Separation and radioassay of fecal cholesterol and coprosterol using thin-layer chromatography*

Separation of fecal neutral sterols by column chromatography[1–4], and by gas–liquid chromatography[5,6] has been reported by several groups of investigators. Separation of synthetic compounds by thin-layer chromatography (TLC) was also described[7,8]. In the present paper, the quantitative separation of fecal cholesterol and coprosterol by TLC is reported.

TLC was carried out according to the procedure of STAHL[9] on 20 × 20 cm plates, 275 mμ thick. The plates were developed with a solvent system containing toluene and ethyl acetate (9:1, v/v) until the solvent front had migrated 12 cm from the origin. The development time was approximately 40 min. The plates were sprayed with a solution of phosphomolybdic acid (10 % w/v) and heated to 110° for detection of sterols. Each unsprayed gel area, corresponding to a migration zone, was introduced into a liquid scintillation counter vial, and ^{14}C and ^{3}H activities were determined.

Coprosterol-^{14}C was prepared in the following manner: cholesterol-4-^{14}C (50 mC/mmol) was suspended in ethyl ether and added to 50 ml of isotonic NaCl solution. The ether was evaporated and the mixture was autoclaved at 125° for 15 min. An aliquot of normal human feces was transferred to the flask and was incubated at 37° with slow shaking for 6 days under nitrogen. The incubate was lyophilized and the powder was extracted in a Soxhlet extractor with ethyl alcohol. Saponification was carried out by KOH, and neutral sterols were extracted with petroleum ether.

TABLE I

AVERAGE C.P.M. PER ZONE (AND STANDARD ERROR OF MEANS) OF TLC SEPARATED NEUTRAL STEROL EXTRACT OF HUMAN FECES AFTER THE ADDITION OF CHOLESTEROL-7α-^{3}H AND COPROSTEROL-4-^{14}C RECOVERED FROM ZONES CORRESPONDING TO CHOLESTEROL AND COPROSTEROL

Amount μl	*Number of spots*		*Cholesterol zone*		*Coprosterol zone*	
			^{3}H (c.p.m.)	*^{14}C (c.p.m.)*	*^{3}H (c.p.m.)*	*^{14}C (c.p.m.)*
5	4	Average	1263 ± 36	0	10 ± 1.5	203 ± 8
		% Distribution	97	—	0.8	100
10	4	Average	2744 ± 55	0	23 ± 3.5	463 ± 9
		% Distribution	98	—	0.8	99.6
20	13	Average	5155 ± 86	0	28 ± 3.6	872 ± 16
		% Distribution	98.7	—	0.5	99.6

The petroleum ether was washed with water, concentrated and chromatographed twice in the system described. The zone corresponding to coprosterol was cut out, the silica gel recovered and extracted three times with ethanol and acetone (1:1, v/v) This procedure resulted in a 30–40 % conversion of cholesterol-zone radioactivity to

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coprosterol-zone radioactivity in the present system. When the latter material was subjected to gas–liquid chromatography (by Drs. E. MOSBACH and S. SHEFER) a single homogeneous peak was obtained in the zone corresponding to coprosterol. Cholesterol-7α-^{3}H (225 mC/mmol) and coprosterol-^{14}C were added to the "cold" cholesterol–coprosterol mixture and to neutral sterol extract of normal human feces before application to the chromatoplates.

The R_F values of the various sterols investigated were: cholesterol 0.30; coprosterol 0.40; epi-coprosterol 0.42; coprostenone 0.41; Δ^7-cholesten-3β-ol 0.26; cholestanol 0.27; 7-dehydrocholesterol 0.28; β-sitosterol 0.30; stigmasterol 0.31; 4-cholesten-3-one 0.50.

All cholestane compounds migrated together with cholesterol, except 4-cholesten-3-one, and all coprostane compounds were recovered with coprosterol. Table I shows the radioactivity recovered from the chromatographed stool extract. The average overlap of the two labels carried by the two compounds varied between 0 and 0.8 %. Since the larger majority of fecal neutral sterols consists of coprosterol and cholesterol, the method described seems satisfactory for the rapid separation of these two classes of material.

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Thin-layer chromatography of steroid conjugates

Various procedures have been reported for the chromatographic separation of steroid conjugates. For instance, group separation of steroid sulfates and glucuronosides may be achieved by adsorption chromatography on alumina[1,2], silica gel[3] and florisil[4] or by ion exchange chromatography on columns of DEAE or ECTEOLA cellulose and DEAE Sephadex[5]. Similar results can be obtained by paper chromatography, employing acidic or alkaline solvent systems on regular[6–10] or ion exchange paper[5]. In the present communication thin-layer chromatography of steroid sulfates and glucuronosides is described.