Separation with uni-dimensional TLC of all neutral lipid classes

A method was devised to separate all classes of neutral lipid (cholesterol ester, triglyceride, fatty acid, cholesterol, 1,3-diglyceride, 1,2-diglyceride and monoglyceride) by TLC in a single dimension. This procedure permitted multiple samples to be applied to each plate.

No solvent system has been reported which will resolve all neutral lipid classes[1]. Solvent systems which separate the more rapidly migrating components leave the diglyceride isomers and cholesterol unresolved. Solvent systems designed to resolve the latter leave the cholesterol esters and triglycerides together at the solvent front. Bi-dimensional TLC has the distinct disadvantage of permitting application of only single samples per plate. The procedure described below utilizes sequential development in two different solvent systems to resolve all neutral lipid components.

Eight-inch square plates of Silica Gel G of 250 μ thickness were prepared on a Shandon spreader from a 2:1 (w/w) water-powder slurry and dried at 110°. Ten to forty μl of lipid sample in chloroform–methanol were applied at 3 cm from the bottom of the plate. The plates were then developed, first in a solvent of petroleum ether–ether–acetic acid (70:30:1, v/v) to a height of 16 cm. The plates were air-dried and then developed in the same dimension to a height of 11 cm (a position just below the triglyceride spot) in a solvent of ether–petroleum ether–acetic acid (70:30:1, v/v).

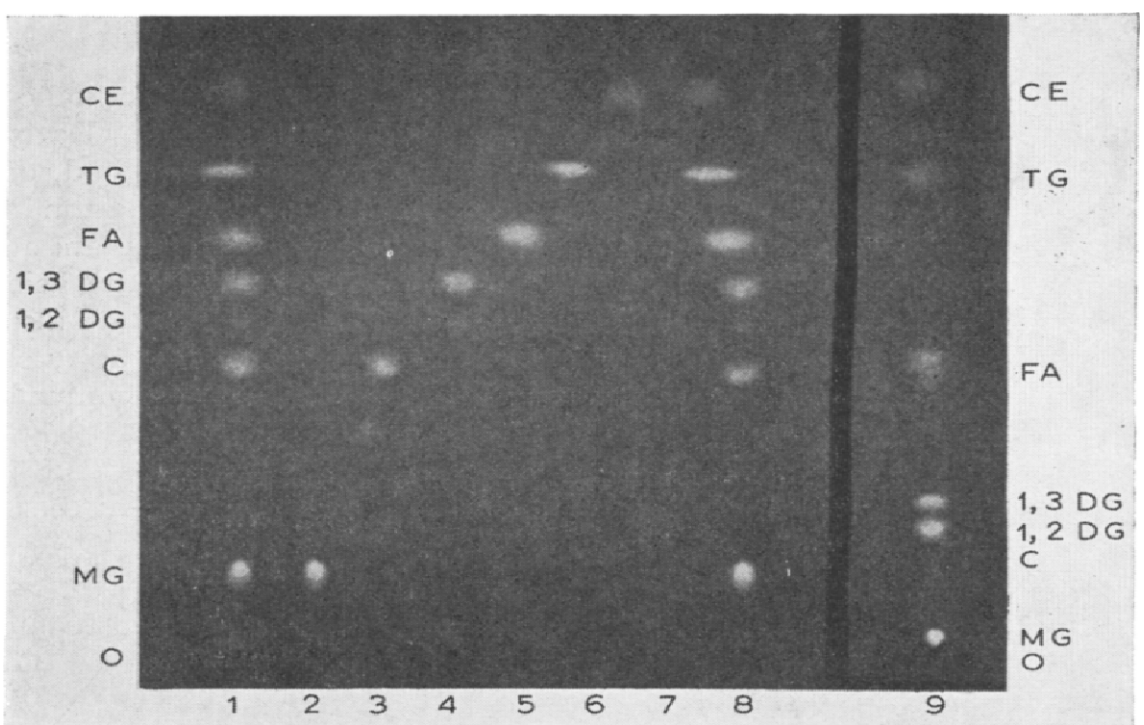

Fig. 1. CE = cholesterol ester; TG = triglyceride; FA = fatty acid; 1,3-DG = 1,3-diglyceride; 1,2-DG = 1,2-diglyceride; C = cholesterol; MG = monoglyceride; 1 and 8 = standard mixture; 2 = monopalmitin; 3 = palmitic acid; 4 = dipalmitin; 5 = cholesterol; 6 = tripalmitin; 7 = cholesterol palmitate; (1) through (8) are developed in both solvent systems; 9 = standard mixture after development in first solvent only. 10 μl of 1 mg/ml solution were applied. Standards were purchased from the Hormel Foundation, Austin, Minnesota, USA. The plate was visualized with 2% dichlorofluorescein in methanol and photographed under U.V. light.

The first solvent served to isolate the cholesterol esters and triglycerides in a position near the top of the plate. The second solvent served to separate cholesterol and the two diglyceride isomers. Phospholipids remain at the origin.

A typical separation is shown in Fig. 1.

This technique was used to chromatograph lipid extracts of serum enzyme digests of ^{14}C-labeled lipid preparations. It was thus possible to observe the localization and appearance of reaction products with considerable accuracy.

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