Quantitative Analysis of Lipids by Thin-Layer Chromatography[1]

M. L. BLANK, J. A. SCHMIT and O. S. PRIVETT, The Hormel Institute, University of Minnesota, Austin, Minnesota

Abstract

A procedure is described for the quantitative analysis of neutral and phospholipids by thin-layer chromatography (TLC) employing densitometry. The chromatoplates are prepared with the usual solvent systems. The spots are charred under standard conditions and analyzed with a Photovolt Corp. densitometer equipped with a special stage designed for holding 20 x 20 cm chromatoplates. Each spot on the chromatoplate gives a peak of density values which is used for quantitative analysis.

Radioactive lipids are analyzed by autoradiography by the densitometry of radiograms of chromatoplates developed from X-ray films.

The precision of the method is demonstrated on model mixtures of mono, di- and triglycerides, neutral and phospholipids and C^{14} labeled lipids.

Results of the analysis of several samples of rat liver lipids compared closely to those obtained by silicic acid column chromatography.

Introduction

SINCE THE FIRST REPORTS of the fractionation of lipids by adsorption chromatography (1,2,3,4,5), this technique has undergone continuous refinement and development for analytical applications. Lipid class analysis by chromatographic methods has been demonstrated by Borgström (6), Fillerup and Mead (7) and Barron and Hanahan (8). The systematic studies by Hirsch and Ahrens (9) brought particular attention to the factors involved in the quantitative analysis of the lipid classes by silicic acid column chromatography and provided a general impetus to its routine application in biological studies.

Column absorption techniques have also been applied extensively to the fractionation of the phospholipids based largely on the studies by Lea et al. (11,12) and Hanahan et al. (13,14,15).

Recognition that no single system was adequate for the complete fractionation of the lipid classes, Rouser et al. (16,17), developed multi-column schemes for the analysis of lipids. Techniques employing a combination of DEAE cellulose, silicic acid and silicic acid-silicate-water columns were described for the analysis of several complex lipids. The general principle involved in the use of a combination of columns is to reduce the complexity of each fraction with each succeeding step until pure components or fractions are obtained.

Since TLC is rapid and efficiently fractionates, a wide variety of lipids, its application to the quantitative analysis of the lipid classes is well indicated. At present, quantitative analysis of lipids by TLC has been carried out mainly by recovering the separated components of a mixture and analyzing them independently by established analytical methods (18, 19,20,21,22,23,24). Snyder and Stephens (25) developed an elegant method for the analysis of radioactive lipids by this technique. The measurement of spot size has also been employed successfully for the quantitative analysis by TLC (26,27,28,29,30). We have employed the densitometry of charred spots for the analysis of mono-, di- and triglyceride mixtures and component triglycerides (31,32). Described here are refinements in this technique and its application to neutral and phospholipids. The quantitative analysis of radioactive lipids by autoradiography by the same general technique is also described.

[1] Supported in part by U. S. Public Health Service. Research Grant HE-05375.

Experimental

Materials. Highly purified reference lipids were obtained from The Hormel Institute, Austin, Minn. Samples of lecithin and phosphatidyl ethanolamine were isolated from egg lipid by the general procedure described by Rhodes and Lea (10,11). Characteristics of these preparations have been previously described (33).

Sphingomyelin was isolated from beef heart by the method of Rapport and Lerner (34). This preparation was homogeneous by TLC analysis and no impurities could be detected in it by IR analysis (35).

Cerebroside was prepared from beef spinal cord employing the general procedure described by Carter (36) and Radin (37). However, repeated crystallization of the preparation as described by these investigators failed to give a chromatographically homogeneous product by TLC, and thus, final purification was carried out by silicic acid chromatography.

Samples of rat liver lipid, for which analyses are reported herein, were obtained from animals receiving different fat supplements in nutritional experiments. The results of these experiments will be reported elsewhere.

Tripalmitin-1-C^{14}, palmitic acid-1-C^{14}, cholesterol-4-C^{14} and cholesterol palmitate-1-C^{14} were obtained commercially and purified by TLC (28). The activity of all solutions was determined with a Packard Tricarb scintillation counter.

General Procedure

Thin-Layer Chromatography. The analyses were carried out on 20 x 20 cm chromatoplates coated with a thin layer of silicic acid containing 10% calcium sulfate as a binder. The chromatoplates for these studies were dried and activated by heating them for 2 hr at 110C in a convection type oven where they were allowed to cool to room temp. No special conditions were used for storage of the plates, but they were used within a day after their preparation. The silicic acid used as an adsorbent for TLC in this study was prepared in our laboratory because commercial products, even those compounded especially for TLC, invariably contained contaminant organic matter which made them unsatisfactory for preparative TLC. There are many aspects to the preparation of silicic acid. A consideration of the properties of

