

# BIOCHEMICAL PROBLEMS OF LIPIDS

EDITED BY

A. C. FRAZER

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VOLUME 1

# BIOCHEMICAL PROBLEMS OF LIPIDS

EDITED BY

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THIS VOLUME CONSISTS OF THE PROCEEDINGS  
OF THE SEVENTH INTERNATIONAL CONFERENCE  
ON BIOCHEMICAL PROBLEMS OF LIPIDS, MEETING ON FAT ABSORPTION,  
HELD IN BIRMINGHAM, JULY 24-27, 1962

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WITH 322 ILLUSTRATIONS AND 123 TABLES

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## PREFACE

The Seventh International Conference on Biochemical Problems of Lipids was held at the University of Birmingham, England, on July 24th-27th, 1962. The main theme of the meeting was the Absorption of Lipids from the Intestine. It was originally hoped that it would be possible for papers to be submitted and circulated in proof form before the meeting; however, several intending contributors were unable to submit their papers before the meeting, so this plan was abandoned. Papers were, consequently, submitted after the meeting. In spite of every effort on the part of the editors and publishers, a considerable delay in publication has occurred. This is regretted. It is felt, however, that rapid publication, which everyone agrees is desirable especially for conference proceedings, can only be achieved with certainty if corrected galley proofs are submitted to the editors at the time of the meeting.

Sixty papers were included in the original programme. Of these, 58 were presented at the meeting. The full text of 54 papers, two of which appeared in the programme of the meeting in two parts, and an abstract of 2 papers are contained in this volume. The main contributions to discussion on each paper are also included.

The Conference was organised in six Sessions as follows: I - Intraluminal aspects of fat absorption; II - Structural aspects of the intestinal cell and absorption studies; III - Enzymatic aspects of intestinal lipid metabolism; IV - Chylomicrons and lipoproteins; V - Mitochondrial and other aspects of lipid metabolism; VI - Wider aspects of lipid metabolism.

In Session I, attention was paid to certain aspects of thin-layer chromatography, a technique of particular value to workers in the field of lipid metabolism. Several papers were dealt with, including various aspects of bile salt metabolism, dispersion of sterols and other lipid-soluble molecules during absorption. The role of bile salts in fat absorption was re-examined in studies involving bile-shunting. A method for the measurement of sterols and bile salts in faeces was described. Two papers dealt with the role of the intestinal flora on faecal fat. The first three papers in Session II involved description and discussion of the ultrastructure of the small intestinal absorbing cell. The other papers in this section were concerned with various studies on fat absorption in animals. In Session III a series of papers dealt with the enzymes in the intestinal mucosa concerned with the hydrolysis or biosynthesis of lipids. The relationship of some of these enzymes to the ultrastructure of the small intestinal cell and the specificity and significance of phospholipid biosynthesis was also discussed. Papers included in Session IV dealt with the study of chylomicrons—their composition, distribution, metabolism and uptake into liver cells.

A point of particular interest was the demonstration of two species of fat particle in the blood stream that could be differentiated by simple flotation in a P.V.P. density gradient. Studies on other serum lipoproteins were also described. Session V was opened by an excellent review of the role of mitochondrial lipid in electron transfer and oxidative phosphorylation. Further papers dealt with mitochondrial activity in the liver cell and mitochondrial and other changes in fatty livers. The Final Session

consisted of thirteen papers, many of great interest. They included a report on studies on the effect of dietary changes on cell membrane composition, on fatty acid biosynthesis in microorganisms, on lipid metabolism in the brain and on the essential fatty acids.

I should like to express my most sincere thanks to all those who helped to make the meeting a success and to all the contributors to the scientific meeting and discussions, to the assistant editors and the publishers for their great assistance in the preparation of this volume.

*Birmingham, July 1963*

A. C. FRAZER

## CONTENTS

Preface . . . . .	v
-------------------	---

### *I. Intraluminal Aspects of Fat Absorption*

Thin-layer adsorption chromatography of lipids by A. F. HOFMANN (Lund, Sweden) . . . . .	1
Quantitative aspects of thin-layer chromatography applied to the lipids of biological materials by J. G. LINES (Birmingham, Great Britain) . . . . .	17
Bile salt metabolism in the rat by G. S. BOYD, M. A. EASTWOOD and BARBARA KERMACK (Edinburgh, Great Britain) . . . . .	24
The dispersion of sterols in the lumen of the intestine and mechanism of their absorption by J. C. DESAI and J. GLOVER (Liverpool, Great Britain) . . . . .	37
The dispersion of lipid-soluble materials in bile by K. BURDETT and W. F. R. POVER (Birmingham, Great Britain) . . . . .	47
Hydrolysis of micellar solutions of long-chain monoglycerides by pancreatic lipase by B. BORGSTRÖM and A. F. HOFMANN (Lund, Sweden) . . . . .	54
The effect of bile diversion on the absorption of [U- <sup>14</sup> C]oleic acid in the rat by D. R. SAUNDERS and A. M. DAWSON (London) . . . . .	55
Some effects of bile shunting in rats by O. C. FORBES and J. G. LINES (Birmingham, Great Britain) . . . . .	60
Direct measurement of sterols and bile acids in human feces by N. SPRITZ and E. H. AHRENS, JR. (New York, N.Y.) . . . . .	66
Intestinal bacteria and faecal fat by P. P. HOET, J. V. JOOSSENS, E. EVRARD, H. EYSSEN and P. DE SOMER (Louvain, Belgium) . . . . .	73
The influence of antibiotics on fecal fat in chicks by P. DE SOMER, H. EYSSEN and E. EVRARD (Louvain, Belgium) . . . . .	84

