Gas-liquid Chromatography of Human Bile Acids.* (26555)

ROLF BLOMSTRAND
Swedish Medical Research Council, Unit for Biochemical Research on Atherosclerosis, University of Lund, Sweden

Recent results from different laboratories (1,2,3) indicate that most of the biologically important steroids may be identified and quantitated with the aid of gas-liquid chromatography (GLC). VandenHeuvel, Sweeley and Horning(4) describe the separation of some bile acids and sex hormones with the aid of GLC and their technic offers a new way to a better understanding of the metabolism of steroids and diseases related to these compounds.

In the present paper, separation of bile acids from human bile with the aid of GLC has been evaluated and preliminary results are reported on the quantitative relationship between the major bile acids in man in some different conditions.

Methods. Bile was collected by aspiration into ethanol from the gallbladder of patients without hepatobiliary disease and also from patients with gallstones and biliary tract disease. Samples of duodenal contents were also collected through polyvinyl tubing introduced nasally. The tubing was introduced in the evening and the sample collected the next morning after intravenous injection of purified preparation of pancreozymin-cholecystokinin, ("Cecekin," obtained from Vitrum, Sweden). All samples were frozen and kept at −16°C.

As a rule 5 ml of the bile and the intestinal contents were extracted with 20 volumes of ethanol, filtered and evaporated to almost dryness *in vacuo*. Water was added to the concentrate and mixed with ethyleneglycol and 4 N sodium hydroxide in the proportions water + bile : ethyleneglycol : sodium hydroxide 2 : 1 : 1, and this mixture was refluxed on a sand bath for 24 hours. After hydrolysis and extraction with ether, the bile acids were partitioned 4 times between 30 ml of petroleum ether and 50 ml of 70% ethanol. The bile acids were then dissolved in methanol and methylated with diazomethane. The methyl esters of the bile acids were then dissolved in cyclohexanol and taken up with a micropipette.

Conditions for Gas-liquid chromatography:
Pye Argon Chromatograph.
Column dimensions: 1300 × 4 mm I.D. straight glass tubing.
Solid support Celite (80/100 mesh), acid washed, alkali treated.
Stationary Phase: Silicone Elastomer, E. 301 I.C.I. Lim. Nobel Division, Glasgow, England, Stationary phase 12% by weight of solid support.
Temperatures: Injection 280°C. Column and detector 245°C.
Carrier gas: Argon at 70 ml/min. Inlet pressure ca. 1.5 atm.
Outlet pressure: atm.
Detector: Beta ionization detector at 1,000 V.
Recorder: Philip recorder, 1 volt full scale.
Sample size: 0.5 μl, 5% solution in cyclohexanol.
Analysis time: ca. 45 min.

The response of the beta ionization detector is primarily a function of molecular weight with argon as carrier gas. Under the conditions used there was a tendency toward somewhat lower values for the slower-moving trihydroxy bile acids and correspondingly high values for the faster moving monohydroxy bile acids. It was therefore necessary to calibrate the instrument with a test mixture of known composition and use the correction factor obtained for the peak areas in order to calculate the relative distribution of the bile acids in human bile.

Results. Fig. 1 illustrates the separation of a test mixture of lithocholic, deoxycholic, chenodeoxycholic, hyodeoxycholic and cholic acid. A good separation was obtained between the 3 dihydroxy bile acids. For clini-

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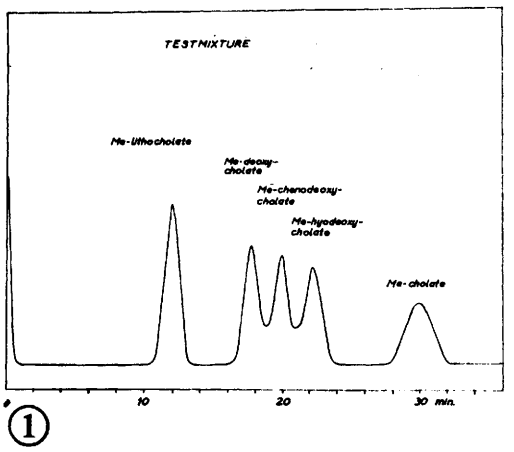

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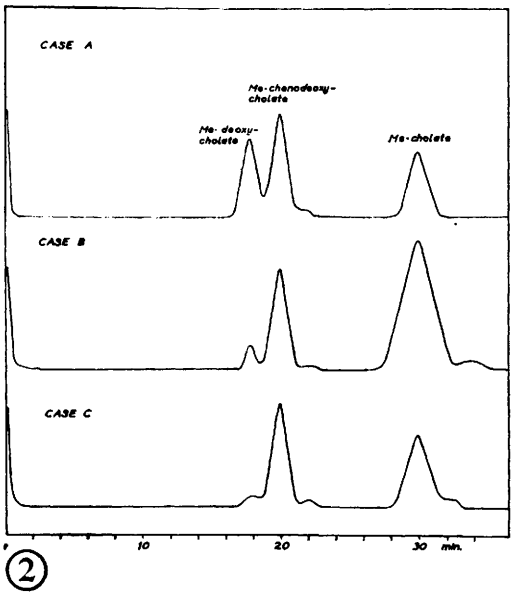

