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Note

Separation of six lipid classes on one thin-layer chromatogram

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Since the concentrations of squalene[1] and cholesteryl esters[2] in the aorta have been related to the severity of atherosclerosis, it seems highly desirable to be able to separate these two lipid classes, along with triglycerides, fatty acids, sterols, and complex lipids, on one thin-layer chromatogram. Such a separation would allow the six lipid classes to be analyzed fluorometrically or densitometrically *in situ* on one chromatogram[3] and prevent losses of microgram quantities of these two lipids when scraped, eluted, and rechromatographed for separation by conventional procedures. Previously, Blank *et al.*[4] separated five lipid classes on a single chromatogram. However, an overlap of the sterol ester and hydrocarbon fractions necessitates the scraping of this region followed by elution and development in a different solvent system (*e.g.* diethyl ether–petroleum ether (b.p. 38.5–40.0°), 7:93) if these two classes are to be studied. This communication describes a technique for the separation of six lipid classes on one chromatogram through the sequential use of two solvent systems.

MATERIALS AND METHODS

Standard lipid mixture

Ten milligrams of the following lipid standards were dissolved in methylene chloride–methanol (2:1) to yield a final concentration of 1 μg of each per μl of solution: L-α-lecithin dipalmitoyl from Schwarz-Mann, Orangeburg, N.Y., cholesteryl oleate and triolein from Applied Science Laboratories, State College, Pa., cholesterol from Steraloids Inc., Pawling, N.Y., palmitic acid from Calbiochem, San Diego, Calif., and squalene from K & K Laboratories Inc., Plainview, N.Y.. If the separated lipids were to be quantitated fluorometrically, the standard solution was hydrogenated by the method of Farquhar *et al.*[5] to prevent quenching of spots[3].

Thin-layer chromatography

Merck chromatoplates (Darmstadt, G.F.R.) (20×20 cm) with a 0.25 mm layer of silica gel G were pre-washed in Solvent I (diethyl ether–glacial acetic acid–petroleum ether (b.p. 38.5–40.0°), 100:3:97) and activated at 110° for 30 min. Fifteen microlitres of the standard lipid mixture was spotted at the origin (2 cm above the plate bottom) with a 50 μl Hamilton syringe and repeating dispenser (Hamilton

Pb-600). Warm air blowers were used to dry the chromatoplates between sample drops in order to minimize the spot size.

After Solvent I had migrated to 9 cm above the plate bottom, the plate was removed from the chamber and allowed to air dry. The chromatoplate was next placed in Solvent II (diethyl ether–petroleum ether (b.p. 38.5–40.0°), 3:97) in a second chamber. Solvent II was allowed to migrate to 15 cm above the plate bottom. The chromatoplate was then air dried and sprayed with Rhodamine 6 G (ref. 6) to visualize the separated lipid classes.

RESULTS AND DISCUSSION

Fig. 1A shows the development of the lipid standards in Solvent I. In this solvent system the phospholipid (1), sterol (2), and free fatty acid (3) separate as

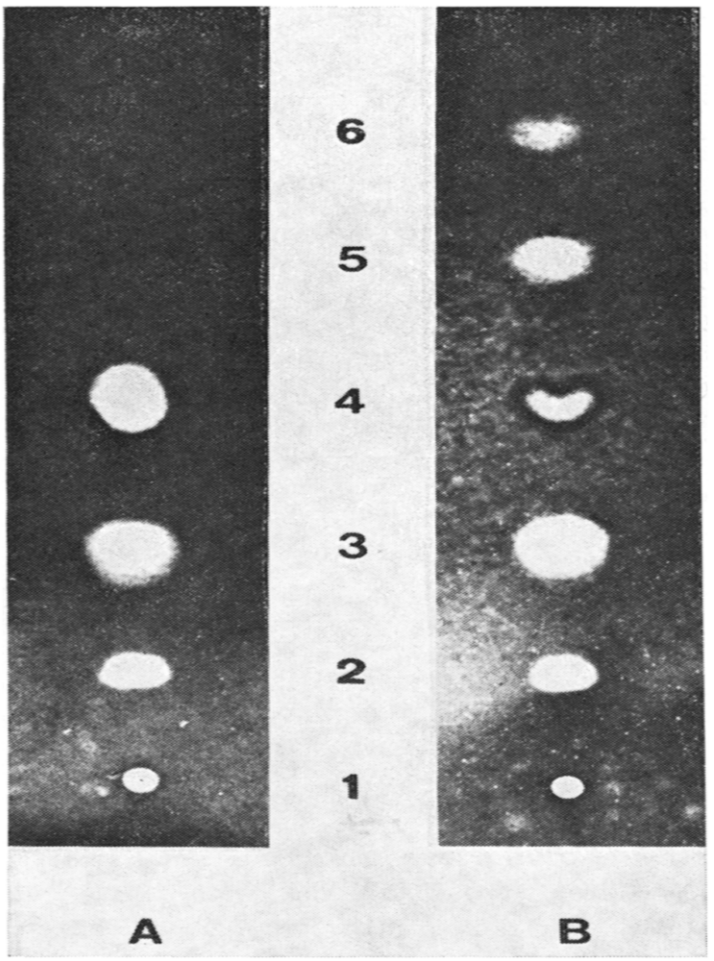

Fig. 1. (A) Thin-layer chromatogram of lipid standards developed in Solvent I (1 = phospholipid at origin; 2 = cholesterol; 3 = free fatty acid; 4 = triglyceride, cholesteryl ester, and squalene). (B) Thin-layer chromatogram of lipid standards after subsequent development in Solvent II. 1 = phospholipid at origin; 2 = cholesterol; 3 = free fatty acid; 4 = triglyceride; 5 = cholesteryl ester; 6 = squalene.

individual spots while the triglyceride, sterol ester and hydrocarbon migrate together forming spot 4. Subsequent development in Solvent II results in a further separation of the latter three lipids into individual spots as seen in Fig. 1B. The substantial distance between each lipid class separated by this method permits *in situ* fluorometric or densitometric analysis of each class or elution and recovery of squalene and cholesteryl esters when radioactive lipids are fractionated.

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