### *II. Structural Aspects of the Intestinal Cell and Absorption Studies*

The fine structure of the columnar epithelium of the mouse intestine, with special reference to fat absorption by F. S. SJÖSTRAND (Los Angeles, Calif.) . . . . .	91
Studies on the structural integrity of the brush border of rat intestinal epithelial cells by P. F. MILLINGTON and J. B. FINEAN (Birmingham, Great Britain) . . . . .	116
Localization of adenosine triphosphatase in cytomembranes involved in active transport by C. T. ASHWORTH, F. J. LUIBEL and S. C. STEWART (Dallas, Tex.) . . . . .	130
The transport of sterols across the mucosal cell by C. GREEN (Liverpool, Great Britain) . . . . .	144
Fat absorption in the frog's stomach isolated <i>in situ</i> by HALINA DOMINAS, J. DOROSZEWSKI and W. NIEMIERKO (Warsaw) . . . . .	150
Absorption and transport of lipids from the small intestine of infant rats by O. KOLDOVSKÝ, P. HAHN, V. MELICHAR, M. NOVÁK, P. PROCHÁZKA, J. ROKOS and Z. VACEK (Prague) . . . . .	162
Absorption des acides gras à chaînes moyennes et courtes par G. CLÉMENT, J. CLÉMENT, E. COUREL, J. KLEPPING et S. BRIET (Dijon, France) . . . . .	172
The <i>in vitro</i> absorption and incorporation of fatty acids in rats at different levels of the small intestine by A. KRONDL, I. SKÁLA, Č. MICHÁLEC, O. ANDRÝSEK and V. HROMÁDKOVÁ (Prague) . . . . .	180
Absorption of dicyclic compounds in lipid solution by P. JOHNSON and W. F. R. POVER (Birmingham, Great Britain) . . . . .	186

### *III. Enzymatic Aspects of Intestinal Lipid Metabolism*

Localisation subcellulaire de l'acyl CoA synthétase de la muqueuse intestinale par G. AILHAUD, D. SAMUEL et P. DESNUELLE (Marseille, France) . . . . .	197
Structural and enzymatic relationships in intestinal fat metabolism by G. HÜBSCHER, B. CLARK, MARGARET E. WEBB and H. S. A. SHERRATT (Birmingham, Great Britain) . . . . .	201
Intestinal utilization of monoglycerides by J. M. JOHNSTON and J. L. BROWN (Dallas, Tex.) . . . . .	211
Specificity of a lipase of the intestinal mucosa by H. C. TIDWELL, J. L. POPE, R. E. ASKINS and J. C. MCPHERSON (Dallas, Tex.) . . . . .	217
Specificity in fatty acid esterification during fat absorption. I. Triglycerides and cholesterol esters by A. KARMEN, D. S. GOODMAN and H. M. WHYTE (Bethesda, Md.) . . . . .	223
Specificity in fatty acid esterification during fat absorption. II. Phospholipids and positional relationships by H. M. WHYTE, D. S. GOODMAN and A. KARMEN (Bethesda, Md.) . . . . .	229
The phospholipid composition and turnover in rat intestinal mucosa during fat absorption by M. I. GURR, W. F. R. POVER, J. N. HAWTHORNE and A. C. FRAZER (Birmingham, Great Britain) . . . . .	236
The mode of action of human pancreatic phospholipase A by G. H. DE HAAS, C. H. T. HEEMSKERK, L. L. M. VAN DEENEN, R. W. R. BAKER, J. GALLAI-HATCHARD, W. L. MAGEE and R. H. S. THOMPSON (Utrecht, The Netherlands) . . . . .	244
The metabolism of cyclopropenyl fatty acids by R. REISER, C. K. PAREKH and W. W. MEINKE (College Station, Tex.) . . . . .	251

### *IV. Chylomicrons and Lipoproteins*

Centrifugation methods for the study of chylomicrons by D. B. ZILVERSMIT (Memphis, Tenn.) . . . . .	257
Thoracic duct lymph lipids of the sheep by A. K. LOUGH, L. FELIŃSKI and G. A. GARTON (Aberdeen, Great Britain) . . . . .	264
The fatty acid composition of thoracic lymph fat of rats fed single triglycerides by J. J. GOTTENBOS and H. J. THOMASSON (Vlaardingen, The Netherlands) . . . . .	272
Les origines du cholestérol du chyle par F. CHEVALLIER et M. VYAS (Saclay, S. et O., France) . . . . .	280
The removal rate and tissue distribution of different doses of chylomicrons in the rat after single injection by B. BORGSTRÖM, P. BELFRAGE and T. OLIVECRONA (Lund, Sweden) . . . . .	295
The behaviour of chylomicrons in the circulation. Observations with the electron microscope by J. E. FRENCH (Oxford, Great Britain) . . . . .	296
Further studies on fat- and carbohydrate-induced lipemia in man. Reduction of lipemia by feeding fat by E. H. AHRENS, JR. and N. SPRITZ (New York, N.Y.) . . . . .	304
Serum lipoprotein changes during fat absorption by A. FASOLI (Milan, Italy) . . . . .	313
Turnover studies of <sup>131</sup> I-labelled $\beta$ -lipoprotein in health and in thyroid disease by P. J. SCOTT, P. W. DYKES, J. DAVIS and K. WALTON (Birmingham, Great Britain) . . . . .	318