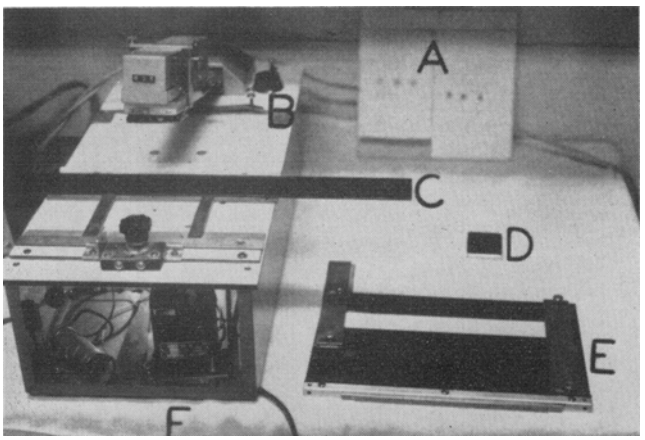

Fig. 1. Modified stage for quantitative TLC.
A. Chromatoplate;
B. Height adjustment for photocell;
C. Strip containing various slit sizes;
D. Slit cap for photocell;
E. Adjustable stage to hold chromatoplates;
F. Motor for continuous stage movement.

these adsorbents for the separation of lipids has been reviewed by Wren (38).

The following procedure was used for the preparation of silicic acid used in this study. Four liters 40% sodium silicate (Sargent Co.) is diluted with 12 liters distilled water, and the silicic acid is precipitated by the addition of 2 liters concen HCl. The excess acid is removed by washing the precipitate with distilled water. When the washings are neutral to methyl orange, the silicic acid (ca. 1400 g) is washed 3 times with ca. 10 liters acetone, then 3 times with ca. 10 liters of a 1:1 mixture of acetone and diethyl ether and, finally, twice more with about 5 liters diethyl ether. The silicic acid is then dried *in vacuo*, ground with glass marbles (1 cm OD) in a small laboratory ball mill, and sieved through a No. 200 mesh screen. The yield of final product was 1100 g. Reagent grade calcium sulfate (10% by wt) was then mixed with it to serve as a binder.

The neutral lipids were separated with various ratios of diethyl ether, petroleum ether and acetic acid, and the phospholipids with chloroform, methanol and water mixtures as described with the results.

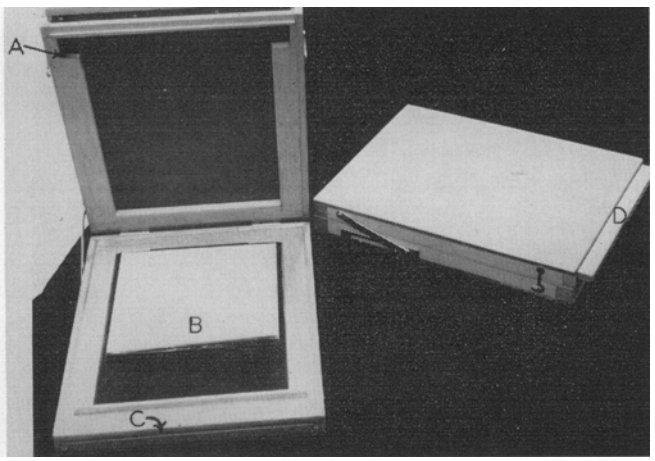

Fig. 2. Film holder for exposing chromatoplates to X-ray film.
A. Slot for X-ray film.
B. Chromatoplate on platform.
C. Slot by which slide D raises chromatoplate against the X-ray film.

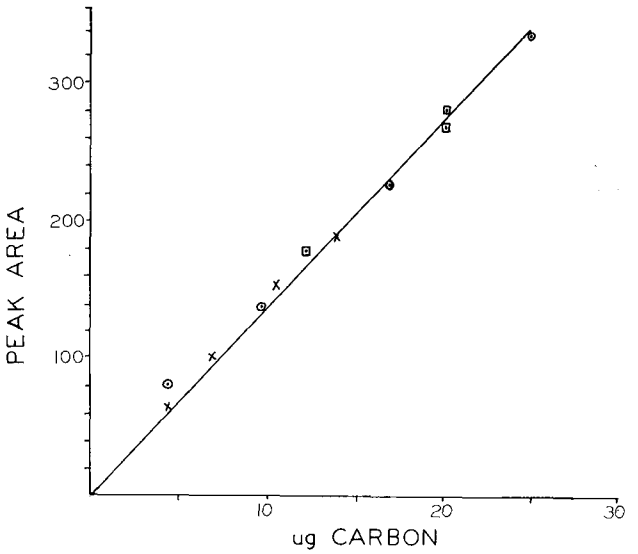

Fig. 3. TLC standard curve showing relationship of peak area vs. μg of carbon.
⊙ Cholesterol palmitate.
⊡ Palmitic acid.
× Tripalmitin.

