Fractionation of Serum Triglycerides on a Silicic Acid-Silver Nitrate Column

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By using silver nitrate-silicic acid columns de Vries has recently separated various lipids differing only in geometry or in number of their double bonds [1]. He applied the new method to several model compounds, but Haahti *et al.* have later extended its use also for natural mixtures of cholesterol esters [2]. The present report describes the application of the new method to the more complex mixture of serum triglycerides.

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The triglycerides were isolated from a pooled sample of postabsorptive donor serum by using conventional chromatographic methods [3]. Their fatty acid "spectrum" was similar to that described by Hallgren *et al.*[4]

The triglycerides were fractionated on a silver nitrate-silicic acid column essentially as described by de Vries [5]. All the fractions in Fig. 1 were practically homogeneous triglycerides according to TLC on Kieselgel G, and they showed fairly correct ester-glycerol ratios [6,7]. Their fatty acid composition is recorded in Table 1.

The glycerides of the first peak in Fig. 1 contained only saturated fatty acids. The second peak contained in average one double bond per glyceride molecule, the front of the third peak two, and the rear of the third peak three double bonds. The double bond content increased also in the last two peaks, which contained more than three olefinic linkages per glyceride molecule.

The fractionation obtained was thus analogous to de Vries' observations on model triglycerides [1]. Therefore it appears very likely that the number of double

Table 1. Approximate composition of the triglyceride fractions *

Sample	Relative amounts of different fatty acids ** (Area %)												Average number of double bonds in triglycerides
	14:0	15:0(?)	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5(?)	22:5	22:6	
Original mixture	5	1	34	5	3	43	5	1	+	+	+	1	2.3
Fr. 6	16	2	71		9								0.0
Fr. 8	14	2	69		10	3							0.1
Fr. 12	10		51	2	6	30							1.0
Fr. 15	8	2	51	4	5	30							1.1
Fr. 22	2		34	3	3	56	2						1.9
Fr. 29	3		28	7		63							2.1
Fr. 31	3	2	25	9		59	3						2.3
Fr. 36	2		19	5		62	13						2.8
Fr. 41	2		15	10		63	10	1					2.9
Fr. 52	1		22	7	2	43	19	3	3				3.3
Fr. 60	2		37	5	3	24	9		3	4	4	7	4.3
Fr. 61	5		36	6	3	22	5		2	2	3	16	5.0

* Analyzed by acid catalyzed methanolysis and subsequent GLC analysis of the methylesters on a polyethylene glycol succinate column at 180°.

** The different fatty acids are symbolized by the chain length and by the number of their double bonds.

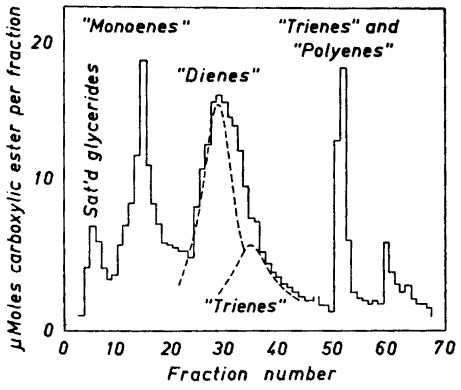

Fig. 1. Chromatography of 117 µmoles of serum triglycerides on a 6.0 g column (13 × 70 mm) of silicic acid-silver nitrate. Fraction volume: 5 ml. Recovery: 106 %. Fractions 1—40 were eluted with petroleum ether containing 0—100 % toluene. The 'polyenoic' fractions were recovered with chloroform and chloroform-methanol (20:1) (v/v).

bonds in each triglyceride molecule is constant within the 'monoenoic', 'dienoic', and 'trienoic' peaks in Fig. 1. This means that the serum triglycerides contained about 6 % saturated, 21 % 'monoenoic', 35 % 'dienoic', and 38 % 'trienoic' and 'polyenoic' glycerides.

Table 1 suggests among other things that the 'dienoic' triglycerides were mainly of 'monopalmito-diolein' type, whereas the amount of the 'dipalmito-monolinolein' type seemed to be surprisingly small. Similar analysis of the 'polyenoic' glycerides is not yet possible because of their incomplete separation. However, it is apparent that they contained rather large amounts both of fully saturated and of tetra-, penta-, and hexaenoic acids.

The monochain lipids are, of course, easier to separate than the polychain lipids. — We have thus obtained very satisfactory separations of, *e.g.* methyl-esters on the silicic acid-silver nitrate adsorbent *. — But nevertheless we think that the real value of de Vries' method lies in its application for the analysis of chain combinations in the polychain lipids.

* 'Monoenoic fatty acids of serum sphingomyelins'; Under preparation.

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The Degradation of Alginates at Different pH Values

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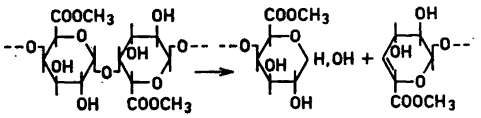
Recent investigations have shown [1-3] that pectin is rapidly degraded both in alkali and in neutral solutions and that this degradation leads to the formation of unsaturated uronic acid derivatives. The carbonyl group of the methyl ester is essential for this reaction and the degradation is regarded as a β-elimination reaction:



Consequently, the free acids are known to be more stable in alkaline solutions, and whether the same reaction mechanism occurs with a free carboxyl group in the 6-position does not seem to be definitely established [4].