### *V. Mitochondrial and Other Aspects of Lipid Metabolism*

Role of lipids in mitochondrial electron transfer and oxidative phosphorylation by D. E. GREEN and S. FLEISCHER (Madison, Wis.) . . . . .	325
Protective action of phosphorylcholine on mitochondrial oxidative phosphorylation by N. SILIPRANDI (Padua, Italy) . . . . .	346
Synthesis of phospholipids during contraction of rat liver mitochondria by PAULINA WŁODAWER and L. WOJTCZAK (Warsaw) . . . . .	352
Reduced plasma lipoprotein production as a factor in the development of fatty livers by D. S. ROBINSON and A. SEAKINS (Oxford, Great Britain) . . . . .	359
Cephalin-cholesterol flocculation reaction observed by complement fixation phenomena by I. HARA, I. KIMURA, K. HOTTA and M. KUROKAWA (Tokyo) . . . . .	367

## VI. Wider Aspects of Lipid Metabolism

<i>In vitro</i> metabolism of L-leucine by rat epididymal adipose tissue by J. CHRISTOPHE and C. WODON (Brussels) . . . . .	373
Fat metabolism as related to carbohydrate metabolism in infant rats by P. HAHN, O. KOLDOVSKÝ, V. MELICHAR and M. NOVÁK (Prague) . . . . .	385
Blood cell lipid metabolism of lipaemic blood <i>in vitro</i> by C. J. MIRAS (Athens) . . . . .	391
Comportement des lipides globulaires au cours de l'hémolyse <i>in vitro</i> par L. DOUSTE-BLAZY, G. SOULA et P. VALDIGUIÉ (Toulouse, France) . . . . .	396
Dietary effects on the lipid composition of biomembranes by L. L. M. VAN DEENEN, J. DE GIER, U. M. T. HOUTSMULLER, A. MONTFOORT and E. MULDER (Utrecht, The Netherlands) . . . . .	404
Fatty acid metabolism in anaerobic yeast by F. MEYER, R. J. LIGHT and K. BLOCH (Cambridge, Mass.) . . . . .	415
Chemistry of firmly-bound cell-wall lipids in gram-negative bacteria by A. NOWOTNY, S. THOMAS and OLGA S. DURON (Duarte, Calif.) . . . . .	422
The fate of radioactive phospholipid precursors injected into the subarachnoid space of the rat by G. B. ANSELL and T. CHOJNACKI (Birmingham, Great Britain) . . . . .	425
Studies on brain phospholipids, with special reference to phosphatidic acid by S. YAMAZOE, K. HAYASHI and T. KANO (Maebashi, Japan) . . . . .	433
On the inability of the cholesterol esters of the aorta wall to be identical with those of the serum by N. T. WERTHESSEN (San Antonio, Tex.) . . . . .	439
Effects of dietary essential fatty acids upon polyunsaturated fatty acids in rat heart tissue by H. MOHRHAUER and R. T. HOLMAN (Austin, Minn.) . . . . .	446
Effect of methyl <i>cis</i> -2-octenoate on essential fatty acid deficiency in the rat by R. R. BRENNER, O. MERCURI and MARIA E. DE TOMÁS (La Plata, Argentina) . . . . .	453
Conversion <i>in vitro</i> of linoleic acid into $\gamma$ -linolenic acid by rat liver enzymes by D. H. NUGTEREN (Vlaardingen, The Netherlands) . . . . .	460
List of participants . . . . .	465
Author index . . . . .	469
Subject index . . . . .	471



## THIN-LAYER ADSORPTION CHROMATOGRAPHY OF LIPIDS

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At the last annual meeting of this society in Marseilles, 1960, the term "thin-layer chromatography" or its more euphonious French equivalent, "chromatographie sur les couches minces", was not mentioned. Now, 2 years later, it is probable that the great majority of my colleagues here do not spend a day in their laboratories without running several thin-layer chromatograms. It will be assumed that everyone here is familiar with the principles of the technique<sup>1-3</sup>, and in view of the sophistication of this group, I shall omit a historical review. However, it might be noted that the method was well described in the American literature in the early 1950's<sup>4</sup>, but that its explosive expansion awaited the vigorous and definitive work of Stahl<sup>1</sup>, now Professor at the University of the Saar. Stahl, after developing an excellent adsorbent and a convenient, well-designed dispenser, realized the method's immense potential, both scientific and commercial, and arranged for its promotion by large laboratory supply houses, notably E. Merck and C. Desaga.

Complete analysis of a lipid sample requires a separation into classes, followed by fractionation of each class into its component homologues. Curiously enough, the seemingly more difficult problem of quantitative homologue analysis was essentially resolved by the remarkable achievement of gas-liquid chromatography by James and Martin<sup>5</sup>. Although column chromatography has been of immense usefulness in class separation, thin-layer chromatography promises eventually to do the same with much more speed, precision, and ease. For the preparative organic chemist, thin-layer chromatography has opened up new vistas of understanding.