Charring of the spots was carried out by heating the plates in an oven for 25 min at 180C, after spraying them lightly with a saturated solution of $K_2Cr_2O_7$ in 70% by volume of aqueous sulfuric acid (39).

Radioactive lipids are analyzed by autoradiography as follows. After the chromatoplates are developed, they are exposed to no-screen X-ray film in the holder shown in Figure 2. The holder is loaded and unloaded in the dark. The chromatoplate is placed on the stage (Fig. 2) and the holder is closed. Then the chromatoplate is brought into contact with the X-ray film by removing the slide from the upper slot and placing it in the lower slot. The length of time the plate is left in contact with the X-ray film will depend on the activity in the spots. With C^{14} labeled lipids, spots with 1000–10,000 dpm can be measured with a 24-hr exposure period. Spots with 100–1000 dpm should be exposed up to ca. 10 days.

After the plates have been exposed for the required period of time, they are developed photographically as described by Mangold, et al. (28,29). The final radiograms are trimmed to 20 x 20 cm and analyzed by densitometry by the same general procedure applied to charred spots on chromatoplates as described below.

Densitometry. A Photovolt Densitometer (Models 52C, 520A Photometer, Photovolt Corp., New York) with a specially designed stage for holding 20 x 20 cm chromatoplates was used for the densitometry. Details of the construction stage show in Figure 1. The sliding metal strip (C, Fig. 1) contains a series of splits of different lengths (0.5 mm wide) to insure high sensitivity regardless of the size of the spot. The metal strip is made of 3/16 in. material to collimate the light. The small attachment (D. Fig. 1) which fits over the photocell also is for this purpose. It is important to eliminate all stray and reflected light; otherwise, standard curves do not pass through the origin. For this reason, also, all parts of the stage are painted black. The stage itself is made out of 1/4 in. Bakelite. The metal guides on the stage (E, Fig. 1) permit adjustment of the plate so that all spots pass directly over the slit. The photo-

TABLE I

Standard Curve Analysis

Compound	Slope
Cholesterol palmitate	.504
Tripalmitin	.504
Tristearin	.504
Triolein	.508
Palmitic acid	.498
Stearic acid	.498
Linoleic acid	.501
Cholesterol	.492
Hydrogenated egg lecithin	.492
Purified egg lecithin	.494
Purified soybean lecithin	.495
Dipalmitin	.508
Monopalmitin	.501
Sphingomyelin	.643
Phosphatidyl ethanolamine	.658

cell support (B, Fig. 1) permits fine adjustment of the photocell above the chromatoplate.

The chromatoplate may be placed either adsorbent side up or down on the stage. When a dark room is available, it is preferable to make the measurements with the adsorbent side up so that the adsorbent layer is not disturbed. After the chromatoplate is properly positioned on the stage, a slit is selected which is ca. 1/4 again as long as the diameter of the spot. The intensity of the light, with no filter on the instrument, is adjusted to 100% transmission when the slit is between the spots, and 0% transmission when the shutter in front of the photocell is closed. Analysis of the spots may be carried out semi-automatically by plotting the readings on the photometer against millimeters of travel or automatically by means of a strip chart recorder (Photovolt Varicord Model 43 recorder). Each spot gives a peak of density values; the area under the peak is a function of the size and density of the spot and is used for quantitative analysis.

Results and Discussion

Analysis of the charred spots of a large number of lipids showed that the peak area vs. the amount of sample gave a linear relationship which passed through the origin, as demonstrated in Figure 3. The amount of sample is expressed as carbon so that all compounds may be equated on the same basis. The proportionality (slope of the curve, Fig. 3) of the relationship of peak area and amount of sample agreed closely for a large number of lipids as shown in Table I. This relationship depends on the extent of the conversion to carbon. Thus, the charring conditions are important. The extent of the conversion to carbon is influenced by structure and mol wt. The conversion to carbon appears to be nearly quantitative for most of the common lipids under the conditions employed. Phosphatidyl ethanolamine, sphingomyelin and methyl esters of long-chain fatty acids gave anomalous high values which cannot be explained at present, however.

It was shown previously (39) that when the charring was carried out at high temp (250C) and with a weak oxidizing agent (50% H_2SO_4) the amount of carbon formed resulted from two reactions, oxidation and evaporation. Accordingly, since unsaturated compounds are oxidized much faster than their saturated analogues, they gave much higher yields of carbon. Surprisingly enough, an appreciable amount of evaporation of tripalmitin also occurred under these conditions.

Variations due to differences in yield of carbon can be normalized by comparison with known amounts of suitable standards applied to the same plate. Apart from small differences due to carbon density, there is little variation in the extent of the conversion of compounds of the same class to carbon, as shown in Table I. Thus, a compound need not be identical with that being analyzed to serve as a standard.

