Fractionation of naturally occurring lecithins according to degree of unsaturation by thin-layer chromatography

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SUMMARY Intact, naturally occurring lecithins were fractionated according to degree of unsaturation by thin-layer chromatography on silica gel plates impregnated with silver nitrate and heated at 175–180° for several hours.

KEY WORDS thin-layer chromatography · lecithins · silver nitrate–silica gel · fractionation · degree of unsaturation

THIN-LAYER CHROMATOGRAPHY on silica gel plates impregnated with silver nitrate has been used successfully for the separation of neutral lipids according to degree of unsaturation (1–3). With this method Renkonen (4) fractionated the acetylated and dephosphorylated residues resulting from acetolytic cleavage of phosphatides. Kaufmann, Wessels, and Bondopadhyaya (5) reported the resolution of soy and egg lecithins into several fractions on silica gel–silver nitrate plates, using chloroform–ether–acetic acid 97.0:2.3:0.5 as the moving phase. Quantitative data on the fatty acid composition of the different fractions were not given. This note reports the separation of intact, naturally occurring lecithins according to their degree of unsaturation by TLC on silica gel impregnated with silver nitrate.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

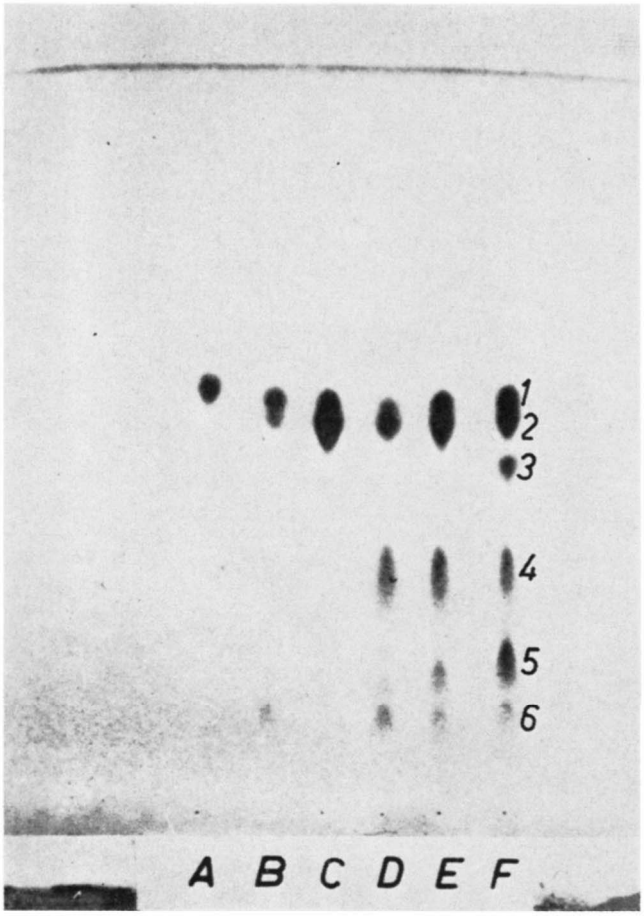

FIG. 1. Thin-layer chromatogram of lecithins on Silica Gel H (E. Merck), impregnated with silver nitrate and heated for 5 hr at 175°. Solvent system chloroform–methanol–water 65:25:4 (v/v/v). *A*, hydrogenated egg lecithin; *B*, egg lecithin; *C*, rabbit liver lecithin; *D*, rat liver lecithin; *E*, pig liver lecithin; *F*, bovine liver lecithin. Total sample load per spot was 0.5–2 μg P, depending on the number of subfractions in the sample. Detection by charring after spraying with 50% sulfuric acid.

Materials and Methods. Naturally occurring lecithins from different sources, with different fatty acid compositions, were used. Lecithins from egg yolk and from the liver of various species were obtained by preparative TLC of the chloroform–methanol extracts, using 1 mm thick layers and the solvent system of Skipski, Peterson, and Barclay (6). Each lecithin sample yielded only one spot on rechromatography, and the molar ratios of ester (7) to phosphorus (8) were between 1.90 and 2.10. Egg lecithin was hydrogenated over palladium on charcoal and the hydrogenated lecithin subsequently purified by TLC. The fatty acids of the product contained 42.4% 16:0, 52.0% 18:0, and 5.6% 18:1.