Mangold, who deserves credit for introducing thin-layer chromatography and lipids to each other<sup>6-8</sup> has made many useful contributions and has thoroughly and imaginatively reviewed its application to lipids<sup>3,9</sup>. Therefore I shall not review the technique extensively but rather report our own experiences. I shall first make some disconnected statements on methodology *per se*, and then discuss separations in three major groups of lipids—glycerides and fatty acids, bile acids, and phospholipids.

I shall deal only with absorption separations and not discuss reversed-phase partition separations. Silica gel chromatoplates impregnated with liquid paraffin or silicone appear useful for reversed-phase partition separations of triglyceride or diglyceride homologues, as well as fatty acids<sup>8,10</sup>. Mangold<sup>9</sup> has prepared polyethylene chromatoplates which serve as both supporting and stationary phase for partition separations using aqueous ethanol-acetone mixtures as developer. Such plates are comparable to the factice columns of Hirsch<sup>11</sup>, and it is probable we can expect more phases of this type to be discovered. The problem of quantitation is still being actively pursued by many laboratories.

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## DISCUSSION OF METHODOLOGY

*Preparation of the plates*

The original Desaga apparatus\* has no provision for preparing layers of varying thickness. A thin, U-shaped strip of brass may be placed around each end of the dispenser to prepare thicker adsorbent layers (J. Elovson, personal communication). We have three pairs of strips of different thicknesses, and these suffice for preparing thick layers for preparative work or when adsorbents of low capacity are used.

Within a few days after purchase of this apparatus, it was realized that the only way the full potential of the technique could be explored would be to run several hundred chromatograms daily. A large sheet of glass was cut to fit the templet, and we then attempted to prepare a layer of microscope slides upon this. After some ludicrous attempts using doubly-faced Scotch tape, we found a few drops of water to be an excellent adhesive. Subsequently, 66 × 66 mm square plates were purchased and we have used these ever since<sup>12</sup>. We did not realize at that time all the advantages of this modification, which conceptually at least, must be considered as trivial. For the first, the time required for a chromatographic run is 5–10 min. Only a few ml of solvent are required. The plates can be heated without breaking on a copper plate directly over a burner flame and substances detected with extreme sensitivity. Lastly and most important, if small spots are applied, the resolution obtained is nearly as good as that obtained on the large plates (Fig. 1). Preparation of 45 square plates requires about 15 min.

We usually purchase several hundred glass plates, sort them into groups of identical thickness using a micrometer, and mark them with a diamond pencil. After the slides have been coated with adsorbent and activated, we store them in square refrigerator jars over silica gel. Such an arrangement is convenient in that each worker in an institute can have his own supply of plates. They are conveniently run in histological staining jars.

*Adsorbents*

The majority of reported thin-layer separations of lipids have been with Silica

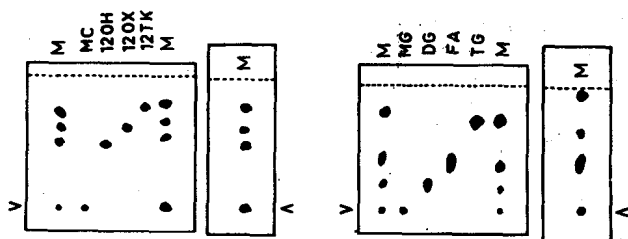


Fig. 1. Bile acid derivatives (left) and glycerides and fatty acid (right) on 66 × 66 mm plates and microscope slides. Adsorbent: Silica Gel G. Activated at 110° for 30 min. Bile acid derivatives<sup>24</sup>. Developer: ethyl acetate-cyclohexane (40:60, by volume). Solvent path: 5 cm. Development time: 8 min. Samples of about 10 µg: mixture (M); methyl cholate (MC); 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (12OH); 3α,7α-diacetoxy-12-keto-5β-methylcholanate (12OX); 3α,7α-diacetoxy-12-thioacetal-5β-methylcholanate (12TK); and mixture (M). Glycerides and fatty acid. Developer: di-isopropyl ether-heptane-acetic acid (40:60:2, by volume). Solvent path: 5 cm. Development time: 8 min. Samples of about 10 µg: mixture (M); monoolein (MG); diolein (DG); oleic acid (FA); triolein (TG); and mixture (M). From ref. 12, published by permission of Academic Press, Inc.

\* C. Desaga GmbH, Heidelberg, Germany.

Gel G\* as adsorbent. This was the first adsorbent for thin-layer chromatography that was commercially available and it is certainly an excellent adsorbent, forming strong, unreactive layers, which give excellent resolution when used with acidic, basic, or neutral solvents in aqueous or non-aqueous systems. However, one finds, in general, if two compounds cannot be separated when a number of reliable solvent systems of appropriate polarity have been used, they are probably impossible to separate on this adsorbent.

We were quite slow to realize the importance of varying the adsorbent as well as the developing solvent. This point is worthy of emphasis, namely, that thin-layer chromatography is useful for characterizing adsorbents *per se*. This was done previously by laborious column chromatographic investigations by such pioneers as Trappe<sup>13</sup>. Now it should be possible to examine rapidly all inorganic salts as adsorbents and experiment will probably precede theory. It is possible that the developing field of inorganic polymers may provide some interesting adsorbents.

