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QUANTITATIVE THIN-LAYER–GAS–LIQUID CHROMATOGRAPHY OF HUMAN BLOOD TRIGLYCERIDES

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SUMMARY

A technique for the quantitative recovery of triglycerides separated on impregnated and non-impregnated layers of silica gel, based on the principle of dry column chromatography, has been developed.

By using this technique, we analysed triglyceride fractions obtained from human blood plasma and erythrocyte lipids. Triglycerides were isolated by column chromatography and by thin-layer chromatography. Further separation according to their degree of unsaturation was achieved on a layer of silica gel impregnated with 3 % of silver nitrate. All of the separated fractions were quantified by the hydroxamic acid method, analysed by gas–liquid chromatography according to their carbon number and converted into their methyl esters with sodium methoxide. The fatty acid compositions of all of the separated fractions were determined by quantitative gas–liquid chromatography.

INTRODUCTION

Studies of the structure and metabolism of triglycerides require more detailed information than it is possible to obtain by the usual triglyceride fatty acid analysis. We can obtain very important data about the molecular weight distribution of a sample by gas–liquid chromatography (GLC) of the intact triglycerides. Although this type of analysis was described several years ago[1–6], it has not found wide application. In 1972, WATTS *et al.*[7] published a paper on the clinical application of direct gas chromatographic (GC) triglyceride analysis. The object of investigations on human blood lipids carried out in our laboratory was to demonstrate the presence of triglycerides in erythrocyte lipids. By GC analysis, differences in the composition of erythrocyte triglycerides were observed in healthy subjects in comparison with patients suffering from arteriosclerosis. At this stage of our study, it was necessary to establish whether the erythrocyte triglyceride is not of artificial plasmatic origin, as is believed by certain workers[8,9]. For this reason, a decision was made to fractionate triglycerides of the plasma and of erythrocytes of the same person under study, according to the degree of unsaturation, on a thin layer of silica gel impregnated with silver nitrate.

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In order to analyze by GC the fractionated triglycerides thus obtained, it was necessary to find, first of all, a method that permits the quantitative recovery of even very small amounts of triglycerides separated by thin-layer chromatography (TLC).

EXPERIMENTAL

Previously described extraction methods[10–12] for the quantitative recovery of non-polar lipids separated by TLC were checked using as the standard pure triolein (Applied Science Laboratories) at several concentrations. The recovery was measured colorimetrically by the hydroxamic acid method against sample that had not been applied to the plate. The results obtained with several types of extraction mixtures and techniques, shown in Table I, were not satisfactory.

TABLE I

RECOVERIES OBTAINED BY EXTRACTION PROCEDURES WITH DIFFERENT SOLVENT SYSTEMS

All values are percentages of the originally spotted sample.

Amount of sample (μmoles)	*Solvent*[a]		
	I	*II*	*III*
0.33	35	68	62
0.66	65	69	87
1.00	86	80	80
1.33	76	73	73

[a] Solvent systems: I = methanol–diethyl ether (1:9); II = methanol–light petroleum (1:9); III = methanol–diethyl ether (1:9) with re-extraction with pure diethyl ether after acidification with 10% HCl.

We therefore tried to improve the elution of the separated triglycerides by a modified technique of dry column chromatography. Material scraped from the plate was poured into a glass column of internal dimensions 8 × 400 mm, at the lower end of which was a glass-wool plug covering a 4-cm layer of Florisil–silica gel (1:1, w/w). Lipids were eluted with 50 ml of several different solvents and solvent mixtures. The best results were obtained with chloroform or light petroleum–diethyl ether (1:1) for layers that were not sprayed with dichlorofluorescein.

Great differences were observed between the recoveries of triglycerides made visible with dichlorofluorescein and with Silica Gel $HF_{254+366}$ (Merck), as demonstrated in Table II.

A mixture of 20% of methanol in chloroform gives quantitative results for layers sprayed with dichlorofluorescein and for impregnated layers. In this instance, the eluted triglycerides have to be re-extracted after the evaporation of the solvents three times with 5-ml portions of light petroleum.

