tendency of an increase of R_F with increasing polarity of the solvent can be observed. An attempt to correlate the R_F or R_M values of these compounds with the number of hydroxyl groups did not give conclusive results.

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A rapid preparative thin-layer chromatographic technique for serum lipids

The multiple development technique[1,2] described here achieves preparative separation of up to 22 lipid fractions from serum within a few hours.

The serum (up to 3 ml) is prepared for chromatography by incubation with β-glucuronidase*[3], after which it is deproteinized with 2:1 chloroform–methanol (1:20). It is then filtered and evaporated *in vacuo*. The residue is dissolved in the same solvent, again filtered to remove precipitated solids, and evaporated to approximately 0.1 ml for spotting.

Materials

Silica gel** in a double thickness man's cotton sock is extracted in a soxhlet —first with heptane, then chloroform, and finally 95 % ethanol, each extraction being carried out overnight. After the final extraction, the air-dried silica gel is dried further at 60° for 24 h, and is then sifted through a 160 mesh sieve. A stock solution of the dye 2,7-dichlorofluorescein[4] (0.04 % w/v in 0.01 *N* NaOH), used for visualizing the lipid fractions on the plate, may be stored in the dark under refrigeration for

* Warner-Chilcott Laboratories, Ketodase.
** Silica Gel G with gypsum binder, Warner-Chilcott Laboratories.

several months. The solvents are all redistilled and stored over anhydrous sodium sulfate to remove water.

Chromatography

The extracted silica gel is prepared by mixing 60 g of the dry powder in a blender (10 sec at high speed and 20 sec at low) with 100 ml of 0.05 *N* NaOH and 5 ml of the stock dye solution. The pH of the gel mixture should be slightly above 7.0 to yield fluorescence of the very dilute dye. Before the plate is poured most of the bubbles are removed with a glass stirring rod as the container is rotated slowly by hand. Remaining bubbles are removed by bumping the plate holder after the film is spread. Clean plates (20 cm square and 3 mm thick, matched for thickness to ensure that a uniform layer of gel may be obtained) are laid end-to-end and wiped with acetone. A 0.75 mm thick layer is spread* and allowed to air dry in the dark until it looks chalky (about an hour). A 5 mm strip of silica gel is removed from around the edges of the plates. Since the dye should not be heated at the usual activation temperature of 110°, activation is accomplished by heating at 60° for an hour. The plates may be