Florisil\*\*, a synthetic mixture of silicon dioxide and magnesium oxide, has been used for the column chromatographic separation of lipids<sup>14</sup>. The manufacturer supplied us with some Florisil of very fine particle size and this forms excellent layers when calcium sulfate is used as a binder. When activated at 110° for 30 min, it is a weaker adsorbent than Silica Gel G, does not give quite so good resolution, and seems limited to the separation of neutral compounds, as acidic solvent mixtures undergo demixion. However, relative  $R_F$ 's of compounds are not the same on Silica Gel G and Florisil, and the adsorbent may be a useful alternative to Silica Gel G.

At the suggestion of Borgström, some weeks were spent preparing thin layers of hydroxyl apatite<sup>15, 16</sup>, a partially hydrolyzed calcium phosphate, for the thin-layer chromatographic separation of proteins. Eventually we were successful and obtained some interesting separations<sup>17, 18</sup>. However, we then discovered that this adsorbent when prepared with calcium sulfate as a binder was most useful for polar lipids. It was weaker than Silica Gel G, had a lower capacity, but separated such compounds as 1- and 2-monoglycerides<sup>19</sup>, isomers which could not be separated on Silica Gel G<sup>20</sup>.

Aluminum oxide with calcium sulfate as binder (Aluminum Oxide G\*\*\*) is available commercially and some separations will be shown. We have had no success with polyamide adsorbents.

In addition to these simple adsorbents, it is possible to prepare the suspensions of adsorbent in a salt solution instead of water. This approach, probably originating from Stahl's original suggestion of preparing Silica Gel G in aqueous oxalic acid rather than water, has been extended by Mangold and Kammereck<sup>9</sup> and appears to be very useful for certain problems, such as phospholipid separations. Barrett and Padley<sup>21</sup> have prepared silver nitrate impregnated plates which separate fatty acids according to number of double bonds and *cis-trans* isomerization.

### Binder

The concentration of the calcium sulfate used as binder is probably quite im-

\* Silica Gel G (for thin-layer chromatography according to Stahl), E. Merck, AG, Darmstadt, Germany.

\*\* Florisil, Floridin Co., Tallahassee, Florida, U.S.A.

\*\*\* Aluminum Oxide G (for thin-layer chromatography according to Stahl), E. Merck, AG, Darmstadt, Germany.

portant for the chromatographic properties of Silica Gel G. With hydroxyl apatite, it was found that for lipids as well as amino acid separations, the resolution improved with increasing calcium sulfate concentration, up to about 8%, then worsened<sup>19</sup>. We have used sugar and an alcohol-soluble nylon, Zytel 61<sup>®</sup>\*, as binding agents<sup>17</sup>. Other workers have used collodion, methyl cellulose, starch, etc.<sup>8</sup>. An ideal binding agent such as calcium sulfate is unreactive and contributes to, or at least does not detract from, the chromatographic properties of the adsorbent. With some adsorbents as, for example, hydroxyl apatite, quite satisfactory layers can be prepared with no binding agent at all<sup>18</sup>.

#### *Preparing the suspension*

For the preparation of good layers, it is essential to sieve the adsorbent through 150 mesh or finer if it contains large particles. Better layers of some adsorbents are often obtained by preparing the suspension of adsorbent in a solvent other than water. Cellulose, for example, flocculates in water, but not in alcohol. The hydroxyl apatite used for the thin-layer chromatography of proteins was suspended in 70% alcohol, as this was an excellent solvent for the polyamide binder used. When we work with new adsorbents, we routinely use a high-speed homogenizer to prepare a uniform slurry.

#### *Developers*

Solvents and solvent mixtures which have been useful in our laboratory for the chromatography of acidic and neutral lipids are listed in Table I in order of increasing polarity. Obviously, other satisfactory solvent combinations exist. For acidic substances, 1% (by volume) acetic acid is added to prevent tailing<sup>7</sup>. This does not influence the mobility of neutral substances significantly.

#### *Detection*

Phosphomolybdic acid (10%, w/v, in ethanol) is an extremely useful detecting agent. When the small plates are heated on a copper plate over an open flame, this agent will detect almost any lipid with a sensitivity of about 0.4  $\mu$ g. The reactivity

TABLE I  
USEFUL SOLVENT SYSTEMS FOR THIN-LAYER CHROMATOGRAPHY OF NEUTRAL  
OR ACIDIC LIPIDS ON SILICA GEL G

1. heptane<sup>a</sup>
2. benzene-heptane<sup>a</sup> (1:1, by volume)
3. ether-heptane<sup>a</sup> (1:9, 2:8, 3:7, by volume)
4. ethyl acetate-heptane<sup>a</sup> (1:9, 2:8, 3:7, 4:6, by volume)
5. ethyl acetate-benzene<sup>a</sup> (2:8, 3:7, 4:6, 5:5, by volume)
6. iso-amylacetate-di-isopropyl ether-carbontetrachloride-benzene-*n*-propanol-acetic acid (40:30:20:10:10:5, by volume)
7. iso-amylacetate-propionic acid-*n*-propanol-water (40:30:20:10, by volume)
8. chloroform-methanol-water (65:25:4, by volume)<sup>20</sup>

<sup>a</sup> 1% acetic acid, by volume, is added to these systems when acidic lipids are to be chromatographed. The solvent combinations are listed in order of increasing polarity. It is obvious that other satisfactory solvent combinations exist.

