Laboratorium studieren wir systematisch die Papier- sowie Dünnschichtchromatographie sowohl verschiedener Handelstypen, als auch verschiedener gewählter Modellfarbstoffe und wollen in weiteren Mitteilungen über diese Resultate referieren.

Dünnschichtchromatographie

20 g Kieselgel G (Merck) wurde mit 40 ml Wasser angerührt und auf Platten (20 × 17 cm) in üblicher Weise verteilt. Die Platten wurden am nächsten Tag lufttrocken verwendet. Die zu chromatographieren Farbstoffe wurden in Form ca. 5 %iger wässriger Lösungen (5 μl) aufgetragen und das Entwickeln geschah aufsteigend in üblicher Weise unter Anwendung des Lösungsmittelsystems *n*-Propanol–Ammoniak (2:1), Pyridin–*n*-Amylalkohol–Ammoniak (1:1:1) u.ä.

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Gel filtration of lipid mixtures*

Gel filtration has proven to be a very useful technique for the fractionation of water-soluble materials but the technique has not been extended to the fractionation of lipids because the polysaccharide derivative generally used as a gel filtration medium does not swell in non-polar solvents. Beads of polystyrene crosslinked with small amounts of divinylbenzene swell extensively in benzene, suggesting the possibility of fractionating benzene-soluble mixtures on columns of these beads. VAUGHN[1] has reported the fractionation of polystyrene on similar columns.

Although simple and complex lipids do not differ greatly in molecular weight, phosphatides associate into micelles in non-polar solvents[2–6], while non-polar lipids, such as triglycerides, sterols and sterol esters apparently have little tendency to do so. Thus the basis exists for a gel filtration separation of lipid mixtures into relatively polar and non-polar fractions. The usual methods for carrying out this separation have been acetone precipitation of phosphatides or chromatography, usually on silicic acid columns[7]. Acetone precipitation often results in inclusion of saturated triglycerides and sterol esters in the "phosphatide" fraction and the galactosylglyceride lipids of plants are found in the acetone-soluble fraction[8]. Column chromatography is relatively time-consuming on a preparative scale and can result in losses due to irreversible adsorption. Dialysis of lipid mixtures through rubber membranes in a non-polar

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solvent has also been used to separate polar and non-polar lipids[9,10]. The technique described here offers advantages of simplicity, speed, mild conditions and good resolution over the other methods mentioned.

Experimental

Lipid samples. Corn "lecithin", a preparation containing a variety of glycolipids and phospholipids from corn germ, was obtained from Corn Products Company, Argo, Ill. beef lung fat, from the VioBin Corporation, Monticello, Ill. Cholesterol stearate was synthesized and contributed by Mr. ALAN MA. Egg lecithin, cholesterol and triolein were standard commercial products.

Preparation of columns. Polystyrene beads with 2% divinylbenzene cross-linkages, 50–100 mesh, were obtained from the Dow Chemical Company, Midland, Mich. One hundred and thirty-nine g of this material was slurried in benzene and packed in a chromatographic column 4.0 cm in diameter to a height of 52 cm. The column was washed with about 1 l of benzene before the first use. Lipid samples were applied to the column in 5–10 ml benzene, and elution was carried out with the same solvent. Approximately 5 ml fractions were collected with a time-operated fraction collector.

Analysis of eluates. Weight curves were obtained by transferring 1 ml portions of each fraction into tared aluminum weighing dishes, evaporating the solvent under reduced pressure in a vacuum desiccator and reweighing. Phosphorus and galactose were estimated on 1 ml portions by the methods of HARRIS AND POPAT[11] and RADIN *et al.*[12] respectively. Infrared spectra of some samples before and after passage over the column were obtained as smears or Nujol mulls using a Perkin-Elmer Model 137 Infracord Spectrophotometer.

Results and discussion

An elution curve for corn "lecithin" is shown in Fig. 1. The mixture is resolved into two well-separated fractions, with all the phosphorus and carbohydrate in the first

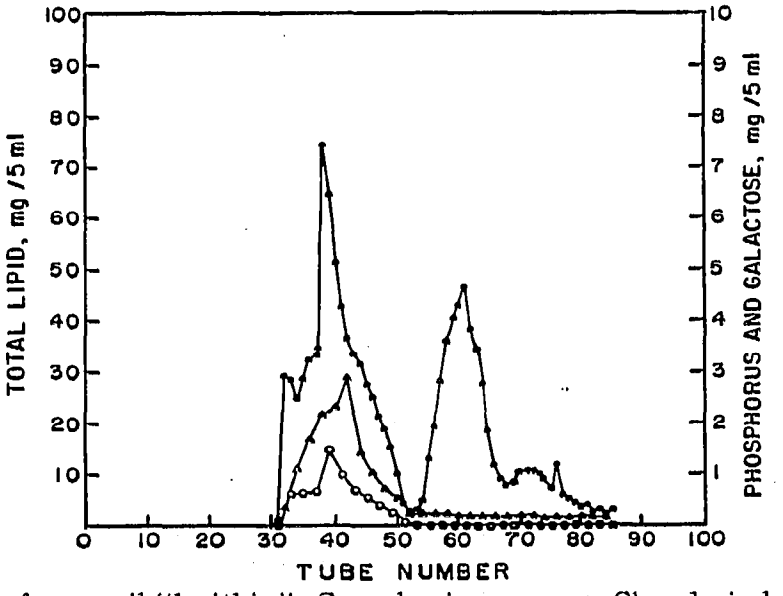

Fig. 1. Fractionation of corn oil "lecithin". Sample size: 1.05 g. Closed circles, total lipid. Open circles, phosphorus. Triangles, carbohydrate.

