New solvent systems for thin-layer chromatography of bile acids

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SUMMARY A modified thin-layer chromatographic technique separates small amounts of glycochenodeoxycholic from glycodeoxycholic acid and the major taurine conjugates and unconjugated bile acids from one another.

KEY WORDS thin-layer chromatography · wedge technique · conjugated · unconjugated · bile acids

OF THE SEVERAL recent methods for thin-layer chromatography of bile acids (1–12) I have found a modification of the Hamilton method as reported by Kritchevsky, Martak, and Rothblat (3) to be more effective than others since it permits separation of up to 12 bile acids in one chromatogram. This modification is reported here.

Methods. Silica Gel G with calcium sulfate binder (Research Specialties Co., Richmond, Calif.) was used although Silica Gel H, binder free (E. Merck, A. G., Darmstadt, Germany), which was investigated later, gave comparable results. It adhered to the chromatoplate more strongly and because of its greater transparency allowed better staining of the bile acids. A slurry of 30 g of Silica Gel G in 58 ml of distilled water was spread 0.2 mm thick with the aid of a commercial apparatus (Microchemical Specialty Co., Berkeley, Calif.) on glass plates measuring 20 by 20 cm.

Commercial cholic acid and deoxycholic acid (Mann Fine Chemicals Inc., New York, N.Y.) and lithocholic, ursodeoxycholic, and chenodeoxycholic acids (Steraloids, Inc., Pawling, N.Y.) were purchased. The conjugated

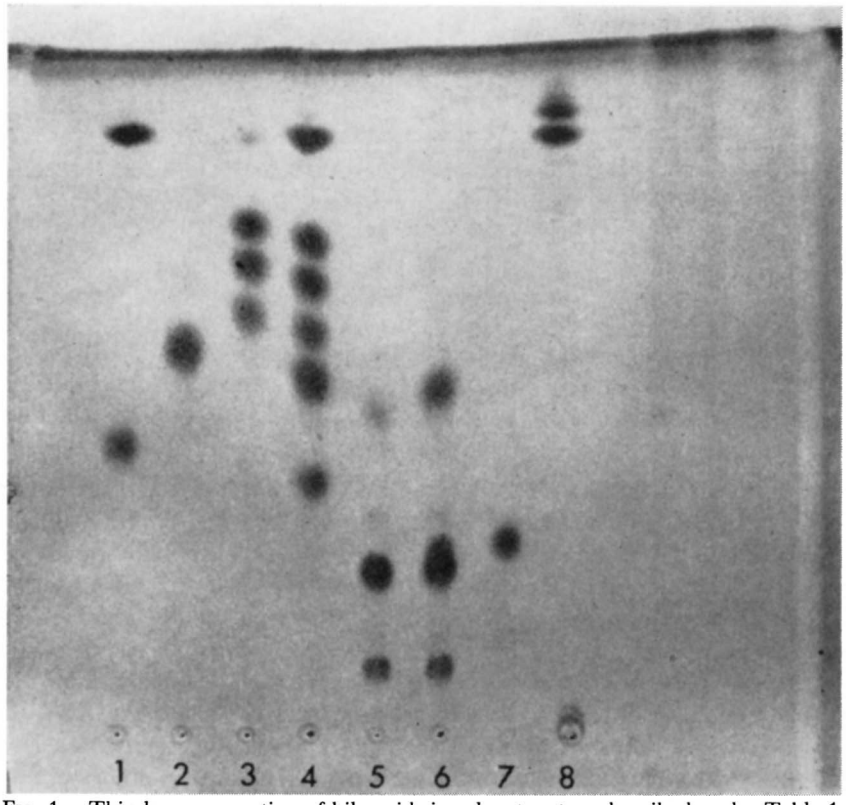

FIG. 1. Thin-layer separation of bile acids in solvent system described under Table 1. *1*, C, LiC; *2*, GLiC; *3*, UDC, CDC, DC; *4*, C, GLiC, UDC, CDC, DC, LiC; *5*, GC, GCD (and impurity); *6*, GC, GCD, GDC, GLiC; *7*, GDC; and *8*, TC, TLiC, LiC (impurity in TLiC).