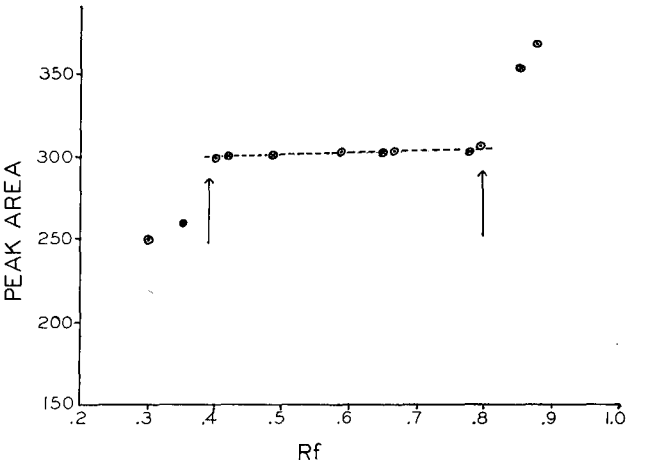

Fig. 4. Relationship between R_f value and peak area with tripalmitin.

Regardless of the technique employed, poorly developed chromatoplates should not be used for quantitative analysis. Samples for quantitative analysis also should be applied to chromatoplates in low boiling petroleum ether or other highly volatile solvents in order to avoid a chromatographic effect within the spot itself.

Influence of R_f Value. Generally, spots become larger the higher they are allowed to migrate on the chromatoplate. The increase in size is compensated by a decrease in density, giving the same densitometrically determined peak area only when the spot is migrated sufficiently to form its spot characteristics and not so close to the front to become distorted. This relationship is illustrated with tripalmitin in Figure 4. These results show that the peak area is essentially constant from an R_f of about 0.3–0.8.

The effect of R_f can also be normalized by the use

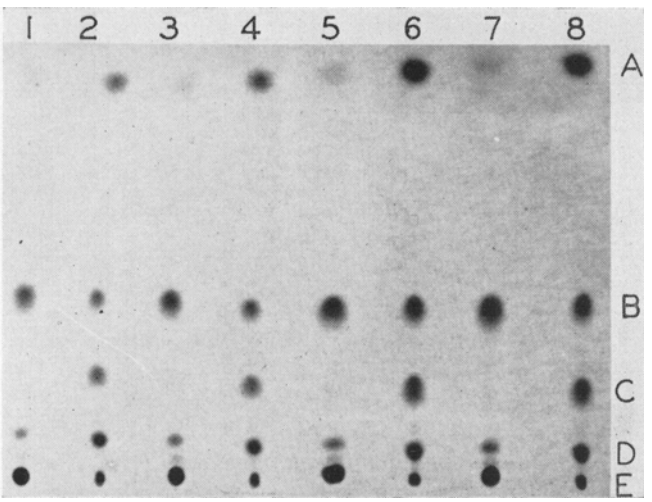

Fig. 5. Chromatoplate of separated major lipid classes of natural and reference mixtures on silicic acid with 15% diethyl ether in petroleum ether + 1.0% acetic acid.

A. Sterol esters;
B. Triglycerides;
C. Free fatty acids;
D. Cholesterol;
E. Phospholipids (even numbers are standard mixtures; odd numbers, samples of natural lipid).

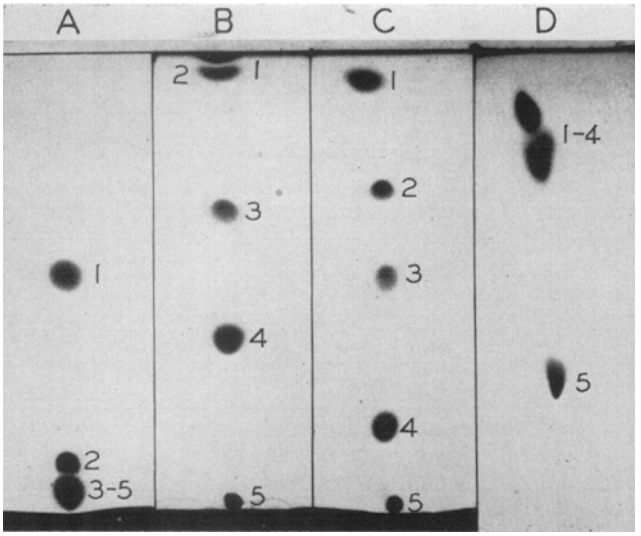

Fig. 6. Chromatoplate of the separated major lipid classes in 4 solvent systems on the same plate.

