels of the small intestine. Cholecystokinin was used to stimulate gallbladder contraction.

The present study provides additional information on the total bile acid distribution by GLC in duodenal and jejunal aspirates from ten normal subjects. The techniques utilized to prepare samples for GLC are different from those used by Mallory et al and this allows comparison of results obtained by two different preparatory methods. In addition, recovery experiments are reported utilizing the STDH assay as an intrinsic bile acid monitoring system and by adding labelled taurocholate. A small meal, rather than cholecystokinin, was used as the physiologic stimulus for gallbladder emptying.
Materials and Methods

SAMPLE COLLECTION

Ten normal subjects, five males and five females, ages 21 to 45 years, were studied. After an overnight fast, a modified Cantor tube was positioned under fluoroscopic guidance at the ligament of Treitz. Samples of duodenal fluid were aspirated thirty minutes after a test meal, consisting of one slice of buttered toast and a cup of tea with sugar. The tube was then passed into the distal jejunum where additional aspirates were obtained. All samples were collected on ice, centrifuged immediately and aliquots of 2.0 ml were frozen at —20° until processed.

TOTAL BILE ACID ANALYSIS BY GLC

Intestinal samples for determination of bile acid distribution were processed as follows: 20 ml of absolute alcohol was added to a 2.0 ml aliquot of aspirate. The sample was placed in a boiling waterbath for ten minutes, cooled and centrifuged. After the addition of 4.6 ml N NaOH, the supernatant was extracted three times with 20 ml of petroleum ether. The combined ether extracts were washed with a mixture of 3.5 ml water and 6.5 ml N NaOH in 50 percent ethanol, this wash being added to the sample. The sample was mixed with 4 ml of 10 N NaOH, transferred into nickel crucibles and the bile acids were hydrolyzed for three hours in an autoclave (15 psi, 122°). After hydrolysis, the pH was adjusted to 1.1 and the sample was stored at 4° overnight. The sample was then sequentially extracted with 35, 50, 35, 50 ml chloroform, passed through anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 0.5 ml methanol and 4.5 ml diethyl ether. The methyl esters of the bile acids were prepared by adding fresh diazomethane until a yellow color persisted and were dried under nitrogen. Trifluoroacetic anhydride, 0.5 ml, was added to each sample and allowed to react for thirty minutes at 38°. The sample was dried and dissolved in 0.3 ml acetone immediately prior to gas liquid chromatography.

GLC analyses were performed on glass columns (4 mm id x 4 ft) packed with 2 percent QF-1 on 100/200 Gas Chrom Q. The operating temperatures were: injection port, 260°; column oven, 245°; hydrogen flame detector, 260°. Helium was used as the carrier gas at a flow rate of 60 ml per min.

The area of each bile acid on the chromatograph was measured by triangulation and expressed as area per μl solution injected. Four injections per sample were assayed and the values averaged. The individual bile acids were calculated as the percentage of the total bile acid content. Standard bile acid solutions were determined daily to confirm retention times. Each bile acid gave a quantitative response between 0.75 and 2.80 μg with standard solutions.

RECOVERY EXPERIMENTS

Intestinal samples from three normal subjects were processed according to the methods cited (vide supra) except that aliquots were taken after protein precipitation for comparison with aliquots taken after chloroform extraction. All aliquots were dried, redissolved in methanol and assayed for bile acid concentration by a modification of the STDH procedure of Iwata and Yamasaki. Intestinal aspirates of two patients, selected because of an abnormal bile acid distribution characterized by absent deoxycholic acid, were similarly monitored.

Radioactive sodium-taurocholate-(Carbonyl 14C) was purified by TLC on Silica Gel G. A 0.2 ml aliquot of the purified material in benzene:ethanol (3:2) containing 500,000 dpm was added to the initial supernatant of the intestinal aspirates of one normal subject and a patient selected because of an abnormal bile acid distribution

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characterized by absent deoxycholic acid. Two aliquots were removed from this supernatant to be compared to duplicate aliquots taken from a methanolic solution after all hydrolysis and extraction procedures. These aliquots were analyzed by STDH and by determining their radioactivity by liquid scintillation spectrometry. Both Aquasol (3.5 ml water and 15 ml Aquasol) and PPO (5 ml ethanol and 15 ml 2,5 diphenyloxazole-5.5 g per liter toluene) were used as solvents.