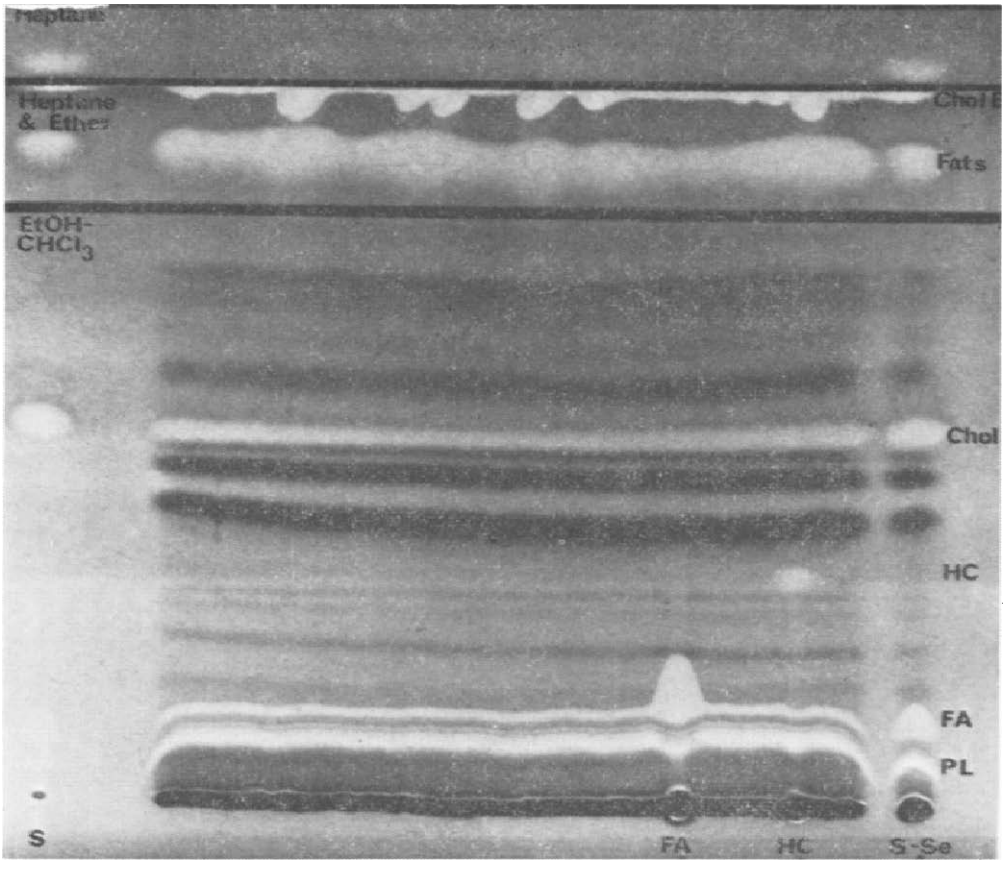

Fig. 1. Photograph under ultraviolet light of a thin-layer chromatogram showing the resolution of lipids achieved by means of Procedure 1. Pure standards (S) are spotted on the left side of the plate and serum + standards (S-Se) are spotted on the right to aid in identification of substances in the serum. For the purposes of this report, a fatty acid mixture was superimposed on the sample at the origin at the FA, and hydrocortisone at HC. The phospholipids, cholesterol, and cholesterol esters are signified by PL, Chol, and Chol E, respectively. The solvents are shown at their respective solvent fronts.

* Desaga-Brinkman apparatus.

stored only for about two days, since the dye rapidly becomes non-fluorescent even in a dark, dry storage cabinet. After storage they must be reactivated in a 60° oven for 10 min before use.

A discrete spot of a standard mixture of lipids is placed 1.5 cm from the left edge of and 1.5 cm above the bottom of the silica gel. A thin streak of the serum extract is placed in a straight line across the plate at the same level as the standard, starting 2 cm from the standard, and ending 1.5 cm from the right edge of the silica. As soon as the first application is dry (speeded by having the plate warm), others are made until the entire sample and washings of the sample container have been transferred quantitatively to the plate. The plate is dried in an oven at 35° for 30 min before development.

Development

A set of small tanks, one for each of the separate solvent systems used, containing anhydrous sodium sulfate and lined with filter paper is prepared with fresh solvent daily. After each development the plate is air dried for about 10 min or until there is no odor of the solvent; then, it is examined under ultraviolet light (2537 Å) to determine if additional development with the same system would improve resolution. When resolution is satisfactory it is placed in the succeeding system.

