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Note

Thin-layer chromatographic method for the separation of conjugated deoxycholic acid from conjugated chenodeoxycholic acid

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The quantitation of individual conjugated bile acids in biological fluids depends largely on the separation of the bile acids by thin-layer chromatography (TLC) before being analysed by enzymatic [1] or gas—liquid chromatographic [2] methods. Gas—liquid chromatographic separation of bile acids usually requires derivatization and had not been successfully achieved with bile salts [3]. Various solvent systems have been proposed for the separation of conjugated bile acids [4—14], but to our knowledge, no solvent system has been able to separate the conjugated dihydroxy isomers (glycochenodeoxycholic and glycodeoxycholic acids) and the corresponding taurine conjugates (taurochenodeoxycholic and taurodeoxycholic acids) by TLC. Attempts have been made to quantify these conjugated dihydroxy isomers by sophisticated differential colorimetric techniques [15, 16] or enzymatic or alkaline hydrolysis [17] of the unresolved isomeric mixtures from a TLC plate followed by chromatographic separation of the hydrolysate with the appropriate solvent system and enzymatic assay. The colorimetric method is tedious and the disadvantages of hydrolysis and TLC procedures are the destruction of conjugates and the loss of time required for hydrolysis and re-chromatography. Recently, a combination of 3α-hydroxysteroid dehydrogenase and 7α-hydroxysteroid dehydrogenase have been used for quantifying the components of binary mixtures of dihydroxy conjugates [18]. High-pressure liquid chromatography has been able to resolve partially the tauro-dihydroxy conjugates [19]. The solvent system described here clearly separates the isomeric conjugated dihydroxy bile acids along with other conjugated bile acids on the same plate. The quantitative analysis of individual bile acids can be carried out by scraping off the appropriate portion of the bile acid from the TLC plate and enzymatic assay by 3α-hydroxysteroid dehydrogenase.

MATERIAL AND METHODS

Glycocholic, taurocholic, glycodeoxycholic, taurodeoxycholic, glycochenodeoxycholic, taurochenodeoxycholic, cholic, chenodeoxycholic and deoxycholic acids were purchased from P-L Biochemicals (Milwaukee, Wisc., U.S.A.), glycolithocholic and taurolithocholic acids from Calbiochem (San Diego, Calif., U.S.A.), and cholesterol from Sigma (St. Louis, Mo., U.S.A.). All solvents were Baker analyzed reagent grade (J.T. Baker, Phillipsburg, N.J., U.S.A.). Methanolic solutions of bile acids and cholesterol were applied as streaks with a Hamilton microliter syringe on 20 × 20 cm pre-coated TLC silica gel 60 plates of 0.25 mm thickness (E. Merck, Darmstadt, G.F.R., distributed by Curtin Scientific, Brisbane, Calif., U.S.A., catalogue No. 5763). The plate was marked at 7 cm and 15 cm from the starting line. Samples (10—15 μg) of bile acids and cholesterol and methanolic solution of hamster gallbladder bile were applied 1.5 cm above the bottom edge of the plate, allowed to dry with cold air and placed in a rectangular glass tank (29 × 9 × 25.5 cm). The solvent was chloroform—isopropand—isobutand—acetic acid—water (30:20:10:2:1) and the plate was developed at room temperature (23—25°). First two successive runs up to 15 cm and the next four successive runs up to 7 cm were allowed. After each run, the plate was dried with cold air by means of a hair drier. Finally the plate was removed from the chromatographic chamber, dried in hot air, sprayed with copper—molybdenum spray reagent [20] and heated for 15 min in an oven at 70—80° in order to make the components visible. Each bile acid can be identified by its characteristic color which is very distinct amongst each others.

DISCUSSION

The positions of different bile acids (conjugated and free) and cholesterol on a TLC plate after its development with the solvent system chloroform—isopropanol—isobutanol—acetic acid—water (30:20:10:2:1) is shown in Fig. 1. It is evident from the figure that the conjugated isomeric mixtures have been clearly separated. The free bile acids and cholesterol run well ahead of the conjugated bile acids. The R_F values of different conjugated bile acids are shown in

TABLE I

R_F VALUES OF CONJUGATED BILE ACIDS ON SILICA GEL

Solvent system: chloroform—isopropanol—isobutanol—acetic acid—water (30:20:10:2:1).