Results
The distribution of the bile acids in intestinal aspirates of ten normal subjects is summarized in table I. The mean percent distribution of the duodenal samples was: deoxycholic acid, (D), 27.7 ± 10.6 percent; chenodeoxycholic acid, (CDC), 34.4 ± 5.5 percent; and cholic acid, (C), 37.3 ± 8.2 percent. The corresponding bile acid distribution of distal jejunal samples (seven subjects) was: D, 23.5 ± 7.5 percent; CDC, 35.9 ± 4.8 percent; and C, 38.6 ± 10.0 percent. Statistical analysis of the data from the seven subjects with both samples showed significant differences in distribution between the duodenal and jejunal bile acids:

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<th>Subject</th>
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<th>CDC</th>
<th>C</th>
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Mean: 27.7 ± 34.4 ± 37.3 ± 2.0 ± 23.5 ± 35.9 ± 38.6
S.D.: ±10.6 ±5.5 ±6.2 ±2.3 ±7.5 ±4.8 ±10.0

TABLE I
Percent Bile Acid Distribution in Intestinal Samples of Normal Subjects

D, 0.05 > p > 0.025; CDC, p = 0.01; and C, 0.005 > p > 0.001.

Overall recovery of the bile acid content in aspirates from normal subjects was calculated by two techniques. Using the STDH enzyme assay to evaluate recovery after the final chloroform extraction, samples from three normal subjects had 100 percent, 100 percent and 99 percent of the STDH activity of the original sample. Two samples containing an abnormal bile acid distribution with absent deoxycholic acid yielded recoveries of 66 percent and 84 percent. By radioactive sodium taurocholate recovery, the final extract in a normal sample contained 92 percent of the initial radioactivity and 98 percent of the original STDH activity, while the abnormal sample contained only 81 percent of the initial radioactivity and 84 percent of the original STDH activity.

Discussion
Bile acid data from GLC analysis of normal human intestinal aspirates are relatively sparse. This paper reports the bile acid distribution in ten normal subjects. The only other comparable study, that of Mallory et al.6 (lithocholic acid (L), 2 percent; D, 20 percent; CDC, 34 percent; C, 44 percent) shows good agreement in duodenal bile acid distribution with data here reported L < 1 percent; D, 28 percent; CDC, 34 percent; C, 37 percent). However, the data of jejunal bile acid distribution in that series (L, 1 percent; D, 17 percent; CDC, 30 percent; C, 52 percent) differs somewhat from this report (L, 2 percent; D, 23 percent; CDC, 36 percent; C, 39 percent). Mean values in the two studies do not reveal a significant difference in bile acid distribution between duodenal and jejunal samples. In the present report, however, statistical analyses of D, CDC and C in individual subjects reveal significant differences in bile acid distribution at the two sites. This difference reflects the metabolism and/or absorption of bile acids during passage through the intestinal tract.
Metabolism and absorption of individual bile acids vary between subjects and, therefore, are not reflected in the mean data.

The presence of L in intestinal aspirates of normal subjects was confirmed by this series. Although Mallory et al obtained collections after cholecystokinin while the physiologic stimulus of a small meal was used in the present study, the duodenal bile acid distribution was similar. The largest discrepancy between the two studies is observed in the reported levels of distal jejunal C. The cause of this discrepancy is uncertain; however, preparation of samples for GLC analysis differed in two respects. In the present study, neutral lipid and steroid materials were removed with petroleum ether before hydrolysis rather than by TLC after hydrolysis; and extraction of hydrolyzed bile acids was achieved with chloroform rather than with diethyl ether. Studies in our laboratory have shown chloroform and diethyl ether to be equally effective, four extractions being required in both cases for complete recovery. If a procedural difference accounts for the variation in the reported cholic acid levels, it most likely reflects the different lipid extraction technique.

The reliability of the bile acid extraction procedure reported here was evaluated by two separate techniques. By STDH assay, the bile acid content of the initial sample was compared with the material after all hydrolysis and extraction steps, yielding recoveries of 100 percent, 100 percent and 89 percent. This indicates no appreciable loss of bile acid. This finding was appreciated by the recovery of added radioactive sodium taurocholate in one sample, 92 percent, in good agreement with the STDH assay of the same sample, 98 percent.

Surprisingly, this excellent level of recovery observed in material with normal bile acid distribution was not duplicated in samples with abnormal bile acid distribution. In two patient samples containing no measurable deoxycholic acid, lower recoveries were obtained. By STDH assay, recoveries after extraction were only 66 percent and 84 percent with the latter sample being confirmed by a radioactive taurocholate recovery of 81 percent. Therefore, bile acid data from patient samples may not be exactly comparable to data from normal subjects owing to losses during extraction.

The present investigation offers a relatively easy and reliable GLC procedure for total bile acid analysis of intestinal fluid. The duodenal bile acid distribution of ten normal subjects and the jejunal bile acid distribution of seven of these subjects is reported adding to the limited available data in normal man.

References