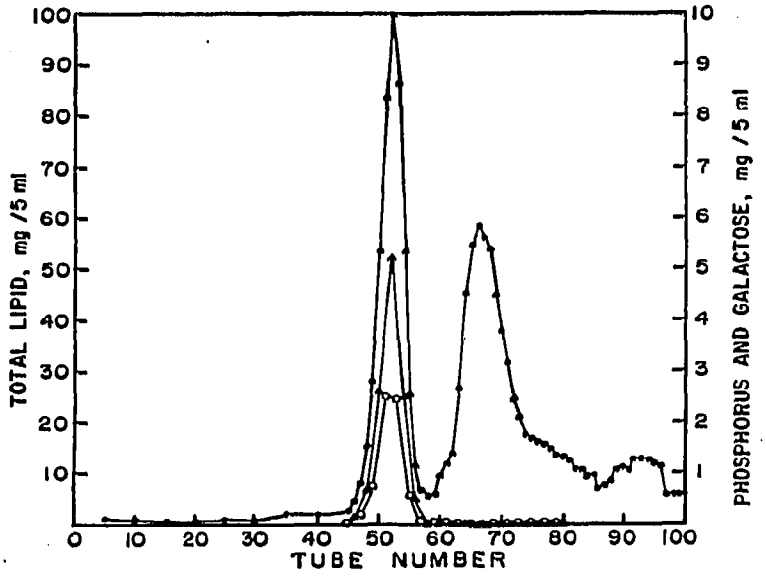

Fig. 2. Fractionation of beef lung fat. Sample size: 1.07 g. Symbols same as Fig. 1.

fraction, as expected from a gel filtration process. Fig. 2 shows an elution diagram for the fractionation of beef lung lipid on the same column. The complex lipids are again eluted first in a well-separated peak. The remaining lipids are partially resolved into three components. Qualitative Liebermann-Burchard tests on the contents of tubes 52, 68, 80 and 95 were positive only on the latter two samples, indicating partial resolution of sterols and sterol esters from triglycerides.

The fractionation of a model mixture containing egg lecithin, triolein, cholesterol stearate and cholesterol is illustrated in Fig. 3. I.R. spectra were obtained for materials from tubes 47, 61–63, 69 and 85 and compared with the spectra of the individual components of the model mixture. These fractions were identified in this way as egg

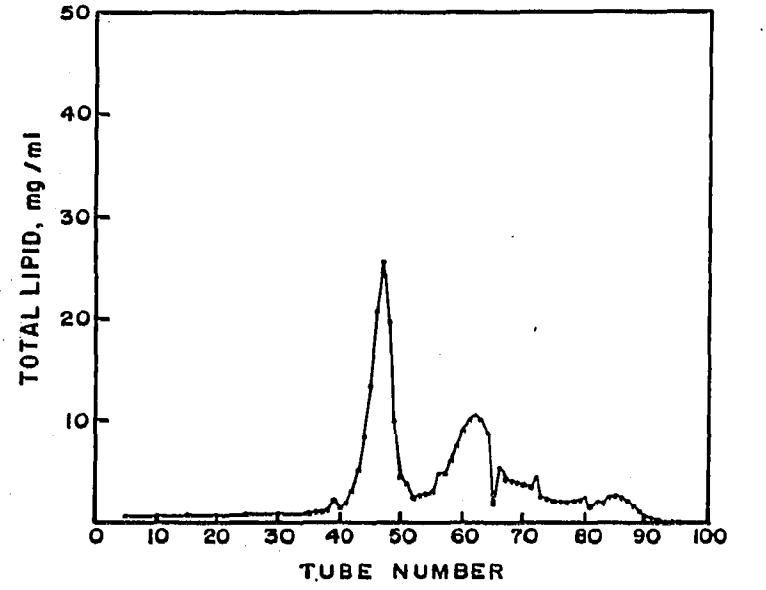

Fig. 3. Weight curve for fractionation of model mixture. Egg lecithin, 0.52 g; triolein, 0.45 g; cholesterol, 0.11 g; cholesterol stearate, 0.08 g.

lecithin, triolein, cholesterol stearate and cholesterol, respectively. Egg lecithin is seen to be well resolved from the remainder of the mixture, while triolein, cholesterol stearate and cholesterol are only partially separated from each other. The spectra of the samples before and after passage over the column were identical with two exceptions. The spectrum of egg lecithin before passage over the column had strong absorption at 1705 cm^{-1} due to free fatty acids. After passage over the column, this absorption was absent in the phosphatide fraction but was found instead in the spectrum of cholesterol stearate, indicating that free fatty acids and sterol esters are eluted together on this column.

The corn "lecithin", beef lung and egg lecithin samples all contained the brown pigment commonly found in lipid samples that have been exposed to the air. This brown material was eluted in each case with the complex lipids, the rest of the fractions being completely colorless. It was also observed that the flow rate of the column, which was set at 2–2.5 ml/min, decreased sharply while the complex lipids were emerging.

Recoveries of lipid samples were approximately 100%. The column is free-flowing with no appreciable tendency of the polystyrene beads to pack more tightly as the column is re-used repeatedly. A complete elution requires about four hours.

It is anticipated that this method will be useful for the preliminary separation of complex and simple lipids for preparative or analytical purposes. Further study of this procedure is now in progress.

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