\* Zytel 61, soluble nylon resin, E.I. du Pont de Nemours & Co., Inc., Wilmington, Delaware, U.S.A.

of phosphomolybdic acid is highly influenced by temperature<sup>19</sup>. Many substances such as keto or acetylated bile acids or saturated monoglycerides react only above 200°. The large plates cannot be heated over an open flame without breaking, but this problem will be solved as soon as large plates of heat-resistant glass become available. At the moment, detection on the large plates is most satisfactorily obtained by heating the plates to 160–180° in an oven, then spraying the plates while still hot. Intense blue spots appear against a vivid yellow background—no reheating of the plates is necessary.

We have also used a mixture of concentrated sulfuric acid, concentrated nitric acid and water (4:3:3, v/v) which works equally well. It might be noted that phosphomolybdic acid does not detect lipids chromatographed on Florisil plates unless they are also sprayed with dilute sulfuric acid.

The illustrations shown are accurate tracings or photographs of chromatograms. In the tracings, the origin is indicated by a horizontal carat or arrow and the front by a dotted line. Dotted or faint spots represent impurities staining moderately heavily; very faintly staining impurities have been omitted from the tracings. All chromatograms are ascending, in an unsaturated atmosphere, and unless noted, the solvent path was 10 cm. Detection was with 10% (w/v) phosphomolybdic acid in ethanol as described, unless noted. The adsorbent and binder composition and activation conditions are described in the legends.

#### DISCUSSION OF SPECIFIC SEPARATIONS

##### *Glycerides and fatty acids*

The separation of mono-, di-, and triglyceride and fatty acid by ether-petroleum ether mixtures (containing 1% acetic acid) was first published by Mangold and Malins<sup>7</sup> and this important separation is shown in Fig. 2. Fig. 3 shows some separations of the lipids of human intestinal content obtained during fat absorption, using the same developer. Although a similar separation of mono-, di-, and triglyceride can

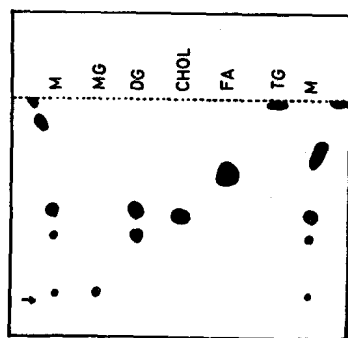


Fig. 2. Glycerides, cholesterol, and fatty acid. Adsorbent: Silica Gel G. Activated at 160° for 30 min. Developer: ether-petroleum ether-acetic acid (20:80:1, by volume). Samples of about 10 µg: mixture (M); monoolein (MG); diolein (DG)—the 1,3 spot, which is larger, runs ahead of the 1,2-diglyceride spot; cholesterol (CHOL); oleic acid (FA); triolein (TG); and mixture (M). The 1,3-diglyceride spot and the cholesterol spot are superimposed in the mixture. The mixtures were run too close to the edge of the plate. The lateral migration of the substances with high  $R_F$  can be prevented by running in a saturated atmosphere<sup>8</sup>.

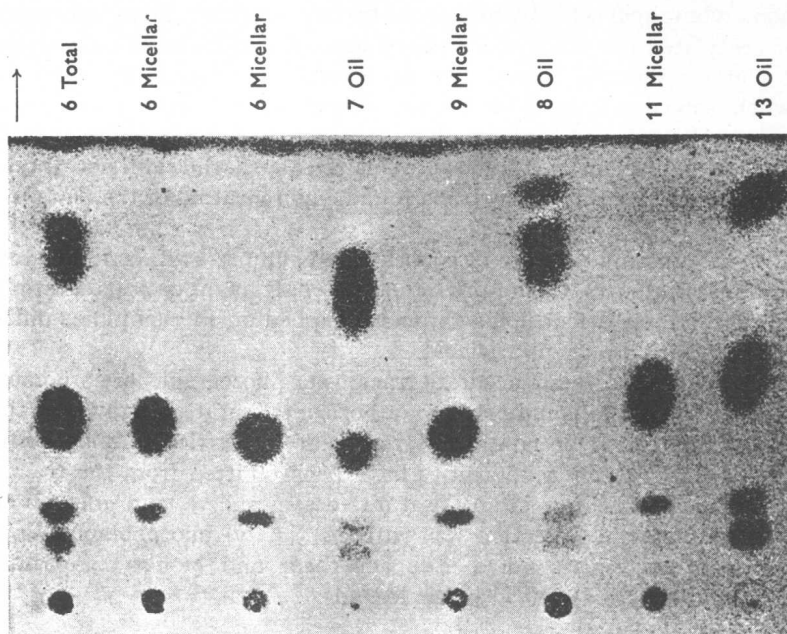


Fig. 3. Lipids from human intestinal content obtained by intubation during fat absorption; for a detailed explanation, see ref. 22. Chromatographic conditions as in Fig. 2, and the general pattern of the spots is as shown there. The spot possessing the mobility of 1,3-diglyceride and cholesterol is chiefly cholesterol, as it is possible to show by other means that little 1,3-diglyceride is present. One of the spots running ahead of the triglyceride is carotene; the other is unidentified. The material remaining at the origin is chiefly monoglyceride and phospholipid. From ref. 22 with permission of the publisher.

be obtained with a great number of solvent systems, with many of these, the  $R_F$  of fatty acid is appreciably less, so that the fatty acid and diglyceride spots overlap. Actually, the separation of glycerides and fatty acids by ether-petroleum ether mixtures (or mixtures of isopropyl ether-heptane) is so satisfactory that there is little point in using any other system. Borgström in our laboratory immediately noted that all diglyceride standards gave rise to two spots, and as is well known now, the 1,2- and 1,3-diglycerides separate cleanly, not only with ether-petroleum ether, but with a large number of other solvent combinations also (Figs. 2, 4 and 5)<sup>19, 20, 22</sup>. We have run two-dimensional chromatography of diglycerides and have not observed any isomerization during chromatography using ether-petroleum ether mixtures at room temperature.