A. 5% ether in petroleum ether
B. 50% ether + 0.5% HAC in petroleum ether
C. 15% ether + 1% HAC in petroleum ether
D. 70:30:4 $CHCl_3$:MeoH:H_2O

1. Cholesterol oleate
2. Triolein
3. Oleic acid
4. Cholesterol
5. Lecithin

of suitable reference standards. Moreover, since most natural mixtures of lipids contain compounds with widely different polarities, the use of reference standards is recommended whenever they are available, because they permit the analysis to be carried out on a single plate as well as eliminating variations due to charring. The general procedure in the analysis of a sample with standards is illustrated in Figure 5. Usually, 4 samples of both reference standard and sample are spotted on a single plate. Since

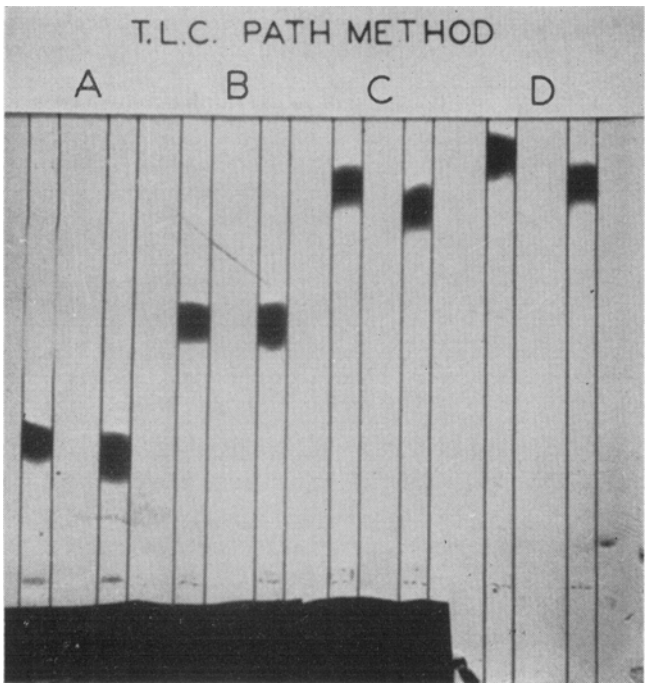

Fig. 7. Chromatoplate illustrating path method for the analysis of lipids with tripalmitin developed to different R_f values.

A. 5% et_2O in petroleum ether.
B. 10% et_2O in petroleum ether.
C. 15% et_2O in petroleum ether.
D. 20% et_2O in petroleum ether.

TABLE II

TLC Analyses of Standard Mixtures of Mono-, Di-, and Tripalmitin

Sample #I

	#1	#2	#3	Avg	Known	Abs. Error
Tri-	68.0	63.5	64.0	65.2	66.7	−1.5
Di-	20.0	24.5	24.4	22.9	23.1	−0.2
Mono-	12.0	12.0	11.6	11.8	10.2	+1.6

Sample #II

	#1	#2	#3	Avg	Known	Abs. Error
Tri-	14.0	11.1	12.2	12.4	11.3	+1.1
Di-	45.6	47.6	48.3	47.2	47.0	+0.2
Mono-	40.4	41.3	39.5	40.4	41.7	−1.3

Sample #III

	#1	#2	#3	Avg	Known	Abs. Error
Tri-	63.0	65.6	66.0	64.9	67.7	−2.8
Di-	13.0	12.6	12.4	12.6	11.6	+1.0
Mono-	24.0	21.8	21.6	22.5	20.7	+1.8

the standard curve for each compound passes through the origin, 4 samples of the same concn can be analyzed and the average of the 4 values compared directly with that of the sample. For example, if the average area for a 10 μg load of the standard triglyceride was 400 and the average of the unknown sample was 200, it could be concluded that the unknown sample contained 5 μg triglyceride. The percentage composition is then calculated from the amount of sample spotted on the plate. In addition to the use of standards, or if standards are not available for all components of a mixture, each compound may be migrated to an R_f value between 0.3–0.8 for direct comparison of areas. In order to eliminate variations from one chromatoplate to another, such an analysis is usually carried out on the same plate by dividing it (scoring the adsorbent layer) into sections, one section for each different solvent system.

This technique is demonstrated on a model mixture of the lipid classes in Figure 6. After the sample in the first section is developed, the adsorbent layer is scraped from the bottom of the chromatoplate above the height of the solvent in the chromatographic jar. Then another sample is spotted in the second section of the chromatoplate. After it has developed, the procedure is repeated until all of the compounds of the mixture have been developed to an R_f in the range of from 0.3–0.8. For the greatest possible accuracy, this technique may be applied along with reference standards.

In the analysis of radioactive lipids, reference standards of known activity must be applied to the same plate as the sample in order to normalize the conditions of exposure to the X-ray film and the photographic development of the film.