Abbreviations. Unconjugated bile acids: *C*, cholic; *UDC*, ursodeoxycholic; *CD*, chenodeoxycholic; *DC*, deoxycholic; and *LiC*, lithocholic. Conjugated bile acids: *GC*, glycocholic; *GCD*, glycochenodeoxycholic; *GDC*, glycodeoxychclic; *GLiC*, glycolithocholic; *TC*, taurocholic; *TCD*, taurochenodeoxycholic; and *TLiC*, taurolithocholic.

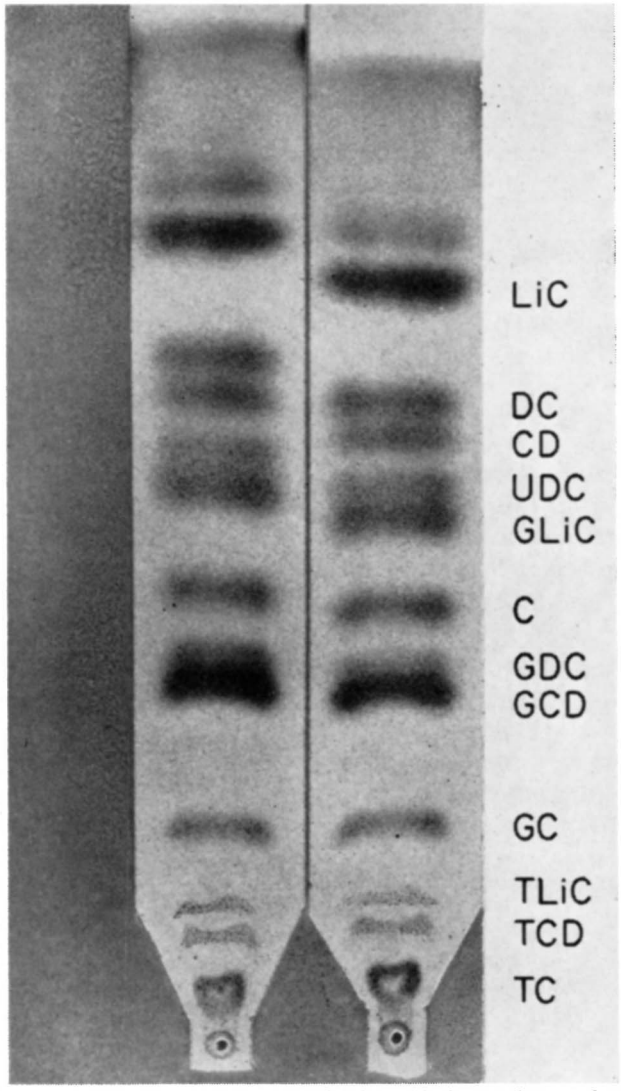

FIG. 2. Thin-layer separation of bile acids by wedge method. Solvent system and abbreviations as in Fig. 1.

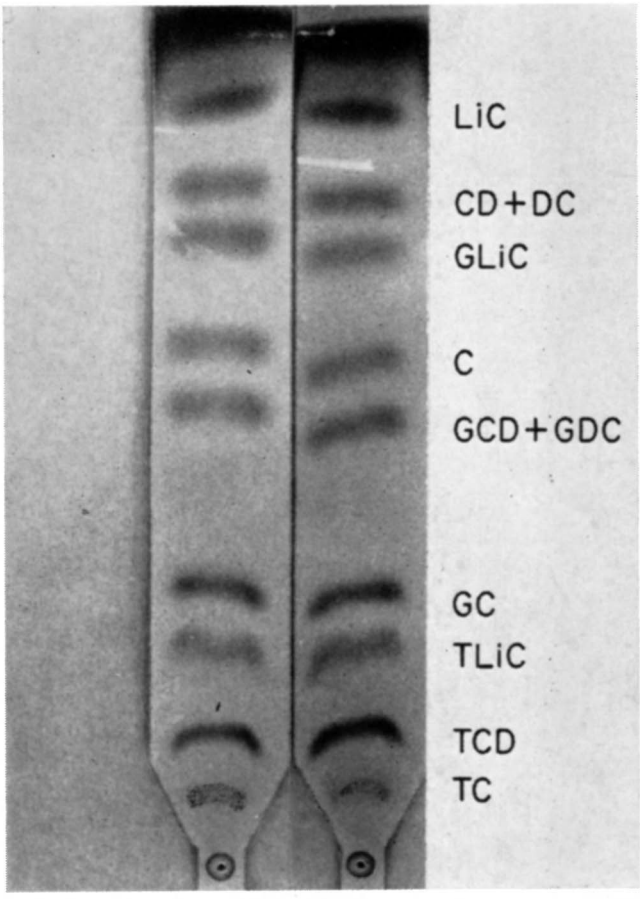

FIG. 3. Thin-layer separation of bile acids in ethylene dichloride–acetic acid–water 1:1:0.1. Abbreviations as in Fig. 1.

