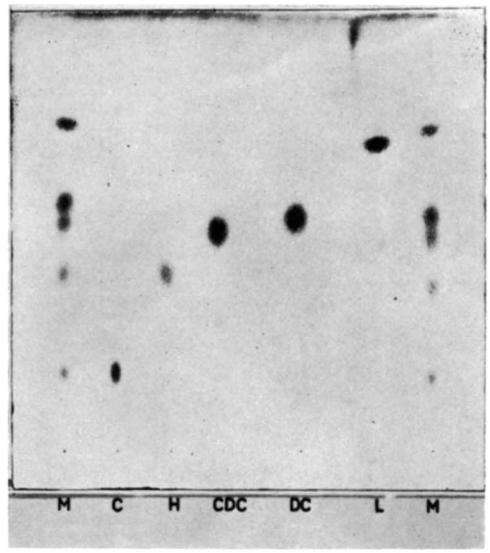

FIG. 1. Free bile acids (10 μg; development time, 1½ hours). From left to right: mixture (M); cholic (C); hyodeoxycholic (H); chenodeoxycholic (CDC); deoxycholic acid (DC); lithocholic (L); mixture (M).

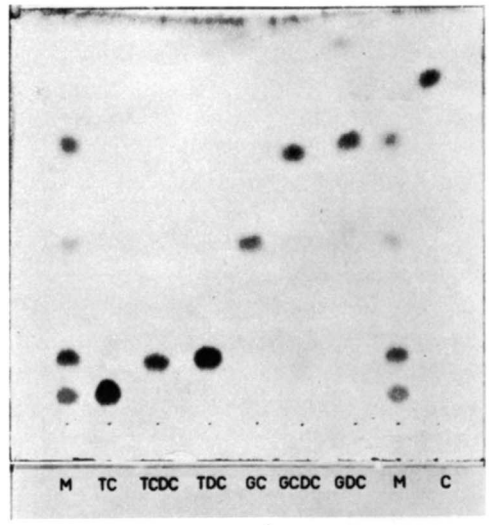

FIG. 2. Conjugated bile acids (40 μg; development time, 3½ hours). From left to right: mixture (M); taurocholic (TC); taurochenodeoxycholic (TCDC); taurodeoxycholic (TDC); glycocholic (GC); glycochenodeoxycholic (GCDC); glycodeoxycholic (GDC); mixture (M); cholic (C). A spot of deoxycholic acid contaminating the glycodeoxycholic acid may be seen near the front.

Thin-layer adsorption chromatography of free and conjugated bile acids on silicic acid

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» Reported here are two solvent systems that have proved useful for the qualitative separation of certain free and conjugated bile acids. Although Gänshirt, Koss, and Morianz (1) have reported other systems, we have found those described here to be more satisfactory.

The thin layers of silicic acid on 20 x 20 cm glass plates were prepared, using a commercial apparatus (thin layer chromatography according to Stahl, C. Desaga GmbH., Heidelberg, Germany) with Kieselgel G (E. Merck, A.G., Darmstadt, Germany), and kept in a desiccator over silica gel. The silicic acid suspension was prepared by adding 58 ml distilled water to 30 g Kieselgel G in a wide-mouthed bottle and shaking vigorously for 15 seconds.

The first solvent system is for free bile acids. In this system, the taurine conjugates remain at the origin. Glycochenodeoxycholic and glycodeoxycholic acid move

slightly but do not separate. Either the acid or the salt form of the bile acid may be applied. If the methyl ester of a free acid or glycine conjugate is used, the R_f increases by about 0.10.

The second system separates certain taurine and glycine conjugated acids. Taurochenodeoxycholic and taurodeoxycholic acid have identical mobilities. Glycodeoxycholic acid moves slightly faster than glycochenodeoxycholic acid but the separation is incomplete.

The compositions of the solvent systems on a volume basis are listed in Table 1 with approximate R_f values. The R_f values vary somewhat with each run, and it is advisable to use standards.

Figures 1 and 2 show typical separations of the bile acids, alone and in mixture. The spots were revealed by spraying the plate, after it had been dried, with 10% phosphomolybdic acid in ethanol and heating it briefly.

The excellent technical assistance of Miss Gunilla Ostberg is appreciated. This work was done while holding a Postdoctoral Fellowship from the National Foundation (USA).

REFERENCE

1. Gänshirt, H., F. W. Koss, and K. Morianz. *Arzneim. Forsch.* **10:** 943, 1960.

TABLE 1. R_F'S OF FREE AND CONJUGATED BILE ACIDS

	R_F Values	
	Solvent I*	Solvent II†
Free bile acids		
Cholic	0.16	0.75
Hyodeoxycholic	0.39	0.80
Chenodeoxycholic	0.53	0.85
Deoxycholic	0.59	0.88
Lithocholic	0.82	0.91
Conjugated bile acids		
Taurocholic	0	0.07
Taurochenodeoxycholic	0	0.16
Taurodeoxycholic	0	0.16
Glycocholic	0	0.48
Glycochenodeoxycholic	0.08	0.68
Glycodeoxycholic	0.08	0.70

* Composition (by volume): 5 acetic acid; 20 carbon tetrachloride; 30 di-isopropyl ether; 40 iso-amyl acetate; 10 *n*-propanol; 10 benzene.

† Composition (by volume): 15 propionic acid; 20 iso-amyl acetate; 5 water; 10 *n*-propanol.