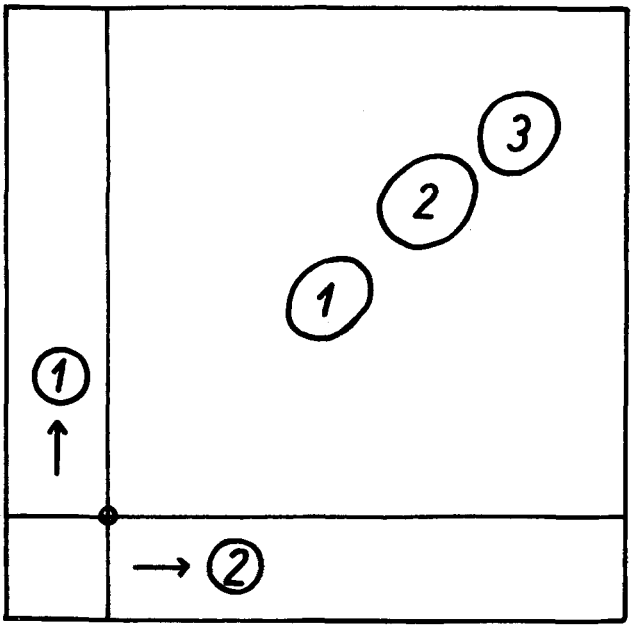

FIG. 1. Two-dimensional chromatography of sterols without bromination on the paper impregnated with aluminum hydroxide. Developing solution, benzene; developing time. 20–30 min.; 5–10 μg of each substance. 1, cholesterol + β-cholestanol; 2, α-coprostanol + β-coprostanol; 3, α-cholestanol.

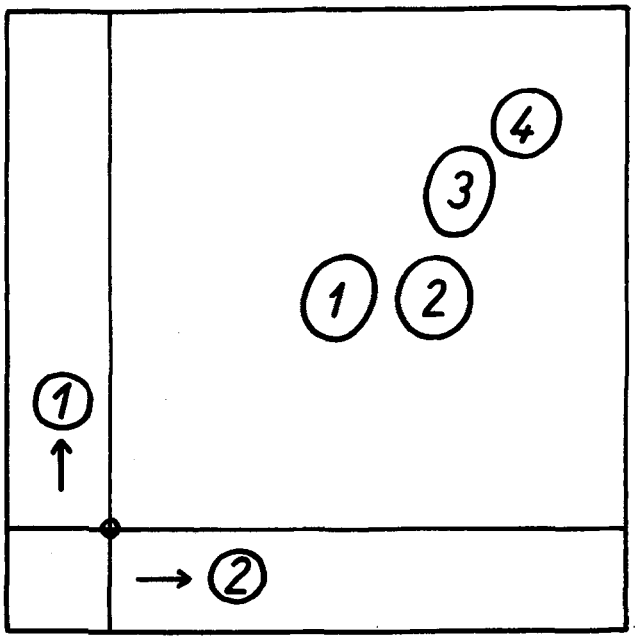

FIG. 2. Two-dimensional chromatography of sterols with bromination on the paper impregnated with aluminum hydroxide. Developing solutions: first run, benzene; second run. benzene–bromine 100:0.5 (v/v). Developing time, 20–30 min. 5–10 μg of each substance. 1, β-cholestanol; 2, cholesteryl bromide; 3, α-coprostanol + β-coprostanol; 4, α-cholestanol.

Paper chromatography of some cholesterol derivatives

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» Although column or thin-layer chromatography has been used for the separation of cholesterol and co-

prostanol and their derivatives (1–3), paper chromatography has not been very successful (4–7), especially for the separation of cholesterol and cholestanol (dihydrocholesterol).

The present paper describes a method for one-dimensional separation of cholesterol, α-cholestanol, α-coprostanol, and β-coprostanol on papers impregnated with aluminum hydroxide or silica gel. Cholesterol and β-cholestanol may also be separated by chromatographing in the second dimension with bromine included in the solvent mixture so that cholesterol is converted to the bromide derivative on the paper.