Bile acid	R_F	
	Glyco-	Tauro-
Cholic acid	0.32	0.09
Deoxycholic acid	0.55	0.22
Chenodeoxycholic acid	0.61	0.25
Lithocholic acid	0.83	0.39

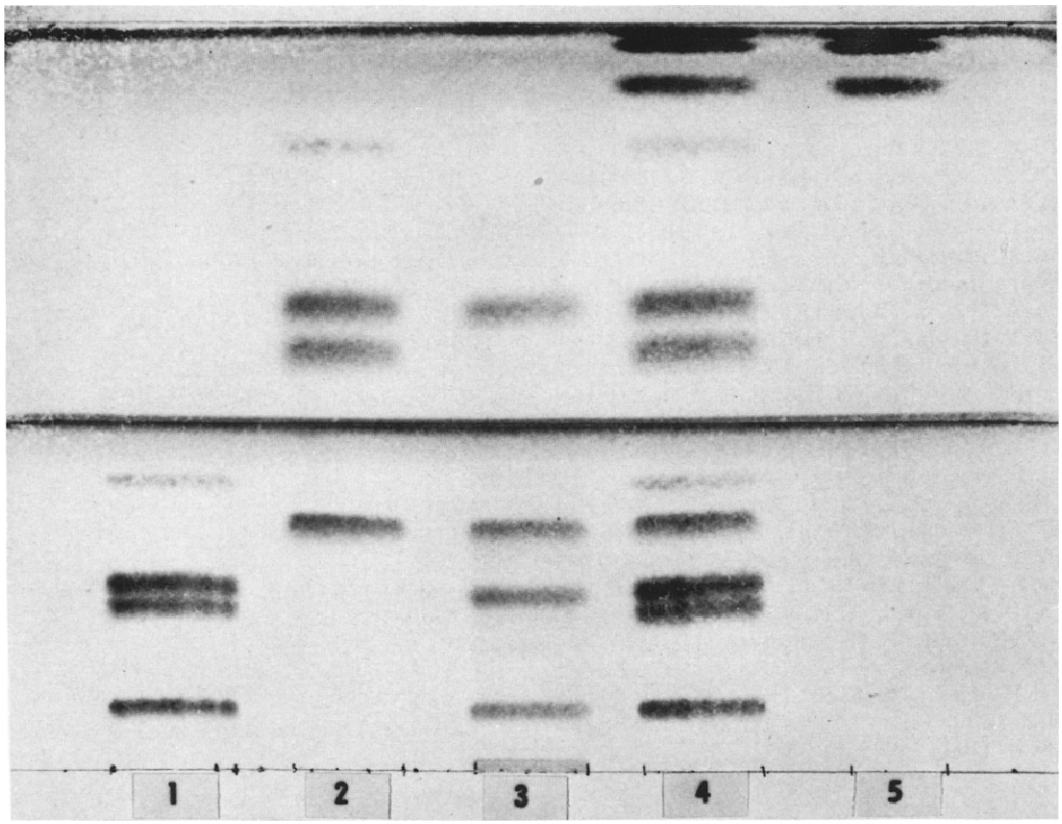

Fig. 1. Thin-layer chromatogram developed in chloroform—isopropanol—isobutanol—acetic acid—water (30:20:10:2:1) and sprayed with copper—molybdenum spray reagent [20]. In ascending order: 1 = taurocholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid and taurolithocholic acid; 2 = glycocholic acid, glycodeoxycholic acid, glycochenodeoxycholic acid and glycolithocholic acid; 3 = methanolic solution of hamster gallbladder bile; 4 = mixture of 1, 2 and 5; 5 = cholic acid, deoxycholic and chenodeoxycholic acid and cholesterol.

Table I. Published methods of chromatography generally fail to separate the taurine- and glycine-conjugated dihydroxy bile acids. This method is therefore unique, i.e. the only method to date to separate the isomeric conjugated dihydroxy bile acids, glycodeoxycholic from glycochenodeoxycholic acid and taurodeoxycholic from taurochenodeoxycholic acid. The advantage of this method is the direct separation of the conjugates in biological samples. It also does not require any hydrolysis of the conjugated dihydroxy isomers (enzymatic or alkaline), solvent extraction or some other laborious procedure. Each individual bile acid can be scraped off the plates and assayed enzymatically by conventional steroid dehydrogenase method [21]. The method when applied will be helpful clinically in understanding bile acid metabolism and the diagnosis of the hepatic and gastrointestinal states [22].

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