Path Method of TLC. This method is illustrated in Figure 7. The sample is developed between lines scored in the adsorbent. The lines fix one dimension of the spots; the other dimension, as well as the maximum density of the spots, is determined by passing the spots over a slit shorter than the distance between the lines. The product of the area of the spot (calculated from the dimensional measurements) and the max density form a linear relationship with the amount of material in the spot. The disadvantage of this method is that the efficiency of separations is decreased because relatively large samples must be applied to the plate. We have applied the principle of the path method in the analysis of triglyc-

TABLE III

TLC Analyses of Standard Mixtures of Lipid Classes

	#1	#2	#3	Avg	Known	Abs. Error
Sample #I						
C.E.	19.5	19.0	18.6	19.0	18.8	+0.2
T.G.	19.2	18.8	20.2	19.4	18.8	+0.6
F.F.A.	23.0	23.3	23.4	23.4	23.2	+0.2
CHOL.	20.0	20.3	19.8	20.0	20.3	−0.3
H.L.	18.3	19.6	18.0	18.6	20.1	−1.5
Sample #II						
C.E.	9.0	8.5	7.0	8.2	7.4	+0.8
T.G.	27.7	26.9	26.8	27.1	26.1	+1.0
F.F.A.	31.6	31.0	30.6	31.1	30.4	+0.7
CHOL.	25.6	26.8	28.3	26.9	28.2	−1.3
H.L.	6.1	6.8	7.3	6.7	7.9	−1.2
Sample #III						
C.E.	10.0	11.1	9.6	10.2	9.1	+1.1
T.G.	46.0	43.3	45.0	44.8	45.9	−1.1
F.F.A.	11.1	11.8	10.8	11.2	10.7	+0.5
CHOL.	10.2	11.7	10.4	10.8	10.0	+0.8
H.L.	22.7	23.1	24.2	23.3	24.3	−1.0

C.E. = Cholesterol Palmitate
T.G. = Tripalmitin
F.F.A. = Palmitic Acid
CHOL. = Cholesterol
H.L. = Hydrogenated Lecithin

eride structure (32). Since the components in the analysis of these compounds have very similar structures, they give spots of the same shape and characteristics. Thus, it is not necessary to develop the spots in channels to control their shape. Although this method has special application in the analysis of triglyceride structure, it has no particular advantage over the normal procedure using a slit just longer than the diam of the spot especially when reference standards are available.

Analysis of Standard Mixtures. The precision of the method for the analysis of the lipid classes which is frequently found as a major component of lipids shows in Tables II, III, IV. The absolute error in these analyses is about ± 1.5%. The solvent systems used for these separations may be varied somewhat from that employed in this study for ideal separations, depending on the activity of the chromatoplates.

Analysis of Natural Fats. Rat liver lipid was selected to illustrate the application of the method to a natural fat because it contains appreciable amounts of both neutral and phospholipids, and because this lipid is frequently the subject of biological studies.

The lipid was extracted from the livers of animals, killed by exsanguination, with chloroform-methanol (2:1, v/v) in a Servall Omni-Mixer. The solutions from 3 extractions were combined and evaporated to near dryness. Then the aqueous fatty residue was dissolved in ca. 100 ml diethyl ether and dried over anhydrous sodium sulfate. By repeating this process, the water, as well as the chloroform and methanol, was removed. The solutions were maintained under an atmosphere of nitrogen throughout all operations to avoid oxidation.

The composition of the neutral lipids was determined first on an aliquot containing a known weight of the sample dissolved in low boiling petroleum ether (35–60C) by the method illustrated in Figure 5. Cholesterol oleate, triolein, oleic acid, cholesterol and hydrogenated lecithin were used as standards for the analysis. The "polar lipids" were estimated by difference because, as indicated above, densitometry of a spot on the origin did not give reliable results. The results of the analysis by the simple system described above show in Table V.

TABLE IV

Silicic Acid Column Chromatography vs. TLC Analyses of Rat Liver Lipids (animals on different fat supplements)

	10% Corn Oil		Fat-Free		10% Lard	
	Column	TLC	Column	TLC	Column	TLC
S.E.	6.2	7.2	7.0	7.5	2.8	3.7
T.G.	48.0	49.4	18.8	20.7	69.5	67.6
F.F.A.	none	none	1.5	2.5	1.0	4.0
Sterols	2.4	3.8	5.8	7.0	3.0	6.5
P.L.[a]	43.4	39.6	66.9	62.3	23.7	18.2

[a] By difference on both column and TLC.
S.E. = Sterol esters.
T.G. = Triglycerides.
F.F.A. = Free fatty acids.
P.L. = Polar lipids (consisting mostly of phospholipids).

The analysis of this fat was also carried out by a corresponding simple silicic acid column chromatographic method. The results also show in Table V. The column analysis was carried out by the stepwise elution of a 2-g sample from a column 20 cm long and 4.7 cm in diam. The elution was started with petroleum ether (bp 35–60C) and continued until all of the sterol ester fraction was eluted as determined by TLC analysis. The elution was then continued with 10% ethyl ether in petroleum ether until all the triglycerides were eluted. The sterol fraction which consisted mainly of cholesterol was eluted next with 50% ethyl ether in petroleum ether. When the free fatty acid content of the lipids is less than 5%, the amount of it which contaminates the other fractions is extremely small, and the bulk of it is eluted in the last fraction with ethyl ether containing 1% acetic acid.