The method has been used in a clinical study of human blood triglycerides. Lipids from plasma and erythrocytes were extracted by the modified methods of FOLCH *et al.*[13] and WAYS AND HANAHAN[14]. We obtained the triglyceride fraction from plasma lipids by TLC and from erythrocyte lipids by a combination of column

TABLE II

DIFFERENCES BETWEEN RECOVERIES OF TRIGLYCERIDES OBTAINED FROM SPRAYED AND NON-SPRAYED LAYERS

Amount of sample (μmoles)	*Solvent*[a]					
	I		*II*		*III*	
	a	*b*	*a*	*b*	*a*	*b*
0.33	100	62	95	60	98	70
0.66	96	56	96	59	95	65
1.00	95	76	103	78	98	76
1.33	94	78	100	78	100	72

[a] Solvents: I = chloroform; II = light petroleum–diethyl ether (1:1); III = diethyl ether. a = Without spraying (substances were made visible by using a fluorescence indicator in Silica Gel $HF_{254+366}$, Merck); b = with spraying with 0.2% of dichlorofluorescein in ethanol.

chromatography and TLC. Triglycerides were further fractionated according to the degree of unsaturation on a thin layer of silica gel impregnated with 3% of silver nitrate.

Quantitative GLC of all of the separated triglyceride fractions was performed under the usual conditions on a 60-cm column packed with 3% OV-1 on Gas-Chrom Q, 100–120 mesh. After the GLC analysis, the triglyceride fractions were quantified by the hydroxamic acid method and converted into their methyl esters with sodium methoxide. The fatty acid compositions of the individual triglyceride fractions were then determined by quantitative GLC. All of the quantitative data were subjected to computer analysis, as previously described[15].

RESULTS

The triglycerides of human blood serum and erythrocytes were analyzed by the method described. The quantitative composition of the different fractions isolated after separation according to the total number of double bonds in the molecule of the triglyceride is shown in Table III. In erythrocyte triglycerides, a relatively

TABLE III

QUANTITATIVE COMPOSITION OF HUMAN BLOOD TRIGLYCERIDES ACCORDING TO THEIR DEGREE OF UNSATURATION

	Triglyceride fraction[a] (% *of total triglycerides*)			
	I	*II*[b]	*III*	*IV*
Plasma	4.8	22.0	42.3	30.9
Erythrocytes	14.0	20.2	42.8	24.0

[a] Fractions: I = saturated triglycerides (correspond to $n = 0$); II = monoenoic triglycerides[b] (correspond to $n = 1$); III = dienoic triglycerides (correspond to $n = 2$); IV = polyenoic triglycerides (correspond to $n = 3$).

[b] Fraction II is probably not pure.

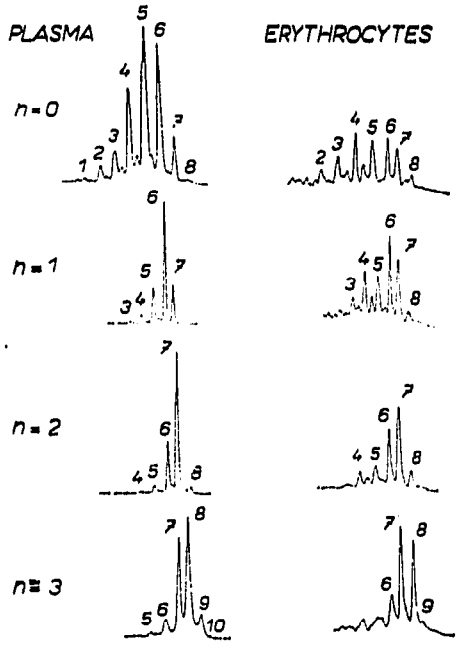

Fig. 1. Fractionation of human blood triglycerides according to their degree of unsaturation. Peaks are identified by the carbon number (the number of carbon atoms in the fatty acid moieties of the triglyceride molecule).

Carbon number	40	42	44	46	48	50	52	54	56	58
Peak number	1	2	3	4	5	6	7	8	9	10

n corresponds to the number of double bonds (except for fraction $n = 1$, which is probably not pure, as indicated by its fatty acid composition listed in Table V).

