

## 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<sup>1</sup>. A preliminary note on the application of this method to bile acids in bile has been published<sup>2</sup>. The method has been used for the determination of bile acids in rat bile<sup>3</sup> 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<sup>1</sup>. Bile from the gall-bladder usually has to be diluted.

Taurine conjugated bile acids are separated with ascending chromatography. Isoamyl 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<sup>1</sup>. Taurocholic acid (TC) is determined in 65% sulfuric acid as described<sup>1</sup>. 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 + TDC. This is done by heating the acids 15 minutes at 50°C in 65% sulfuric acid when TD and TDC show the same molar extinction at their maximum at 305 m $\mu$ . This is reproducible within  $\pm 5\%$  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<sup>4</sup>. 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  $\pm 5\%$ . Except in cases with extraordinarily large amounts of bile pigments no ethanol correction has to be made.

Within the errors of the method (approximately  $\pm 15\%$  for each bile acid in biological material) the ratio of glycine to taurine conjugation was the same for trihydroxy- as for dihydroxycholic 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 hydroxycholic 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.

1. Sjövall, J. *Arkiv Kemi* 8 (1955) 317.
2. Sjövall, J. *Acta Chem. Scand.* 9 (1955) 1034.
3. Eriksson, S. *Acta Chem. Scand.* 10 (1956) 156.
4. Sjövall, J. *Arkiv Kemi* 8 (1955) 299.

## 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<sup>1</sup>.