Separation of lipids by Silica Gel G
column chromatography

QUINCY E. CRIDER,* PETAR ALAUPOVIC, JOE HILLSBERRY, CATHY YEN, and REAGAN H. BRADFORD
Cardiovascular Section, Oklahoma Medical Research Institute and Department of Biochemistry, Oklahoma University School of Medicine, Oklahoma City, Oklahoma

SUMMARY A column chromatographic procedure utilizing Silica Gel G is described for separating lipid components of serum and lipoproteins into individual fractions containing normal hexane (bp 40–60°), Phillips Petroleum Co.; methanol (redistilled before use), chloroform (redistilled before use), ethyl acetate (reagent grade), and glacial acetic acid (reagent grade), Fisher Scientific Co.; diethyl ether (USP), Mallinckrodt; and absolute ethanol, U.S. Industrial Chemical Co.

Column Preparation and Elution. Silica Gel G, suspended in hexane, was packed to a height of 5 cm in a 1.8 X 45 cm column. Applying slight air pressure during packing resulted in a uniform distribution of adsorbent, which was supported and covered by small glass wool plugs.

About 10–15 mg of lipid mixture per g of Silica Gel G was generally an optimal column load; when a markedly disproportionate quantity of one lipid class was present only 7–10 mg of lipid per g of adsorbent was applied.

The lipid components of whole serum or serum lipoproteins were extracted with chloroform–methanol 2:1 (v/v) (4) and added in 5–10 ml of petroleum ether to the adsorbent column. Separation into individual fractions containing hydrocarbons (I), cholesterol esters (II), triglycerides (III), cholesterol (IV), free fatty acids (V), and phospholipids (VI). Silica Gel G required no pretreatment except adjustment of moisture content to 10%. The method affords a rapid, complete separation of all major lipid classes except diglycerides. Recoveries of serum and tissue phospholipids were approximately 60–80%, whereas those of the other major lipid classes were essentially quantitative.

Research studies under way in our laboratory required a rapid procedure whereby the lipid classes in human serum and lipoprotein fractions could be separated completely and obtained in adequate amounts for subsequent quantitative chemical and radioactivity analyses. One or more characteristics of the available methods utilizing silicic acid (1, 2) or Florisil (3) tended to preclude their use for this purpose, particularly when numerous samples were to be separated. A column chromatographic procedure utilizing Silica Gel G as adsorbent was therefore developed.

Adsorbent. Silica Gel G¹ (E. Merck, Darmstadt, West Germany) has the following specifications, according to the manufacturer: CaSO₄·½H₂O, 13%; chloride (maximum), 0.02%; iron (maximum), 0.015%; pH, 6.7 ± 0.3%; density, approximately 30 g/100 ml; grain size, 5–20 μ; drying loss after 3 hr. at 150°, 3.5 to 6%; and surface loss at 900° for 1 hr, 7.8 (average) to 9%. The Silica Gel G in each container was analyzed using an Ohaus moisture balance and sufficient water was added to obtain a moisture content of 10%. The contents of each container were shaken thoroughly, stored in tightly stoppered bottles, and used without further treatment.

Solvents. The following were used: petroleum ether (bp 30–75°, redistilled before use), Baker Chemicals; methanol (reagent grade), Fisher Scientific Co.; ethyl acetate (reagent grade), Mallinckrodt Chemical; normal hexane (bp 40–60°), Phillips Petroleum Co.; chloroform (redistilled before use), Fisher Scientific Co.; diethyl ether (USP), Mallinckrodt; and absolute ethanol, U.S. Industrial Chemical Co.

Notes on Methodology

1. Work done in part as a Cardiovascular Research Trainee, Oklahoma Medical Research Institute supported by PHS Grant HTS-5403, and as Postdoctoral Research Fellow of the Oklahoma Heart Association.

2. Silica Gel G is distributed in the United States by Brinkmann Instruments, Inc., Great Neck, L.I., N.Y. and by Terra Chemicals, Inc., New York, N.Y.
Thin-layer chromatographic analysis of the eluates from a serum lipoprotein extract. A 25 µl aliquot of each eluate fraction was placed on the chromatoplate, developed with a mixture of 5% diethyl ether in petroleum ether, sprayed with 50% sulfuric acid, and charred on a hot plate to demonstrate any lipid material. Tubes 1–6 contained the 10-ml fractions eluted with petroleum ether; no hydrocarbons (I) were demonstrated. Tubes 7–17 contained the 5-ml fractions eluted with 6% diethyl ether in petroleum ether; cholesterol esters (II) were demonstrated in tubes 9–16. Tubes 18–34 contained the 10-ml fractions eluted with 10% ethyl acetate in petroleum ether; triglycerides (III) were demonstrated in tubes 21–23 and cholesterol (IV) in tubes 29–33. Tubes 35–40 contained the 10-ml fractions eluted with diethyl ether; free fatty acids (V) were present in tubes 36 and 37.