TABLE IV

COMPOSITION OF TRIGLYCERIDE FRACTIONS ACCORDING TO THEIR CARBON NUMBER

All values listed are in % (w/w).

Carbon number	*Fraction*[a]							
	I		*II*		*III*		*IV*	
	P[b]	*E*[c]	*P*	*E*	*P*	*E*	*P*	*E*
40	Trace	Trace	—	—	—	—	—	—
42	2.46	6.28	—	Trace	—	—	—	—
44	4.87	9.97	1.51	5.29	—	—	—	—
45	0.99	4.11	—	Trace	—	—	—	—
46	15.94	17.39	3.74	13.60	Trace	5.11	—	—
47	1.17	6.22	—	4.59	—	Trace	—	—
48	37.20	18.63	20.42	14.16	3.95	8.04	1.35	Trace
50	28.30	17.45	52.50	27.80	19.69	26.71	5.92	2.57
52	9.02	15.15	21.76	30.00	71.80	50.90	34.20	49.40
54	Trace	4.75	—	4.39	4.47	9.21	47.50	48.00
56	—	Trace	—	—	—	Trace	10.87	Trace
58	—	—	—	—	—	—	Trace	—

[a] Designation of fractions as in Table III.
[b] P = plasma triglycerides.
[c] E = erythrocyte triglycerides.

high content of saturated fraction I and a low content of polyenoic fraction IV are interesting in comparison with the composition of plasma triglycerides. The molecular compositions of the individual fractions of both types of triglycerides studied, obtained by GC, are shown in Fig. 1.

Quantitative analyses from Fig. 1 are presented in Table IV.

The fatty acid composition of the individual fractions, which is also the criterion of purity of these fractions, was studied again by quantitative GC of the methyl esters of fatty acids obtained by transesterification of the individual triglyceride fractions. The results of the analyses are given in Table V.

TABLE V

FATTY ACID COMPOSITION OF INDIVIDUAL TRIGLYCERIDE FRACTIONS

All values in % (w/w).

Fatty acid	*Fraction*[a]							
	I		*II*		*III*		*IV*	
	P[b]	*E*[c]	*P*	*E*	*P*	*E*	*P*	*E*
12:0	Trace	Trace	—	—	—	Trace	—	Trace
14:0	10.64	2.78	—	Trace	1.91	Trace	Trace	0.78
15:0[d]	0.15	6.45	Trace	2.23	Trace	2.59	Trace	2.69
16:0	66.80	41.37	29.21	21.41	26.93	25.16	13.35	13.32
16:1	—	—	1.10	4.12	6.00	8.46	3.48	4.03
17:0[d]	Trace	9.10	Trace	5.76	Trace	3.29	Trace	3.81
17:1	—	—	Trace	3.86	Trace	2.83	Trace	2.92
18:0	21.24	40.32	21.13	22.88	2.98	9.19	5.08	9.59
18:1	—	—	48.50	39.69	62.10	48.40	37.80	35.40
18:2	—	—	—	—	Trace	Trace	22.40	20.97
18:3	—	—	—	—	—	—	3.93	Trace
20:0	—	—	—	—	—	—	2.08	4.50
20:2	—	—	—	—	—	—	1.91	—
20:3	—	—	—	—	—	—	Trace	—
20:4	—	—	—	—	—	—	9.82	1.95

[a] Designation of fractions as in Table III.
[b] P = plasma triglycerides.
[c] E = erythrocyte triglycerides.
[d] Including branched-chain acids with corresponding number of carbon atoms.

The knowledge of the fatty acid composition of individual triglyceride fractions makes it possible to determine the main molecular types of triglycerides and to calculate their relative proportions.

The fatty acid compositions of triglyceride fractions of plasma and erythrocytes are similar, but different proportions of the individual molecular types must be assumed. All of these findings are indicative of different origins of plasma and erythrocyte triglycerides and support the hypothesis of the existence of trace amounts of triglycerides in the erythrocyte lipids.

From the present analytical study, it is not yet possible to determine the role played by these substances in erythrocyte metabolism.

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