The polar lipids were eluted with methanol; but, since frequently the complete recovery of these compounds was not practical, they were estimated by difference. Comparison of results in Table V shows that the results of the TLC method agree well with those of column method and indicate that TLC may be used for the quantitative analysis of the neutral lipids.

The neutral lipids of some fats may contain appreciable amounts of lipids other than those listed above; for example, some fish oils contain appreciable amounts of glycerol ethers. These may be analyzed by the same general technique using the appropriate standards. It may, of course, be desirable to use more than one solvent system to fractionate all the components. In this case, one component which gives a measurable spot in both solvent systems is used to normalize all the values. Separations in different solvent systems may be carried out on the same plate by employing the technique illustrated in Figure 6.

Polar lipids are analyzed in the same manner as the neutral lipids with the appropriate solvent system on a small sample isolated by preparative TLC. The recovery of the polar lipids is usually carried out by scraping this fraction from a plate while it is still wet with solvent. The material scraped from the plate is slurried with water and placed in a small

TABLE V

TLC Analysis of a Standard Mixture of Polar Lipids

	#1	#2	#3	Avg	Known	Abs. Error
Cerebrosides	23.3	21.9	20.6	21.9	20.8	+1.1
Phosphatidyl ethanolamine	24.9	23.9	24.2	24.3	23.3	+1.0
Lecithin	41.2	42.4	42.0	41.9	44.8	−2.9
Sphingomyelin	10.6	11.8	13.2	11.9	11.1	+0.8

TABLE VI

Analysis of Rat Liver Polar Lipids
(10% corn oil supplemented diets)

	Percent Composition	
	Silicic Acid Column	TLC
Phosphatidyl ethanolamine	26.0	31.0
Lecithin	52.4	50.6
Sphingomyelin	14.0	16.0
Others	2.7	2.4
Loss	4.9	....

sintered glass funnel through which it is filtered by means of suction. When most of the water has been filtered, the adsorbent is washed with methanol several times and finally with chloroform. The sample may be recovered as previously described or by evaporation of the solvent under reduced pressure.

To illustrate the application of the method to a natural mixture of the polar lipids, a sample isolated from the livers of the corn oil supplemented group of rats was analyzed (Table VI). For comparison, a sample also was analyzed by silicic acid column chromatography by essentially the same elution scheme described by Rhodes and Lea (10,11). In this scheme, the elution was started with chloroform which removed traces of unknown material. The phosphatidyl ethanolamine fraction was eluted next with a 9:1 (v/v) ratio of chloroform-methanol. When all but a trace of this fraction had emerged, the elution was continued with a 7:2 (v/v) ratio of chloroform-methanol. A small intermediate fraction was obtained, after which the main bulk of the lecithin was eluted with a 7:3 ratio of these solvents. Toward the end of the elution of the lecithin, some sphingolipids started to emerge from the column. These fractions were collected separately, then the sphingolipids were eluted with methanol. The entire fractionation was followed by TLC analysis. Analysis of the mixed fractions was determined by the quantitative TLC technique, as well as by IR spectral analysis, as described by Smith and Freeman (40). The results in Table VI were calculated from the weights of the pure fractions corrected for the amounts in the mixed fractions. The values obtained by the two methods agreed well, and indicated that the method is applicable to polar lipids.

No attempt was made to make a detailed analysis of the components of the liver polar lipids because resolution of many acidic from nonacidic lipids cannot be effected on silicic acid (16). However, since such separations are readily performed on DEAE columns (17) the problem of the analysis of complex lipids is being attacked by a combination of this technique and quantitative TLC in a collaborative effort by the authors with George Rouser and his co-workers.

Analysis of Radioactive Lipids. Results in Figure 8 demonstrate that the activities in the spots form a linear relationship with peak area of the spots on the X-ray film determined by densitometry as described above. Thus, it is possible to determine the distribution of radioactivity among the components of the mixture by the same general technique employed with charred spots.

By combining the charring technique with autoradiography, specific activity can be determined. When care is taken to insure against saturation of the X-ray film, the radioactivity of unknown compounds can also be determined. Radiometric methods of analysis used in conjunction with TLC have the

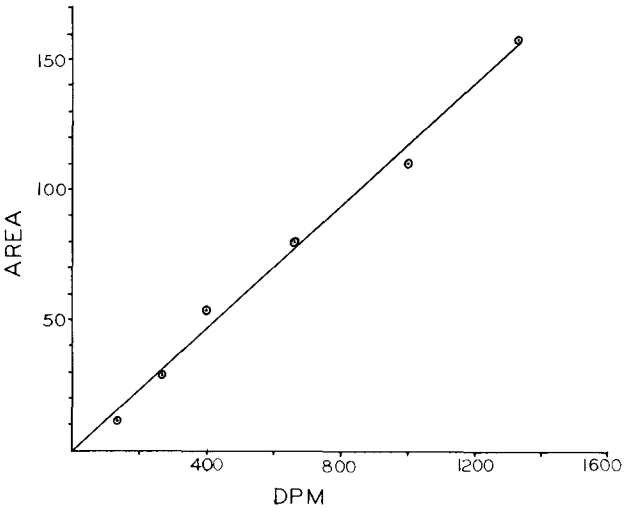

Fig. 8. Standard curve of cholesterol palmitate-1-C^{14} showing relationship between peak area and radioactivity.

