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Fatty acid composition of serum cholesteryl ester fractions isolated by thin-layer chromatography

Paper and thin-layer chromatographic methods have been devised for routine studies of the fatty acid pattern of cholesteryl esters of serum[1,2]. With appropriate solvents the cholesteryl esters can be separated into five well defined bands (Fig. 1).

In an extensive study of the fatty acid changes of serum cholesteryl esters in alcoholics[3] it was desirable to estimate fatty acid composition by a more rapid and simple method than gas–liquid chromatography (GLC). A modified thin-layer chromatographic (TLC) method was developed[3], but before it was applied routinely, the five cholesteryl ester bands were isolated, and their fatty acid composition was investigated.

Experimental

0.5 ml of serum from normal adults[3] was extracted with chloroform–methanol (1:1, v/v). The polar lipids of the extract were removed by passing the total lipids, dissolved in chloroform, through a 1 g silicic acid column. The chloroform eluate was evaporated under nitrogen to a small volume and spotted as a 10 cm long band on a 0.25 mm thick thin-layer plate of Silica Gel G. The plate was developed in *n*-heptane–toluene (60:25, v/v) three consecutive times, as described elsewhere[3]. The cholesteryl ester bands were visualized by spraying the plate with bromthymol blue reagent. The bands were scraped off, transferred to centrifuge tubes, and dried *in vacuo*. Methyl esters of the fatty acids were made by transmethylation with 0.1 *N* sodium methylate in dry methanol at 100° for 2 h. The methyl esters were assayed by GLC, using a Perkin-Elmer Model 880 apparatus, on a 15% diethyleneglycol–succinate

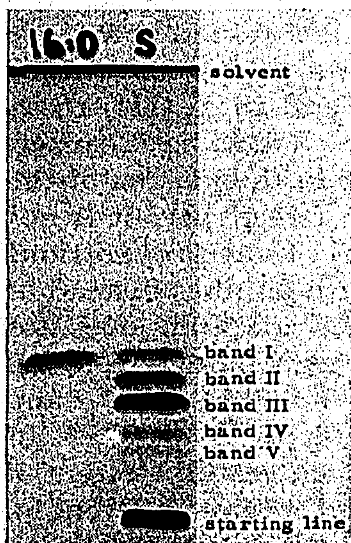

Fig. 1. Thin-layer chromatography of cholesteryl esters in human serum. S = Serum lipids, 16:0 = reference standard. The lipid extract was spotted as a 1.5 cm long line. The plate was developed as described in the text. The spots were visualized by spraying with 50% sulphuric acid and heating at 180° for 30 min.

polyester (DEGS) column, coated on Gas-Chrom Q, 80–100 mesh. The methyl esters were identified as earlier described[4].

Results and discussion

The separation of the serum cholesteryl esters under the present conditions is entirely dependent on the degree of unsaturation of the fatty acids. This is evident from Table I which shows that in each of the five bands there is only one major fatty

TABLE I

PERCENTAGE OF THE FIVE DIFFERENT CHOLESTERYL ESTER BANDS OF HUMAN SERUM ISOLATED BY THIN-LAYER CHROMATOGRAPHY

Fatty acid	*Band*				
	I	*II*	*III*	*IV*	*V*
14:0	3.7	0.8	—	—	—
16:0	86.0	2.6	—	—	0.9
16:1	—	6.0	—	—	—
18:0	8.5	—	—	—	0.5
18:1	1.8	90.6	—	0.3	0.7
18:2ω6	—	—	100.0	2.8	0.8
18:3ω6	—	—	—	7.3	—
18:3ω3	—	—	—	6.2	0.3
18:4ω3	—	—	—	0.3	1.5
20:3ω6	—	—	—	2.8	—
20:4ω6	—	—	—	80.4	2.7
20:5ω3	—	—	—	—	77.5
22:6ω3	—	—	—	—	15.3

acid, namely 16:0, 18:1, 18:2, 20:4, 20:5, respectively. The uniformity in fatty acid composition of each band might explain why it is possible to obtain such a sharp separation between them. Fatty acid compositions of cholesteryl ester bands separated by TLC, have been presented earlier by ZÖLLNER[5] but it was not mentioned how these figures were obtained. He gave the following data for fatty acid composition, arranged according to decreasing R_F value: (I) 16:0 and 18:0, (II) 16:1 and 18:1, (III) 18:2, (IV) 20:3 and 20:4, (V) 20:5, 22:5 and 22:6. Similar but less detailed data have earlier been reported on the fatty acid composition of cholesteryl esters in the plasma of the *Cebus* monkey[7]. Since no quantitative figures were given in the two papers it is not possible to make any detailed comparison. However, the trienes of our preparations were composed mainly of 18:3 of both the linoleate and linolenate families instead of 20:3 and we were able to identify 22:5 only in trace amounts. Overlapping does not exceed 3.4% of the total fatty acid content of any band except for band V (7.4%). This band contains only 2–4% of the total cholesteryl esters of human serum[2,3] and the risk of admixture is thus greater.

These results are valid only for the fatty acid pattern of cholesteryl esters of human serum. The fatty acid composition of the cholesteryl esters are different in other fluids and tissues[6]. Thus, it is necessary to determine the fatty acid composition of the cholesteryl esters from every new source by GLC before the assay of chromatographically separated cholesteryl esters can be used as an index of their fatty acid composition.

Conclusion

Cholesteryl esters were separated into five well defined fractions by TLC. Their fatty acid composition was quantitatively determined by GLC. It was found that each fraction obtained only one major fatty acid, 16:0, 18:1, 18:2, 20:4 and 20:5, respectively, which means that the separation was only dependent on the degree of unsaturation of the fatty acids.

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Délipidation totale des extraits plasmatiques contenant les 17 cétostéroïdes (déhydroépiandrostérone, androstérone, étiocholanolone)*

Pour évaluer les 17 cétostéroïdes plasmatiques, il est indispensable de les séparer des lipides que contiennent les extraits totaux. Cette délipidation doit retirer des quantités importantes de cholestérol libre et estérifié, triglycérides, acides gras, phospholipides et, éventuellement, diglycérides et monoglycérides. Pour la réaliser de nombreuses méthodes ont été préconisées, qui ont toutes des inconvénients: la partition entre solvants (p. ex., méthanol/eau 70:30–hexane 1:1) est fastidieuse et laisse un résidu lipidique non négligeable[1–6]; la précipitation à basse température (−15 °) durant une quinzaine d'heures dans des mélanges complexes à base de méthanol–eau 90:10[7], 70:30[8], 80:20[9] ne débarrasse qu'incomplètement les 17 cétostéroïdes des lipides les accompagnant; le recours à une purification sur colonne de florisil[10–12], d'alumine[13–15], de silicagel[16,17] ou d'Amberlite[18] ne résout pas davantage le problème et introduit, en plus, des pics artefacts dans la chromatographie gazeuse ultérieure[19]; le passage de l'extrait plasmatique sur deux colonnes successives chargées d'un

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