Separation of Serum and Tissue Lipids. The individual lipid classes were eluted from the column in distinct bands without trailing or overlapping (Fig. 1). Representative recoveries of non-phospholipids from serum and an artificial lipid mixture are presented in Table 1. Results obtained when a mixture containing cholesterol, monopalmitin, and 1,2-dipalmitin was eluted with 10% ethyl acetate in petroleum ether indicated that dipalmitin was eluted as a contaminant in the last several cholesterol fractions, whereas monopalmitin was obtained subsequently as a distinct fraction.

Column chromatographic separation with Silica Gel G has proved equally successful for separating tissue lipids, although good recoveries were obtained only for the “neutral” lipids. Representative recovery data for the lipids extracted from rat liver with an ethanol–petroleum ether mixture 10:8.5 (v/v) (12) are presented in Table 1.

**TABLE 1** RECOVERY OF LIPIDS AFTER SILICA GEL G COLUMN CHROMATOGRAPHY

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cholesterol Esters (II)</th>
<th>Triglycerides (III)</th>
<th>Cholesterol (IV)</th>
<th>Free Fatty Acids (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Charge</td>
<td>Recovery</td>
<td>Charge</td>
<td>Recovery</td>
</tr>
<tr>
<td>Whole Serum “A”</td>
<td>13.7</td>
<td>13.00</td>
<td>94.9</td>
<td></td>
</tr>
<tr>
<td>Whole Serum “B”</td>
<td>5.3</td>
<td>5.40</td>
<td>101.9</td>
<td></td>
</tr>
<tr>
<td>Artificial Mixture†</td>
<td>6.0</td>
<td>5.80</td>
<td>96.7</td>
<td></td>
</tr>
<tr>
<td>Palmitic Acid</td>
<td>0.0</td>
<td>0.00</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Rat Liver</td>
<td>0.62</td>
<td>0.61</td>
<td>98.4</td>
<td></td>
</tr>
</tbody>
</table>

— indicates no analyses were performed.
* Values are reported as equivalent milligrams of palmitic acid.
† Contained cholesterol stearate, tripalmitin and cholesterol.

480  **JOURNAL OF LIPID RESEARCH**  Volume 5, 1964  *Notes on Methodology*
No phospholipid contamination of the hydrocarbon fraction was observed when artificial lipid mixtures or samples of serum and liver were separated, in contrast to the reports of Lovern (13) and Riley and Nunn (14) that petroleum ether eluted phospholipids from the silica gel preparation they employed (British Drug Houses, Ltd.). An incomplete recovery of phospholipids extracted from serum and tissue was, however, repeatedly obtained. Even using the very polar mixture methanol-acetic acid-water 8:1:1, only 60–80% of the lipid phosphorus extracted from serum or tissue could be eluted. On the other hand, this mixture eluted cephalin and sphingomyelin, individually applied, almost quantitatively. Since in our studies the phospholipids are normally precipitated from the lipid extract by acetone, further investigation of this difficulty was not undertaken.

The Silica Gel G column chromatography procedure for separating serum and tissue lipids offers, in our hands, several advantages over established methods using silicic acid. The pretreatment procedure required with silicic acid is quite laborious and time consuming (1, 2). Silica Gel G, in contrast, requires no pretreatment except adjustment of moisture content to 10%. The degree of hydration is not as critical for Silica Gel G as for silicic acid; complete separation of the lipid classes was obtained when the moisture content was varied between 5 and 11%. Comparable results have been obtained using a number of different batches of Silica Gel G during a period of 18 months. Complete separation (as judged by thin-layer chromatography) of each major serum lipid class except diglycerides can be achieved, and the time required for column preparation and development is substantially less than for other adsorbents. The free fatty acid fraction obtained by this procedure is separated from other lipid fractions without the overlapping which has been observed with the silicic acid and Florisil methods and the eluting solvent does not interfere with subsequent titrimetric analyses (11), as obtained with the Florisil method (3).

The authors are grateful for the technical assistance of Mrs. Joyce Carson, Mr. John Freidenberger, Miss Alice Fryer, Mr. John Kelley, and Mrs. Linda Prather and for the valuable assistance of Mrs. Merlene Wuerflein in typing the manuscript.

A preliminary report of these studies was presented at the Eighth Tetrasectional Meeting of the American Chemical Society, Oklahoma State University, Stillwater, Oklahoma, March 3, 1962. The studies were supported in part by PHS Research Grants H-1889, H-2528, and H-6915 from the National Heart Institute, by the American Heart Association (61G14), and by the Oklahoma Heart Association.

Manuscript received December 30, 1963; accepted February 25, 1964.

References