Quantitative Determination of Bile Acids in Human Bile

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The methods used for the determination of bile acids in bile have usually been based on different colour reactions. These methods have mostly been limited to the determination of only one or a few of the bile acids in the bile. Since the different bile acids under various conditions may be excreted independently of each other it is desirable to have a method where all the bile acids can be determined simultaneously.

Synthetic mixtures of bile acids can be determined after separation by paper chromatography [1]. A preliminary note on the application of this method to bile acids in bile has been published [2]. The method has been used for the determination of bile acids in rat bile [3] and has now been adapted to the determination of bile acids in gall-bladder bile, fistula bile and duodenal contents of man.

The fluid to be analyzed is applied directly on the starting line of the chromatogram as described for synthetic bile acids [1]. Bile from the gall-bladder usually has to be diluted.

Taurine conjugated bile acids are separated with ascending chromatography. *Iso*amyl acetate:heptane 85:15 saturated with an equal volume of 70 % (v/v) formic acid is used as moving phase. The papers are equilibrated for 1/2 hour in the atmosphere of the moving phase. The chromatograms are then run for 20 hours. This procedure gives well defined spots of the bile acids even when large amounts of other bile constituents are present.

After localization the spots are cut out and eluted [1]. Taurocholic acid (TC) is determined in 65 % sulfuric acid as described [1]. Since taurodeoxycholic (TD) and taurochenodeoxycholic (TDC) acids often occur together in human bile and since they do not separate in these chromatograms they are determined as the sum TD+TCD. This is done by heating the acids 15 minutes at 50° C in 65 % sulfuric acid when TD and TCD show the same molar extinction at their maximum at 305 mμ. This is reproducible within ±5 % with synthetic acids and the acids follow Beer's law singly and in mixtures.

Bile constituents other than bile acids (mainly bile pigments) also contribute to the light absorption of the samples in sulfuric acid. When this contribution is between 10 and 20 % of the total light absorption it can be corrected for by subtracting the light absorption of a corresponding sample in 80 % ethanol where bile acids have no absorption at the wave lengths used.

Glycine conjugated bile acids are separated with descending chromatography using ethylene chloride:heptane 50:50 as moving phase [4]. The filter paper used for the chromatography is cut as described but since the bile acids travel different distances on the different strips in descending chromatography one strip on each side of the strip to be eluted has to be sprayed in order to localize the bile acids. Glycocholic (GC), glycochenodeoxycholic (GCD) and glycodeoxycholic (GD) acids are separated and can be eluted and determined. Synthetic mixtures of GCD and GD are determined within ±5 %. Except in cases with extraordinarily large amounts of bile pigments no ethanol correction has to be made.

Within the errors of the method (approximately ±15 % for each bile acid in biological material) the ratio of glycine to taurine conjugation was the same for trihydroxy- as for dihydroxycholanic acids. In most human samples the glycine conjugated bile acids dominated. No free bile acids were detected It has been confirmed that cholic and chenodeoxycholic acids are the main hydroxycholanic acids in human bile. In several bile samples no conjugated deoxycholic acid could be found. With the method used glycodeoxycholic acid would have been detected when present as 2 % of the total amount of bile acids.

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Quantitative Aspects of Bile Acid Formation in Man

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Our knowledge of the amount of bile acids taking part in the enterohepatic circulation is incomplete and based on determinations of concentration of bile acids in bladder or fistula bile. For a review see Josephson [1].

We have attempted to determine the total amount of circulating cholic acid by an isotope dilution *in vivo*. By determining the fall of specific activity with time information has been gained on the rate of formation of this compound. 3—4 μC of cholic acid-24-^{14}C was given orally as sodium salt to healthy male medical students. At different time intervals bile was obtained through a duodenal tube after administration of 1—3 mg of a purified pancreozymine-cholecystochinine preparation (kindly supplied by Professor Erik Jorpes, Stockholm). Cholic acid was isolated and the specific activity determined. A bile sample was used for a quantitative determination with a paperchromatographic technique of the concentration of various bile acids. These determinations were kindly carried out by Dr. J. Sjövall [2]. By the use of the figures thus obtained the total amount of circulating acids was calculated. In the table the results obtained in three subjects are given:

Subject	Cholic acid g	Total amount of of bile acids g	"Half-life" of cholic acid in days
B	0.575	1.85	1.2
P	0.92	3.65	2.5
S	1.29	3.55	3

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On the Metabolism of Bile Acids in Germ-free Rats

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The bile of rats contains taurocholic and taurochenodeoxycholic acids [1]. With the aid of 24-^{14}C-labelled acids it has been shown that these acids are extensively modified during the passage down the intestinal tract [2]. When the fecal acids were chromatographed these modifications were found to include:

1) a splitting of the peptide bond,
2) an attack on the bile acid moiety leading to a variety of mostly less hydrophilic compounds.

It has been found [3] that in rats treated with intestinal chemoterapeutics no such modifications occur making it highly probable that microbial enzymes are responsible for the reactions. Furthermore several strains of bacteria that can split the conjugated bile acid *in vitro* have been isolated from the rat intestine [4]. We have earlier reported on the turnover of bile acids in normal rats and in animals treated with chemoterapeutics [5]. The half-lives were found to be about 2.5 and 6—7 days, respectively. In order to study the importance of intestinal microorganism for the elimination of bile acids we have administered 24-^{14}C-labelled cholic acid to germ-free rats and to a rat that had been infected with *Aspergillus niger* and then further with *Clostridium perfingens* type E. The rats were reared with slight modifications in the technique of Gustafsson [6]. The time course of excretion was followed and the acids in the feces fractionated with chromatography. One rat was finally transferred from the apparatus to normal environments and the elimination rate investigated.

The germ-free rats were found to excrete unchanged taurocholic acid in their feces. This was also the case in the rat infected with the mould. When the same rat was also infected with the *Clostridium* strain, however, about 25 % of the labelled acids appeared in the feces in the free form, mainly as cholic acid. The chromatographic pattern was in this case similar to that found after *in vitro* incubation of taurocholic acid with the same strain of bacterium [4].

In all rats studied the excretion rate was quite slow with a half life-time of about 7 days. It may be concluded that the state of the intestinal flora greatly influences the rate of elimination from the enterohepatic circulation. As, however, neither the mould nor the peptide bond-splitting *Clostridium* increases the excretion rate it seems probable that other microorganisms are of importance for the rate of elimination of bile acids.

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