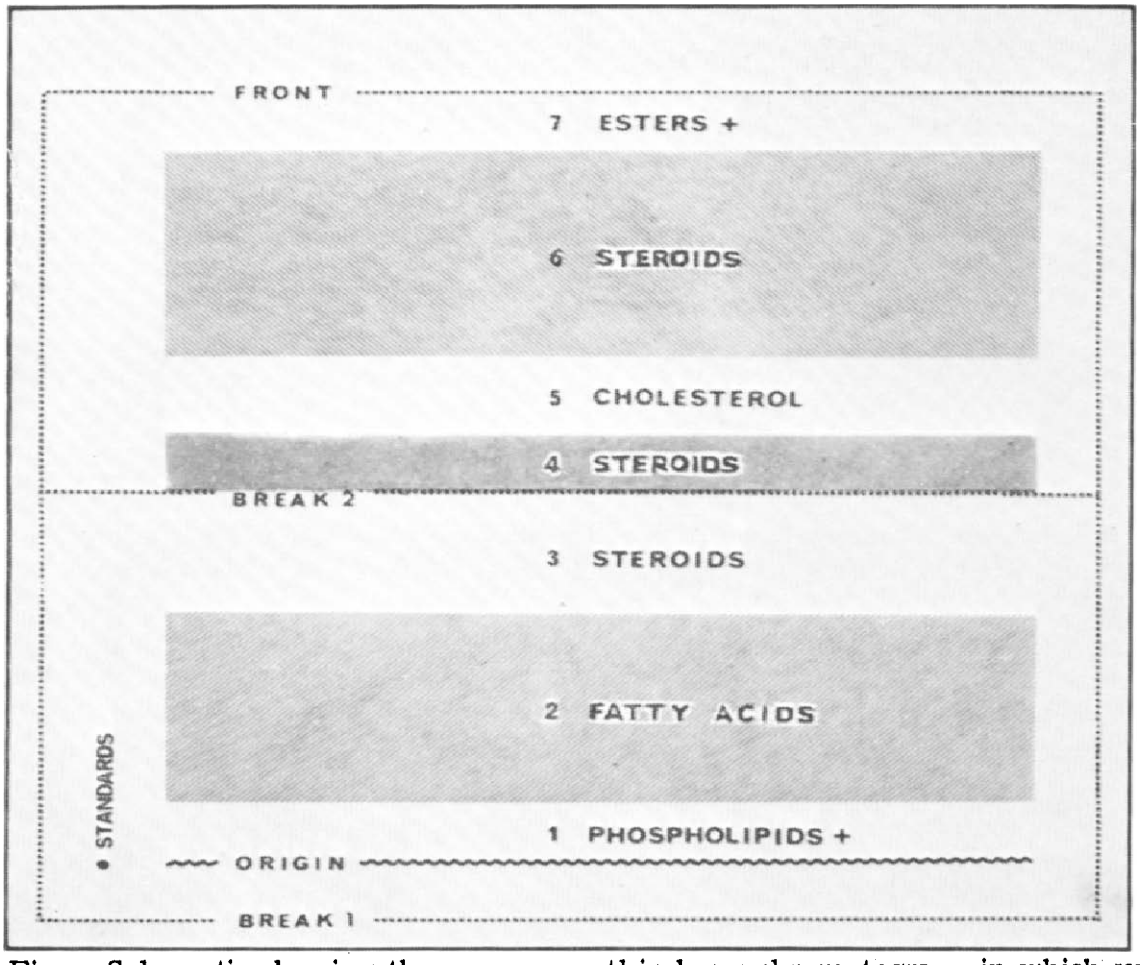

Fig. 2. Schematic showing the areas on a thin-layer chromatogram in which various classes of lipids are resolved by means of Procedure 2.

Two procedures for the development are presented; the first achieves separation of the hydrocarbons, fats, and cholesterol esters with a consequent loss in resolution of the other lipids, while the second simply moves these three substances to the top of the plate so that the entire plate now becomes available for achieving better resolution of the remaining lipids.

Procedure 1. The plates are developed twice in the first solvent, *n*-heptane, to move the hydrocarbons to the solvent front. A 1 mm horizontal strip of the silica is removed from just beneath the hydrocar bons.Then the plates are developed to this strip once with heptane–diethyl ether (70:30) to resolve and move the fats and cholesterol esters toward the top. Another horizontal strip of silica is removed from just beneath the fats, and the plate is then developed once in chloroform–absolute ethanol (80:20) for the final separation of cholesterol, steroids, fatty acids, and phospholipids (Fig. 1).

Procedure 2. The plates are developed twice with chloroform–benzene–absolute ethanol (48.5:48.5:3.0) to move the hydrocarbons, cholesterol esters, and fats to the top of the plate. At this point the cholesterol has moved to an R_F of 0.6 with various steroids distributed above and below it. A 1 mm strip of the silica is removed from just beneath the lowest steroid spot, and the plates are developed to this strip once with chloroform–absolute ethanol (65:35) to resolve the fatty acid group, other steroids, and the phospholipids (Fig. 2).

Preparation for final separation and quantitation

After verification under ultraviolet light of the positions of the lipids in the serum, the standards are removed. Each serum fraction is then outlined and subsequently removed with a razor blade. The fractions may be stored in this form in tightly stoppered vials in a dark deepfreezer. Elution is accomplished with 4:1 chloroform–methanol which dissolves very little of the dye.

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