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A Method for the Separation of Glycine-Conjugated Bile Acids as a Group from Taurine-Conjugated Bile Acids

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Ileal disease, liver disease, and other pathologic conditions have been shown to affect the glycine:taurine ratio (1). The procedure used for determining the glycine:taurine ratio is to separate the individual conjugated bile acids on thin-layer chromatograms and estimate the quantities of each bile acid to get the ratio (2, 3). Sometimes two or more solvent systems are needed to separate the individual bile acids for this purpose (4, 5). Recently, a nonchromatographic colorimetric method has been reported for the estimation of taurine-conjugated bile acids (6). The glycine:taurine ratio by this method is determined by estimating the total conjugated bile acids after separation on thin-layer chromatograms and finding the difference between total and taurine conjugates, giving the value of glycine conjugates and thereby the ratio. All of these methods are time consuming and laborious. The method described here separates all of the glycine-conjugated bile acids in one band and the taurine-conjugated bile acids in another, thus separating the glycine derivatives from taurine derivatives on the same plate in a single run. The bile acids are close together in each band to permit recovery from the chromatograms for quantitative analysis.

MATERIALS AND METHODS

Cholic acid, taurocholic acid, glycocholic acid, glychodeoxycholic acid, lithocholic acid, glycolithocholic acid, taurolithocholic acid (Calbiochem, San Diego, California), taurodeoxycholic acid, glycochenodeoxycholic acid, taurochenodeoxycholic acid, deoxycholic acid, chenodeoxycholic acid, and cholesterol (Sigma Chemicals, St. Louis, Missouri) were used. The solvents used were ethanol (Commercial Solvents Corp., Terre Haute, Indiana), isopropyl alcohol (Baker Analyzed Reagent, J. T. Baker, Phillipsburg, New Jersey), ethyl acetate, and isooctane (Matheson, Coleman, and Bell, Norwood, Ohio).

Cholesterol and bile acid solutions in methanol were applied on a 20 ×

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20-cm precoated thin-layer silica gel plate 0.25 mm thick (E. Merck, Dramstadt, Germany, distributed by Brinkmann Instruments, Westbury, New York, Catalog No. 5763). Ten- to twenty-microgram samples were applied as spots of 4 mm diameter 1.5 cm above the bottom edge of the plate. One-half to one microliter of hamster gallbladder bile was applied directly to the plate by means of a Hamilton microliter syringe. The plate was then placed in a commercial chromatographic chamber (Gelman Instruments, Ann Arbor, Michigan) which was saturated with the solvent system ethanol–isopropyl alcohol–isooctane–ethyl acetate (25:10:10:10) and run for 2 hr at room temperature (23–25°C). The plate was then removed from the chamber, dried, and sprayed with copper–molybdenum spray reagent (7) or developed in iodine vapor for visualization of the spots.

RESULTS AND DISCUSSION

The separation of cholesterol and bile acids (glycine conjugates, taurine conjugates, and free) is shown in Fig. 1. It is evident from this figure that all of the glycine-conjugated bile acids lie in one band and the taurine-conjugated bile acids lie in another. The free bile acids form a third band

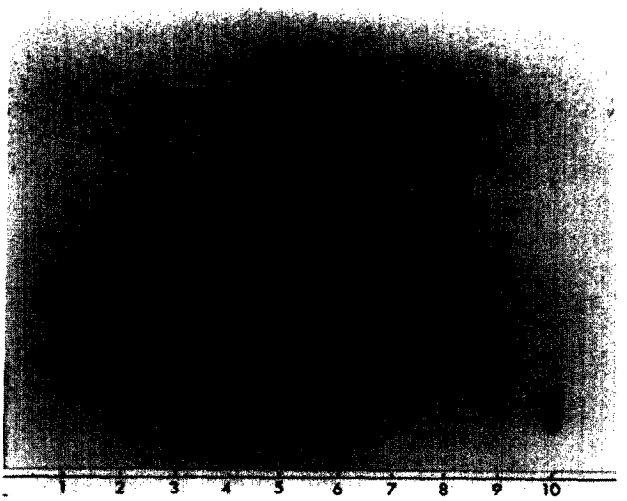

FIG. 1. Thin-layer chromatogram of bile acids, cholesterol, and hamster bile in a solvent system of ethanol–isopropyl alcohol–isooctane–ethyl acetate (25:10:10:10) and sprayed with copper–molybdenum spray reagent (7). 1, Glycholitho (bottom) and taurocholic acid (top); 2, taurolithocholic acid; 3, taurochenodeoxycholic acid; 4, glycocholic, glycodeoxycholic, glycochenodeoxycholic, and glycolithocholic acid; 5, mixture of 4, 7, and 8; 6, hamster bile; 7, taurocholic, taurodeoxycholic, taurochenodeoxycholic, and taurolithocholic acid; 8, cholic, deoxycholic, chenodeoxycholic, lithocholic acid, and cholesterol (top); 9, glycochenodeoxycholic acid; 10, glycocholic acid.

well separated from glycine and taurine conjugates as well as from cholesterol. The hamster bile has also been clearly separated. The R_f values of different bile acids are given in Table 1. Cholesterol has an R_f value of 0.82 and runs well ahead of all the bile acids, free and conjugated. During the course of the experiment, it was found that pesticide-quality and completely denatured ethanol (Matheson, Coleman, and Bell, Norwood, Ohio) may be used instead of 200-proof ethanol.

TABLE 1
R_f VALUES OF BILE ACIDS[a]

Bile acid	Free	Glyco-	Tauro-
Cholic acid	0.68	0.16	0.42
Deoxycholic acid	0.72	0.24	0.49
Chenodeoxycholic acid	0.72	0.24	0.49
Lithocholic acid	0.74	0.26	0.54

[a] Solvent system used: ethanol–isopropyl alcohol–isooctane–ethyl acetate (25:10:10:10).

This solvent system thus separates the different bile acids as a group, e.g., glycine conjugates, taurine conjugates, and free, in three different bands. For quantitative analysis of the glycine:taurine ratio, different bands corresponding to the glycine and taurine regions can be scraped from the chromatogram after visualization with iodine vapor, dissolved in methanol, and analyzed enzymatically (8). This method does not require the laborious extraction procedure and is a very simple, time-saving method for measurement of the glycine:taurine ratio in biological materials.

SUMMARY

A thin-layer chromatographic method for bile acids has been described which separates glycine conjugates, taurine conjugates, and free bile acids into three separate bands. The solvent system used for this purpose is ethanol–isopropyl alcohol–isooctane–ethyl acetate (25:10:10:10). This method is time saving and very simple for quantitative estimation of the glycine:taurine ratio in biological specimens.

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