Column Chromatographic Fractionation of Plasma Lipids

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PARTIAL dehydration of silicic acid will increase its adsorptive property for fats to a maximum. Further dehydration will decrease the adsorptive properties. The anhydrous acid has practically no adsorptive properties.[1]

The degree of hydration not only affects the amount of fats which each gram of the acid may adsorb, but also the type of solvent needed for their elution. A specific example is that of cholesterol esters; when added to a column of silicic acid containing from 20 to 22 per cent moisture, the esters may be quantitatively eluted with straight petroleum ether. However, cholesterol esters added to a column of silicic acid containing 15 per cent or less water of hydration cannot be eluted with petroleum ether alone but must be eluted with a mixture of petroleum ether and diethyl ether.

Since there are no simple methods for controlling the degree of activation, we have found that the most reliable and reproducible results may be obtained by using silicic acid as it is generally obtained from the manufacturer. This contains 20 to 22 per cent moisture. It is well to specify that the acid on ignition shall have a loss of from 20 to 22 per cent by weight.

The columns used consist of 25 ml burettes with self-lubricating teflon plug assemblies* and 125 ml flasks fused to the top of the burettes as reservoirs. A glass wool plug is introduced, and a mixture of equal parts by weight of silicic acid and celite is added, with tamping, to a height of 10 cm. Plasma lipids dissolved in small amounts of petroleum ether containing not more than 30 mg may be added to this column. The tube containing the lipids is washed three times with 2 ml portions of petroleum ether which are added to the column and allowed to flow in without pressure until the liquid has become completely absorbed.

CHOLESTEROL ESTERS

Cholesterol esters are eluted with a total volume of 100 ml petroleum ether under slight positive pressure to maintain a flow rate of approximately 1 ml/minute. The efficiency of the separation is checked by comparing the values for free and total cholesterol determined on the eluate and those obtained by direct analysis of the original plasma lipid filtrate and with values for total fat determined by Bragdon's method on the eluate.[2] In a series of 29 eluates the values differed from the direct determination of esterified cholesterol on the same filtrates by an average of 7.3 ± 5.1 mg % which was 4.8 ± 3.7 per cent of the direct values.† No free cholesterol was present in the eluates nor were other lipid fractions eluted with the cholesterol esters. This last fact was confirmed by the good agreement shown between values for the total fat determined on

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* Ultramax-Fischer, and Porter Co.

† Standard error of the mean.

the aliquots of the eluates and the total fat values calculated from the ester cholesterol using the factors given by Bragdon for cholesterol esterified with a C_{18} fatty acid. In the same series, the mean difference between total fat determined directly and calculated was 9.00 ± 3.00 mg % or 6.2 ± 1.12 per cent of the direct value.

SEPARATION OF FREE CHOLESTEROL, TRI-, DI-, AND MONOGLYCERIDES

Free cholesterol and purified mono-, di-, and triglycerides were added separately and also as mixtures to the columns and different mixtures of petroleum ether and diethyl ether were used to determine the optimum mixture for their separation. If not more than 30 mg of glycerides are added to the column, 50 ml of 3 per cent diethyl ether in petroleum ether will quantitatively remove all the free cholesterol and triglycerides, but none of the mono- or the diglycerides. Ten per cent diethyl ether will elute the diglycerides. One hundred per cent diethyl ether will elute the monoglycerides quantitatively. Excellent recoveries were obtained when a mixture containing equal amounts of each of the glycerides was added to the column, and eluted successively as noted above. When mixtures of the glycerides were added to plasma lipid filtrates they were quantitatively recovered by appropriate elution.

In one such experiment pooled filtrates equivalent to 2 ml of plasma were run on duplicate columns using the solvents specified above. To check recovery of added glycerides, three other pairs of columns were run with 5 mg of either tripalmitin, dipalmitin, or monopalmitin added to the filtrate before evaporation. A fifth pair of columns was run with 5 mg of each added. Results of this experiment are shown in Table I. Variations in recovery are within the error of the method used to determine total fat.[2]

TABLE I

Recovery of Glycerides Added to Pooled Acetone-Alcohol Filtrate of Plasma

Results are expressed as mg of total lipid in the pooled eluate from two columns, equivalent to 4 ml plasma

No. of filtrate:	PF-I	PF-II	PF-III	PF-IV	PF-V
Substance added:	None	Tripalmitin	Dipalmitin	Monopalmitin	Tri-, di-, and monopalmitin
Petroleum ether	11.15	11.15	11.05	11.85	11.55
3% Diethyl ether in PE	9.90	18.65	11.05	9.65	20.40
10% Diethyl ether in PE	0.50	1.15	8.65	0.50	10.00
100% Diethyl ether	0	0.65	0.50	9.40	10.00

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