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Letter to the Editor

Elimination of phospholipid interference in biliary bile acid determination by thin-layer chromatography—densitometry

Sir,

Recently, Takikawa et al. [1] reported a simple thin-layer chromatographic (TLC) procedure using phosphomolybdic acid as a spray reagent for the determination of bile acids in bile. During our studies on the influence of spices on bile composition and secretion in rats [2] using their procedure, it was found that phospholipids present in bile interfered in the quantitation of bile acids. We would like to present a modified procedure by which interference by phospholipids can be eliminated.

Bile acids in bile and methanolic layer of Bligh and Dyer [3] lipid extract of bile were determined by thin-layer chromatography as described by Takikawa et al. [1]. The chromatoplates were scanned using an automatic Camag TLC scanner (Turner Assoc.), attached to a W+W Model 1100 recorder (Scientific Instruments, Switzerland) under the following conditions: lamp, No. 110-852; primary filter, 110-820; secondary filter, 110-823 (10% neutral density); plate speed, 2 cm/min; chart speed, 1 cm/min. Quantitation was done by calculation of areas after triangulation and comparison with authentic individual bile acid standards.

Preliminary experiments on the separation of biliary bile acids by TLC as described by Takikawa et al. [1] showed that some phospholipids also moved along with bile acids and reacted with the phosphomolybdic acid spray reagent. This resulted in an overestimation of bile acids.

As indicated in Fig. 1, phospholipids move very closely with bile acids; in particular, lysolecithin comigrates with taurodeoxycholic acid. The presence of phospholipid in the taurodeoxycholic acid spot was confirmed by determination of phosphorus. Eleven samples of bile were subjected to TLC and the magnitude of interference by phospholipid (estimated by the method of Marinetti [4] was evaluated; the data are presented in Table I. These results showed that about 15% of phospholipid was present in the bile acid spot. This was further confirmed by a nearly 11% higher value for bile acid content in bile than in the phospholipid-free bile extract. These observations

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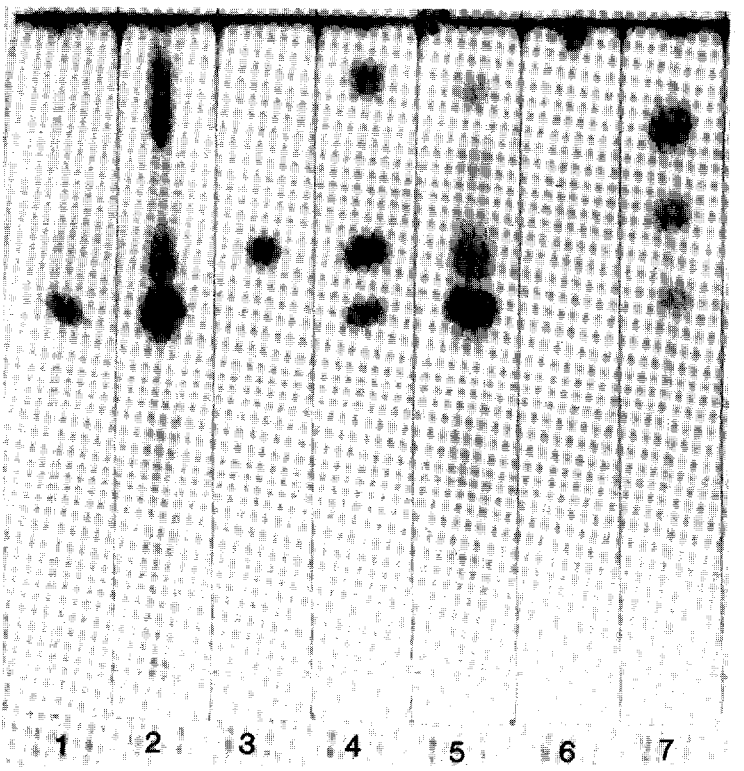

Fig. 1. Thin-layer chromatography of bile acids. 1 = Taurocholic acid; 2 = bile; 3 = taurodeoxycholic acid; 4 = mixture of bile acids (taurocholic, taurodeoxycholic and glycocholic acids in order of increasing R_F); 5 = methanolic extract of bile; 6 = glycocholic acid; 7 = phospholiplids (lysolecithin, phosphatidyl ethanolamine and phosphatidyl choline in order of increasing R_F). Solvent: chloroform—methanol—acetic acid—water (65:24:15:9).

TABLE I

ESTIMATION OF INTERFERENCE BY PHOSPHOLIPIDS IN BILE ACID DETERMINATION

Values are mean ± standard error of the mean (S.E.M.) of eleven samples.

	Phospholipid concentration (μmol/ml)		Phospholipid in bile acid spot (%)	Bile acid concentration (μmol/ml)		Interference (%)
	Bile	Taurodeoxycholate spot		Bile	Methanolic layer	
Mean	1.92	0.290	14.5	4.79	4.33	11.1
S.E.M.	0.15	0.050	2.3	0.40	0.39	1.7

emphasized the need for a method of removal of phospholipids in bile before estimation of bile acids by the TLC—densitometric procedure.

Interference by phospholipids can be eliminated if they are selectively removed from bile before TLC. For this purpose the procedure of Bligh and Dyer [3] used for lipid extraction was found suitable; in this procedure, bile

acids are extracted into the upper methanolic phase and phospholipids along with other lipid components go into the lower chloroform layer. The methanolic layer did not have any phosphorus. Further, spots corresponding to bile acids after TLC of the methanolic layer did not show the presence of any phospholipid when assayed for phosphorus.

Thus, direct TLC—densitometry of bile samples as recommended by Takikawa et al. [1] leads to an overestimation of bile acids. It is therefore suggested that the upper phase (methanolic layer) of the Bligh and Dyer [3] lipid extract be used for separation of individual bile acids by TLC for quantitation.

Biochemistry Section,
Department of Food Chemistry,
Central Food Technological Research Institute,
Mysore 570 013 (India)

K. SAMBAIAH
B. GANESH BHAT
N. CHANDRASEKHARA*

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