bile acids, with the exception of taurolithocholic acid (Steraloids, Inc.), were synthesized by the method of Norman (13) and the bile acids were purified by reverse phase chromatography (14), thin-layer chromatography, or crystallization. The taurolithocholic acid was not purified. The bile acids were applied either as the acid or in the salt form.

Isooctane and isopropyl ether (Fisher Scientific Co.), certified acetic acid (E. I. du Pont de Nemours & Co., Inc.), and isopropyl alcohol (Mallinckrodt Chemical Works, Pharmaceutical Div.) were distilled before use. The bile acids were dissolved in ethanol (1 μg/μl) and 10–20 μg was applied with a sharpened micropipette. The chromatoplates were activated by heating at 100°C for 40–60 min, cooled, and chromatographed. Whether the plates were activated before or after application of the bile acids made no difference to their mobility, but heating after application could conceivably result in some degradation of the bile acids. Later work has shown activation to be unnecessary.

All chromatography was done at 23–25°C in glass tanks of volume 5600 ml. The solvent (see below) was poured into the tank, which was lined with Whatman 3 MM filter paper, and the chromatoplate was inserted without further equilibration. The tank was then covered with a glass plate and sealed with masking tape. The plates were removed when the moving front was 1–2 cm from the top (1–2 hr). After chromatography the plates were dried and sprayed with a 10% solution of phosphomolybdic acid in alcohol and heated for 15 min in an oven at 110°C until the characteristic blue spots appeared. (Detection with a saturated solution of antimony trichloride in chloroform, 50% aqueous sulfuric acid, and iodine was also found to be satisfactory.)

Results. Representative chromatograms after use of the solvent system (modified Hamilton method) isooctane–isopropyl ether–glacial acetic acid–isopropyl alcohol 2:1:1:1 in the standard and wedge methods (15) of chromatography are shown in Figs. 1 and 2 and

TABLE 1 R_f VALUES OF FREE AND CONJUGATED BILE ACIDS

	Method	
	Standard	Wedge
Free bile acids		
Cholic	0.42	0.45
Ursodeoxycholic	0.61	0.58
Chenodeoxycholic	0.67	0.63
Deoxycholic	0.74	0.68
Lithocholic	0.90	0.80
Conjugated bile acids		
Taurocholic	0.0	0.06
Taurochenodeoxycholic	0.0	0.12
Taurolithocholic	0.03	0.16
Glycocholic	0.10	0.22
Glycochenodeoxycholic	0.26	0.36
Glycodeoxycholic	0.30	0.39
Glycolithocholic	0.53	0.55

Adsorbent: Silica Gel G. Solvent mixture: isooctane–isopropyl ether–glacial acetic acid–isopropyl alcohol 2:1:1:1 (see Figs. 1 and 2).

the average R_f values from these two methods are listed in Table 1.

The addition of isopropyl alcohol to the Hamilton system increases the R_f values of all compounds and preserves the excellent separation of the unconjugated dihydroxycholanic acids. The use of straight-chain alcohols in this system was less satisfactory. Although the taurine conjugates remain at or near the base line in the standard method, they move off the point of application in the wedge method and there is complete separation of taurocholic, taurochenodeoxycholic, and taurolithocholic acids. In addition, there is complete separation of glycochenodeoxycholic from glycodeoxycholic acid when their sum is 20 μg or less. Separation of these compounds is incomplete when 30 μg is chromatographed as in Fig. 2.

Another useful solvent system for the chromatography of bile acids by groups is ethylene dichloride–acetic acid–water 1:1:0.1. Development is more rapid than with Gänshirt, Koss, and Morianz's developer and the mixture contains fewer solvents than some other methods. A chromatogram employing this system and the wedge method is shown in Fig. 3.

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