With ether-petroleum ether as developer, cholesterol, which is often present in biological samples, has an  $R_F$  very close to that of the 1,3-diglyceride. In Fig. 3, these compounds are completely superimposed. In Fig. 4, separation of glycerides, fatty acid, and cholesterol by 1,2-dichloroethane<sup>23</sup> is shown. The cholesterol mobility is now significantly less than that of the 1,3-diglyceride, and the cholesterol spot lies between the 1,2- and 1,3-diglyceride spots. Fig. 5 shows a chromatogram of these compounds run with an iso-amylacetate-heptane mixture. With this developer, the cholesterol runs still more slowly, now being superimposed on the 1,2-diglyceride spot. It is

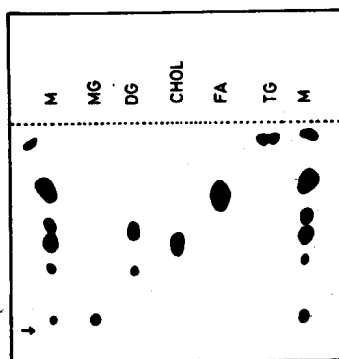


Fig. 4. Glycerides, cholesterol, and fatty acid. Adsorbent: Silica Gel G. Activated at  $160^{\circ}$  for 30 min. Developer: 1,2-dichloroethane-acetic acid (100:1, by volume). About  $10\text{ }\mu\text{g}$  of each sample applied. Abbreviations as in Fig. 2. With this developer, the cholesterol runs as a distinct spot between the isomeric diglycerides.

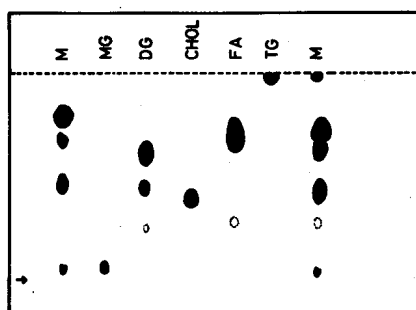


Fig. 5. Glycerides, cholesterol, and fatty acid. Adsorbent: Silica Gel G. Activated at  $160^{\circ}$  for 30 min. Developer: iso-amylacetate-heptane-acetic acid (30:70:1, by volume). About  $10\text{ }\mu\text{g}$  of each samples applied. Abbreviations as in Fig. 2. With this developer, there is excellent resolution of the 1,2- and 1,3-diglycerides. The cholesterol moves more slowly than with any of the developers described in Figs. 2-4, and the 1,2-diglyceride and cholesterol spots are now superimposed.

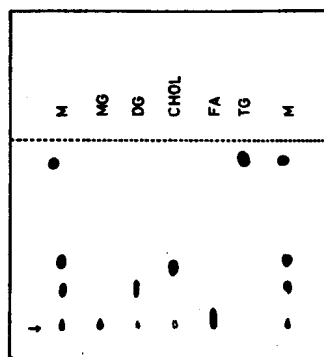


Fig. 6. Glycerides, cholesterol, and fatty acid. Adsorbent: Florisil (fine mesh). Binder: calcium sulfate, 8%, w/w. Activated at  $160^{\circ}$  for 1 h. Developer: ether-petroleum ether (20:80, by volume). About  $10\text{ }\mu\text{g}$  of each sample applied. Abbreviations as in Fig. 2. The 1,2- and 1,3-diglycerides are not separated, but cholesterol moves faster than either. Fatty acid remains near the origin—see text. The spots were detected by spraying the plate, which had been previously heated to  $160^{\circ}$ , with 10% ethanolic phosphomolybdic acid, then 50% (v/v) sulfuric acid.

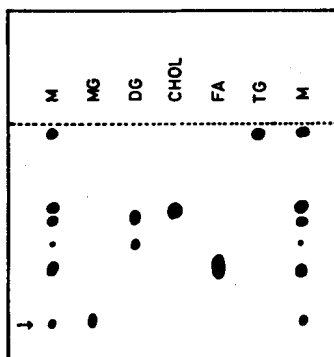


Fig. 7. Glycerides, cholesterol, and fatty acid. Adsorbent: hydroxyl apatite, prepared according to Anacker and Stoy<sup>18</sup>. Binder: calcium sulfate, 8%, w/w. Activated at 160° for 30 min. Developer: acetone-heptane (12:88, by volume). Samples of about 5  $\mu$ g. Abbreviations as in Fig. 2. There is a satisfactory resolution of 1,2- and 1,3-diglyceride and cholesterol. Note that here fatty acid moves more slowly than diglyceride; on Silica Gel G with acidic solvents, it moves with or faster than diglycerides.

evident that with all three of these solvent combinations there is obtained a good resolution of 1,2- and 1,3-diglyceride.