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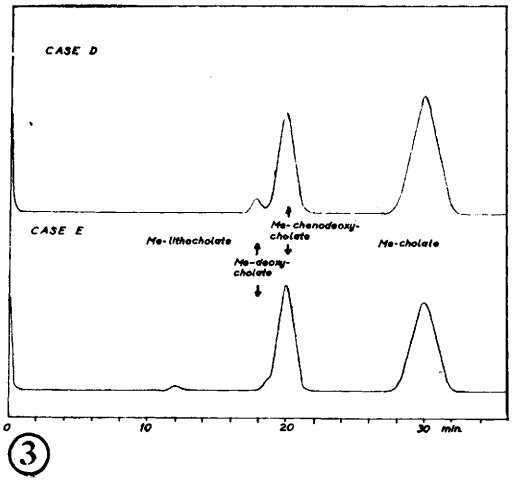

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TABLE I. Bile Acids in Gall-Bladder Bile of Patients with Different Diseases of Liver and Biliary Tract. In patient D hepatic bile was analyzed. In patient E duodenal content was analyzed for bile acids.

Patient	Sex	Age	Diagnosis	Ratio, C : CD : D
A	♂	46	Duodenal ulcer	.9 : 1 : .7
B	♀	37	Cholelithiasis	2.8 : 1 : .2
C	♂	45	Common duct stone with subsiding jaundice	1.1 : 1 : .2
D	♀	75	Cholelithiasis; choledochostomy performed; drainage bile analyzed	1.9 : 1 : .1
E	"	67	Familial hypercholesterolemia; free diet	1.1 : 1 : .02
F	"	53	Cholelithiasis	1.1 : 1 : 2.2
G	♂	62	"	.5 : 1 : 1.1

cal application of this technic it might be valuable to add hyodeoxycholic acid as internal standard both for qualitative and quantitative determinations. The results are very reproducible if all conditions are properly standardized.

In Fig. 2 some gas chromatograms from different patients are shown. A is a normal patient, case B is a patient with gallstones and a normal gall-bladder and case C is a patient with gallstone and subsiding jaundice. Apparently there is variation in the proportions of the different bile acids, which is most pronounced in the concentration of deoxycholic acid. In case B and C some unidentified bile acids are also present on the gas-chromatogram.

FIG. 1. Elution curve of a known mixture of methyl esters of bile acids. The stationary phase was a silicone rubber gum E. 301 maintained at 245°C on a 4-foot column. Flow rate was 70 ml argon per min. Samples were injected in a cyclohexanol solution. Recorder sensitivity 1 volt, full scale.

FIG. 2. Gas chromatogram of bile acid methyl esters in human gall-bladder bile. Operating conditions as described in Fig. 1, Case A. Gall-bladder bile acid methyl esters from a patient without known hepatobiliary disease. Case B. Gall-bladder bile acid esters from a patient with cholelithiasis but normal gall-bladder function. Case C. Gall-bladder bile acid esters from a patient with a common duct stone and subsiding jaundice.

FIG. 3. Case D. Gas chromatogram of bile acid methyl esters in hepatic bile from a patient without jaundice. Case D. Gas chromatogram of bile acid methyl esters in intestinal content of a patient with hypercholesterolemia.

In 2 patients hepatic bile was analyzed and in both patients only traces of deoxycholic acid were found. One of these chromatograms is given in Fig. 3 case D. Case E in Fig. 3 is a patient with familial hypercholesterolemia. The 2 major bile acids were chenodeoxycholic acid and cholic acid. Only traces of deoxycholic acid were found in this patient together with small amounts of lithocholic acid.

Conclusions. The major bile acids in samples of bile as determined with the aid of gas-liquid chromatography were cholic acid, chenodeoxycholic and deoxycholic acid. Regarding the proportions of the different bile acids, the results from this small group agree with those of Wootton and Wiggins(5), Isaksson(6) and Sjövall(7,8). Deoxycholic acid seemed to disappear in obstructive jaundice. In hepatic bile obtained from patients without jaundice only very small amounts of deoxycholic acid were found. Similar results have been reported by Sjövall(7) and Rudman and Kendall(9).

An interesting finding is the very small amount of deoxycholic acid present in a patient with familial hypercholesterolemia. This patient had been on a general mixed diet without the consumption of highly unsaturated fats or any other treatment of the hypercholesterolemia. Sjövall(7) has reported a similar bile acid pattern in a patient with hypercholesterolemia but on a formula diet. Further studies are in progress on the bile acid pattern in diseases with an altered cholesterol metabolism.

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Effect of Citrus Bioflavonoids on Metabolism of Hydrocortisone in Man.* (26556)

THEODORE E. WEICHSELBAUM AND HARRY W. MARGRAF

Dept. of Surgery, Washington University School of Medicine, and Surgical Metabolism Laboratories, of Barnes and Homer G. Phillips Hospitals, St. Louis, Mo.

It has been recognized for some time that the class of compounds generally termed bioflavonoids may be of physiological importance(1,2). Our previous observations of the relatively transient rises in plasma 17-hydroxycorticosteroid levels following major elective surgery(3) have interested us in possible pharmacological agents that would decrease the rate of metabolic inactivation, *i.e.*, Δ^4-3-keto reduction, or, due to other factors, impede removal from plasma of hydrocortisone and possibly other adrenocortical steroids. Masri and DeEds(4) observed that administration of certain bioflavonoids to rats produces involution of the thymus gland in the presence of the adrenal glands, but *not* after their removal, suggesting mediation of the physiological effects of bioflavonoids through an action on the pituitary-adrenal

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