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.

Thin layers of anion exchange cellulose (MN-300 G/DEAE and MN-300 G/ECTEOLA of Macherey, Nagel and Co., Düren, Germany) were prepared with regular equipment of Desaga, Heidelberg, Germany, using mixtures of 15 g DEAE cellulose (ion exchange capacity: 0.7 mequiv./g) or ECTEOLA cellulose (ion exchange capacity: 0.35 mequiv./g) in 80 and 75 ml water respectively. After 2 h at room temperature all plates were kept in an oven at 50° for 45 min. 25 μg of the different conjugates:

estrone sulfate*
dehydroepiandrosterone sulfate*
androsterone sulfate*
pregnenolone sulfate
17-hydroxypregnenolone sulfate
dehydroepiandrosterone glucuronoside**
androsterone glucuronoside**
etiocholanolone glucuronoside**,

dissolved in 0.01 ml methanol, were applied to the plates. The following solvent systems proved effective for adequate separation by ascending chromatography in an S-chamber:

(1) 0.5 *M* acetate buffer, pH 4.25
(2) 0.5 *M* acetate buffer, pH 4.75
(3) 1.0 *M* acetate buffer, pH 4.75
(4) 1.5 *M* acetate buffer, pH 5.00
(5) isopropanol–water–formic acid (65:33:2 v/v)
(6) ethanol–water–acetic acid (80:15:3 v/v)
(7) methanol–water–acetic acid (75:15:10 v/v).

While steroid conjugates with a 17-keto group were detected on dried (80°) plates by spraying with Zimmermann reagent, consisting of 2 vol. 2 % *m*-dinitrobenzene in 95 % ethanol and 1 vol. 15 % potassium hydroxide in 95 % ethanol, and subsequent heating to 75°, Δ^5-3β-hydroxysteroids were revealed by treatment with OERTEL-EIK-NES reagent[11], prepared from 1 vol. 95 % ethanol and 2 vol. conc. sulfuric acid.

All of the R_F values, given in Tables I and II, represent the mean from three chromatograms. As can be seen, satisfactory resolution of steroid sulfates and glucuronosides may be obtained by use of the solvent systems listed above. Lowering the pH or increasing the molarity of the buffer solution results in higher mobilities of steroid glucuronosides as well as steroid sulfates. Likewise, an increase in the acid concentration of the aqueous organic solvents leads to a rise in the mobility of steroid conjugates, especially pertaining to steroid glucuronosides, whereas the mobility of steroid sulfates is only slightly affected.

The application of thin-layer chromatography on anion exchange cellulose to the isolation of steroid conjugates in purified plasma extracts confirmed previous findings[11] for non-polar, solvolysable complexes, which differ markedly from authentic steroid sulfates, inasmuch as they remain at the origin of the thin-layer chromatograms.

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TABLE I

THIN-LAYER CHROMATOGRAPHY OF STEROID CONJUGATES ON DEAE CELLULOSE

Conjugate	R_F value in solvent system						
	1	2	3	4	5	6	7
Estrone sulfate	0.08	0.03	0.07	0.08	0.08	0.02	0.03
Dehydroepiandrosterone sulfate	0.19	0.09	0.14	0.20	0.22	0.07	0.06
Androsterone sulfate	0.23	0.15	0.22	0.24	0.33	0.08	0.07
Pregnenolone sulfate			0.27				
17-Hydroxypregnenolone sulfate			0.09				
Dehydroepiandrosterone glucuronoside	0.54	0.38	0.41	0.56	0.72	0.29	0.36
Androsterone glucuronoside	0.59	0.45	0.48	0.62	0.77	0.35	0.46
Etiocholanolone glucuronoside	0.56	0.40	0.45	0.58	0.75	0.31	0.39

TABLE II

THIN-LAYER CHROMATOGRAPHY OF STEROID CONJUGATES ON ECTEOLA CELLULOSE

Conjugate	R_F value in solvent system						
	1	2	3	4	5	6	7
Estrone sulfate	0.08	0.05	0.08	0.07	0.54	0.05	0.04
Dehydroepiandrosterone sulfate	0.16	0.14	0.23	0.15	0.79	0.11	0.08
Androsterone sulfate	0.23	0.18	0.29	0.21	0.85	0.13	0.09
Pregnenolone sulfate			0.34				
17-Hydroxypregnenolone sulfate			0.12				
Dehydroepiandrosterone glucuronoside	0.65	0.45	0.61	0.64	0.90	0.64	0.48
Androsterone glucuronoside	0.72	0.49	0.67	0.70	0.91	0.77	0.59
Etiocholanolone glucuronoside	0.67	0.47	0.64	0.65	0.91	0.71	0.55

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