These same compounds may also be separated on Florisil, omitting acetic acid from the solvent mixture, and such a chromatographic separation is shown in Fig. 6. With this adsorbent, there is little separation of the isomeric diglycerides, for, as noted, this adsorbent does not generally offer the resolution of Silica Gel G. Cholesterol now moves well in front of the 1,3-diglyceride. The fatty acids remain near the origin, probably indicating salt formation and in agreement with the observations of Carroll<sup>14</sup> who used this adsorbent for the column chromatography of lipids.

These compounds may also be resolved nicely on hydroxyl apatite using acetone-heptane mixtures (Fig. 7). The fatty acid moves slowly as on Florisil. Thick plates must be used for this separation (or alternatively, very small samples applied) because of the low capacity of the hydroxyl apatite. Hydroxyl apatite has the potentially useful property of dissolving in dilute acid or when shaken with a cationic exchange resin in the acid form. This should mean that quantitative recovery of chromatography lipids will be quite simple.

As pointed out in the original paper of Mangold and Malins<sup>7</sup> and pictured in detail by Kaufmann<sup>23</sup>, straight-chain fatty acid homologues of chain length greater than  $C_{12}$  move with identical  $R_F$  using ether-petroleum ether on Silica Gel G. With homologues of chain length less than  $C_{12}$ , the  $R_F$  decreases as the chain length is shortened. Krabich in our laboratory recently prepared  $\alpha,\alpha$ -dimethyl-decanoic acid as well as di- and triglycerides of this branched-chain compound. The  $R_F$ 's of all these compounds were significantly higher than those of their respective straight-chain alkyl homologues when developed with ether-petroleum ether systems.

Because of our interest in fat absorption and the action of pancreatic lipase, we have realized for some time that a separation of 1- and 2-monoglyceride would be useful. Privett and Blank<sup>20</sup> had reported that these isomers could not be separated on Silica Gel G plates, and we had also tried several hundred solvent systems, both aqueous and non-aqueous without success. By accident, we ran some lipids including



1- and 2-monoolein on some hydroxyl apatite plates and observed a clear separation of the 1- and 2-isomers. Subsequent work showed that they could be separated with almost any solvent system of appropriate polarity, but that considerable isomerization took place. When chromatography at low temperature was performed, isomerization was virtually absent, but the separation worsened. Eventually, methyl iso-butyl ketone was found to give good resolution without isomerization<sup>19</sup>. A typical separation is shown in Fig. 8.

### Bile acids

Much of our work in Lund has been concerned with the preparation of pure free and conjugated bile acids<sup>24</sup>. Thin-layer chromatography is far superior to paper chromatography for the assay of compound purity because of its sensitivity and capacity. Our major effort therefore in the early months after we started with this technique

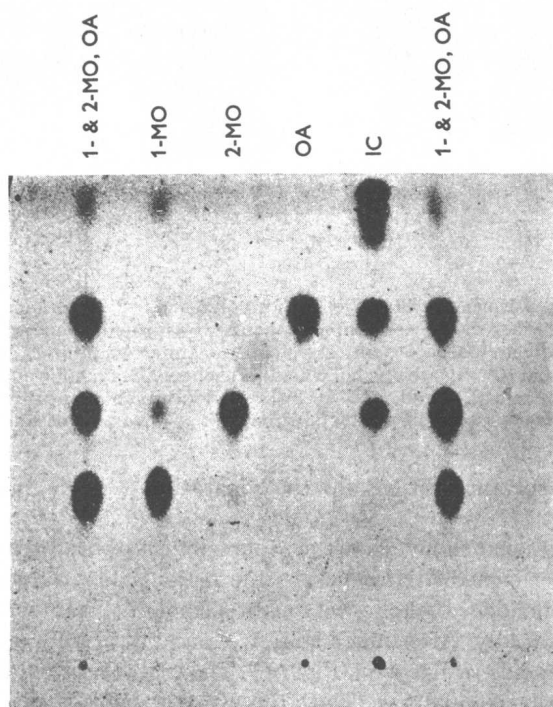


Fig. 8. 1- and 2-Monoglyceride, fatty acid, intestinal content. Adsorbent: hydroxyl apatite prepared according to Anacker and Stoy<sup>16</sup>. Binder: calcium sulfate, 8%, w/w. Activated at 160° for 1 h. Developer: methyl iso-butyl ketone at +10°. Samples of about 10 µg: mixture of 1- and 2-monoolein, oleic acid (1- & 2-MO, OA); 1-monoolein (1-MO); 2-monoolein (2-MO); oleic acid (OA); lipids from fresh human intestinal content obtained by intubation during absorption of a meal rich in lipid—2 ml of intestinal content was collected in 6 ml of heptane-ether-ethanol (1:1:1, v/v), and a 5-µl aliquot of the upper phase applied to the plate (IC); mixture as described (1- & 2-MO, OA). The plate was pre-developed with acetone to remove substances present in the adsorbent which move with the front but react strongly with phosphomolybdic acid. There is a faint spot of 2-monoolein in the 1-monoolein standard and *vice versa*. With this adsorbent and developer, cholesterol, fatty acids, di- and triglycerides move near the front; phospholipids remain at the origin. The monoglyceride present in the sample of fresh intestinal content appears to consist entirely of the 2-isomer. From ref. 19 with permission of the publisher.

References p. 15, 16