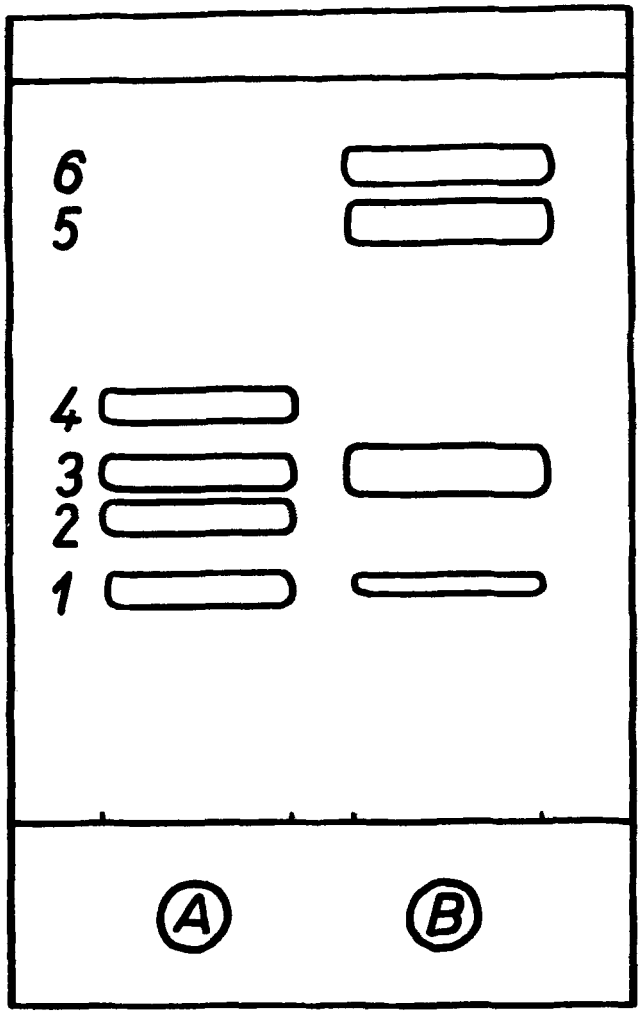

FIG. 3. One-dimensional chromatography of sterols on the glass fiber paper impregnated with silica gel. Developing solution, 5% diethyl ether–petroleum ether (60–90°). Developing time, 20 minutes. A. Reference compounds: 1, cholesterol + β-cholestanol; 2, α-coprostanol; 3, β-coprostanol; 4, α-cholestanol. 5 μg of each sterol. B. Human fecal sterols: 1, cholesterol + β-cholestanol; 3, β-coprostanol; 5 and 6, unidentified substances.

After spotting of the substances on Whatman cellulose paper loaded with aluminium hydroxide (ca. 7.5% Al_2O_3; made by W. and R. Balston, Ltd., Springfield Mill, Maidstone, Kent, England, and delivered by H. Reeve Angel and Co., London, E.C.4.), the chromatography was carried out with benzene at room temperature using an ascending technique. This system is suitable for the separation of cholesterol + β-cholestanol, α-coprostanol + β-coprostanol, and α-cholestanol (Fig. 1). The overlapping mixture of cholesterol and β-cholestanol is separated on aluminum hydroxide paper by developing the second dimension with benzene–bromine 100:0.5 (v/v). Because cholesteryl bromide has the same R_f value as the mixture of coprostanols, it is necessary to perform two-dimensional chromatography (Fig. 2).

The second system employs glass fiber (Whatman GF/A) or cellulose paper (Whatman No. 3) impregnated with silica gel according to Hamilton *et al.* (8). Development with 5% diethyl ether–petroleum ether (60–90°) permits separation of cholesterol + β-cholestanol, α-coprostanol, β-coprostanol, and α-cholestanol (Fig. 3). The mobilities vary somewhat, and it is very useful to use standards.

The spots were detected with a 10% solution of phosphomolybdic acid in ethanol or a 0.001% solution of Rhodamine B in 0.25 M dipotassium hydrogen phosphate. The latter staining technique is very sensitive and less than 1 μg of one sterol can be detected in the presence of a 500-fold excess of another.

After chromatography on glass fiber paper impregnated with silica gel and development with Rhodamine B, components could be determined semi-quantitatively by densitometry.

These simple and rapid techniques have been applied to the characterization of human fecal sterols. It has been ascertained that this material contains a large amount of β-coprostanol and little cholesterol or β-cholestanol. Further results will be reported elsewhere (9).

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