The lecithins were methanolyzed using 2.5% methanolic H_2SO_4 at 65° for 5 hr in stoppered tubes. The extracted methyl esters were purified on thin-layer plates prior to GLC analysis. [This step, recommended by Mangold (9), was found to be advisable with small quantities of methyl esters to avoid the appearance of spurious peaks in GLC which are possibly derived from the methanol used as solvent. Checks were made to insure that no methyl esters were preferentially lost.] The stationary phase for GLC was ethylene glycol succinate polyester, 10% on washed Celite, 100–120 mesh. An electronic integrator was used. Quantitative results with National Heart Institute Fatty Acid Standard F agreed with the stated composition data with a relative error of less than 2% for major components (>10% of total mixture) and less than 5% for minor components (<10% of total mixture).

The adsorbent layers were prepared by mixing 40 g of binder-free Silica Gel H (E. Merck, A. G., Darmstadt, Germany) with 110–115 ml of water containing 12 g of $AgNO_3$ and spreading the thin slurry on 20 × 20 cm glass plates by means of a stainless steel applicator constructed according to the principles of Stahl (10). The thickness of the layer was 0.35 mm. The plates were dried at room temperature (shielded from direct sunlight) for 24 hr, heated either at 175° for 5 hr or at 180°

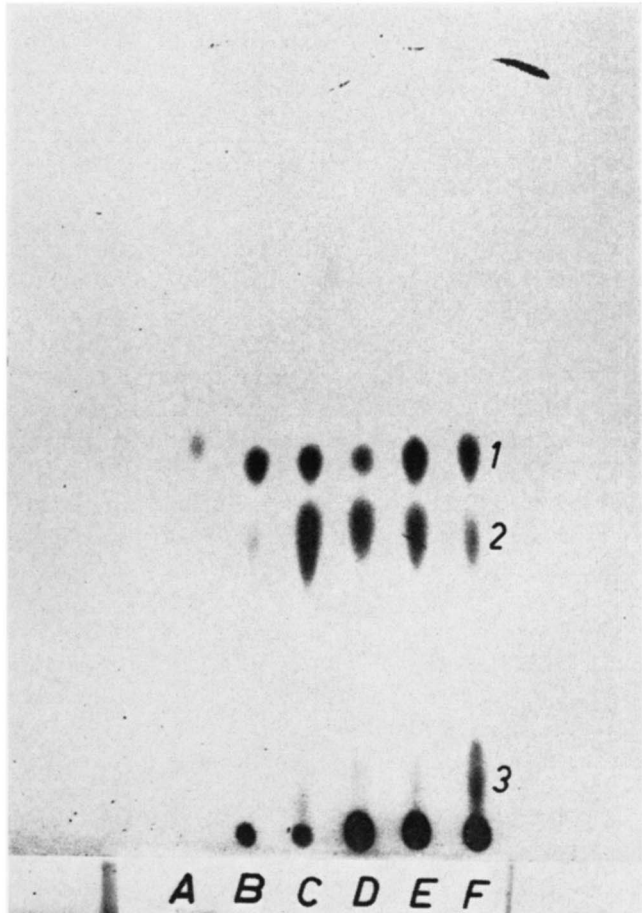

FIG. 2. Thin-layer chromatogram of lecithins on Silica Gel H (E. Merck), impregnated with silver nitrate and heated for 24 hr at 180°. Solvent system, samples, and detection method as in Fig. 1.

TABLE 1 FATTY ACID COMPOSITION (WEIGHT %) OF ISOLATED LECITHINS AND OF THE MAIN SUBFRACTIONS OBTAINED BY TLC

Fatty Acid	Egg Yolk			Rabbit liver			Rat liver				
	Total	1*	2	Total	1*	2	Total	1*	2	4	6
16:0	32.8	38.9	35.0	29.3	36.0	26.6	29.2	45.8	32.8	20.2	33.5
16:1	1.7	1.1	—	1.8	0.5	1.3	1.0	1.9	0.5	0.7	—
18:0	14.5	9.5	14.2	17.3	14.7	19.8	22.4	7.7	15.6	32.7	20.4
18:1	33.4	50.5	0.9	16.6	48.8	3.0	7.9	44.6	2.2	1.1	—
18:2	13.8	—	50.0	35.0	—	49.3	11.5	—	48.9	—	—
20:4	3.8	—	—	†	—	—	21.8	—	—	45.3	—
22:6	†	—	—	†	—	—	6.1	—	—	—	46.1

* Numbers 1, 2, 4, and 6 refer to spot number in Figs. 1 and 2.
† Not determined.

TABLE 2 FATTY ACID COMPOSITION (WEIGHT %) OF ISOLATED LECITHINS AND OF THE MAIN SUBFRACTIONS OBTAINED BY TLC

Fatty Acid	Pig Liver						Bovine Liver						
	Total	1*	2	4	5	6	Total	1*	2	3	4	5	6
16:0	24.8	30.9	23.7	16.7	20.2	25.5	16.1	23.4	12.7	5.3	11.7	12.6	15.6
16:1	1.3	1.0	0.8	1.2	1.7	2.0	2.4	1.8	1.9	1.1	1.3	1.6	2.0
18:0	28.8	22.1	25.7	36.5	30.3	24.2	35.3	27.0	32.8	39.9	42.3	38.1	38.2
18:1	14.7	46.0	6.4	1.6	2.1	3.3	16.6	47.8	6.5	7.8	2.8	3.1	3.5
18:2	13.3	—	43.5	—	2.4	—	9.2	—	46.1	6.4	—	—	—
18:3	tr.	—	—	—	—	—	1.3	—	—	—	3.1	—	—
20:3†	1.5	—	—	—	—	—	4.4	—	—	39.6	—	—	—
20:4	10.9	—	—	44.0	4.5	—	5.9	—	—	—	32.1	3.4	—
20:5†	1.2	—	—	—	9.1	—	1.2	—	—	—	—	—	6.0
22:5†	2.2	—	—	—	29.6	—	5.8	—	—	—	—	41.2	10.1
22:6	1.3	—	—	—	—	45.1	0.9	—	—	—	—	—	24.6
Unidentified	—	—	—	—	—	—	1.0	—	—	—	6.6	—	—

* Numbers 1–6 refer to spot number in Figs. 1 and 2.
† Tentative identification.

for 24 hr, and stored in a desiccator over phosphorus pentoxide. Samples were applied in chloroform solution with a micropipette, either as spots or, when larger quantities were required, as bands. Plates activated in both ways were developed at room temperature for 60 min in chloroform–methanol–water 65:25:4 (v/v/v). Spots were located under ultraviolet light after spraying with a 0.2% solution of dibromo-R-fluorescein (British Drug Houses, Ltd., Poole, Dorset, England) in ethanol. They were then scraped off either into tubes for methanolysis or into small glass columns for elution of the component by hot methanol for rechromatography.

Results. Figures 1 and 2 illustrate the separations obtained on the two types of plates with six different lecithin preparations. In Tables 1 and 2 the fatty acid compositions of the main fractions are given. It is apparent that separation occurs according to number of double bonds in the lecithin molecule, although the separation of fully saturated lecithins from the monoene fraction was poor. The data obtained are in close agreement with the assumption that the majority of the lecithin molecules contain one saturated and one unsaturated fatty acid, but do not permit any conclusion concerning the influence of the *position* of the unsaturated fatty acid on the separations. This could be decided only by fractionating mixtures of synthetic lecithins of known structure. When the fractions were eluted from the silver nitrate-impregnated plates and rechromatographed on Silica Gel H using the system of Skipski et al. (6), each fraction gave one spot which had the same R_F value as the original lecithin and stained positively with the Dragendorff reagent.

The proportions of chloroform, methanol, and water in the solvent system used for the silver nitrate–impregnated plates could be varied within certain limits. Raising the concentration of methanol increased the R_F values of the monoene and diene fractions, while those of the more highly unsaturated fractions were dependent mainly on water concentration. Streaking occurred when water was excluded. The best-defined spots were obtained with solvent systems close to the borderline between the monophasic and biphasic areas in the ternary phase diagram of chloroform, methanol, and water. The saturation of the atmosphere in the chromatographic chamber with solvent greatly influenced the separations. The most satisfactory results were obtained by ascending chromatography in unequilibrated chromatographic tanks.

A comparison of Figs. 1 and 2 shows the effect on R_F values of the duration and temperature of activation

of the adsorbent. The mechanical strength of the thin layers was increased considerably by heating at temperatures above 150°. The adsorbent usually turned black on heating for more than 24 hr at temperatures above 190°; a slight discoloration did not interfere with the separations obtained. The plates could be stored for several weeks in a desiccator over phosphorus pentoxide. They were not very sensitive to daylight.

The fine particles passing a 400 mesh sieve from Mallinckrodt Silicic Acid and binder-free Silica Gel SG 41 (Whatman) gave the same results as Silica Gel H when used with silver nitrate for the described fractionation of lecithins. Silica Gel G (E. Merck, A.G.), containing 13% calcium sulfate binder, could be used for the fractionation of the more unsaturated lecithins, but the resolution of mono- and dienes was poor. This was apparently due to the presence of gypsum, since Silica Gel H to which a corresponding amount of $CaSO_4 \cdot 2H_2O$ was added gave the same results as Silica Gel G.

Construction and manufacture of the stainless steel applicator was accomplished by the very skillful work of Mr. J. I. Johansson.

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