advantage of speed and greater sensitivity when scintillation counters are available. However, in many instances, particularly with poorly resolved or complex mixtures, it is necessary to locate the positions of radioactive lipids by autoradiography. Thus the above procedure serves as a good adjunct to radiometric methods, and, in combination with carbon analysis as described above, permits the determination of specific activity.

REFERENCES

1. Trappe, W., Z. Physiol. Chem. *273*, 177 (1942).
2. Brockmann, H., and F. Volpers, Ber. *80*, 77 (1947).
3. Trappe, W., Biochem. Z. *305*, 150 (1940).
4. Trappe, W., *Ibid. 306*, 316 (1940).
5. Kaufmann, H. P., Fette u Seifen *46*, 268 (1939).
6. Borgström, B., *Acta Physiol. Scand. 25*, 111 (1952).
7. Fillerup, D. L., and J. F. Mead, Proc. Soc. Exptl. Biol. Med. *83*, 574 (1953).
8. Barron, E. J., and D. S. Hanahan, J. Biol. Chem. *231*, 493 (1958).
9. Hirsch, J., and E. H. Ahrens, Jr., *Ibid. 233*, 213 (1958).
10. Rhodes, D. N., and C. H. Lea, Biochem. J. *65*, 526 (1957).
11. Lea, C. H., D. N. Rhodes, and R. D. Stoll, Biochem. J. *60*, 353 (1955).
12. Lea, C. H., "Biochemical Problems of Lipids," Butterworths Scientific Publications, 1956, p. 81–90.
13. Hanahan, D. J., M. B. Turner, and M. E. Jayko, J. Biol. Chem. *192*, 623 (1951).
14. Hanahan, D. J., and M. E. Jayko, J. Am. Chem. Soc. *74*, 5070 (1952).
15. Hanahan, D. J., J. Biol. Chem. *211, 313, 321*, (1954).
16. Rouser, G., A. J. Bauman, G. Kritchevsky, D. Heller, and J. S. O'Brien, JAOCS *38*, 544 (1961).
17. Rouser, G., G. Kritchevsky, D. Heller, and E. Lieber, *Ibid. 40*, 425 (1963).
18. Vioque, E., and R. T. Holman, *Ibid. 39*, 63 (1962).
19. Bird, H. L., H. F. Brickley, J. P. Comer, P. E. Hartsaw, and M. L. Johnson, "Quantitative Thin-Layer Chromatography," Eli Lilly and Co., Indianapolis.
20. Malins, D. C., Chem Ind. 1359 (1960).
21. Malins, D. C., and H. K. Mangold, JAOCS *37*, 576 (1960).
22. Morris, L. J., R. T. Holman and K. Fontell, J. Lipid Res. *2*, 68 (1961).
23. Morris, L. J., R. T. Holman, and K. Fontell, JAOCS *37*, 323 (1960).
24. Kirchner, J. G., J. M. Miller, and R. G. Rice, J. Ag. Food Chem. *2*, 1031 (1954).
25. Snyder, F., and N. Stephens, Anal. Biochem. *4*, 128 (1962).
26. Purdy, S. J., and E. V. Truter, Chem. Ind. 506 (1962).
27. Seher, A., Mikrochim. Acta. *1961*, 308.
28. Mangold, H. K., R. Kammereck, and D. C. Malins, Microchem. Techniques 697 (1962).
29. Mangold, H. K., JAOCS *38*, 708 (1961).
30. Mangold, H. K., and R. Kammereck, Chem. Ind. 1032 (1961).
31. Privett, O. S., M. L. Blank, and W. O. Lundberg, JAOCS *38*, 312 (1961).
32. Privett, O. S., and M. L. Blank, *Ibid. 40*, 70 (1963).
33. Privett, O. S., M. L. Blank, and J. A. Schmit, J. Food Sci. *27*, 463 (1962).
34. Rapport, M., and B. Lerner, J. Biol. Chem. *232*, 63 (1958).
35. Nelson, G. J., and N. K. Freeman, *Ibid. 234*, 1375 (1959).
36. Carter, H. E., W. J. Haines, W. E. Ledyard and W. P. Norris, *Ibid. 169*, 77 (1947).
37. Radin, N. S., Biochem. Prep. 7, 31 (1960). Ed. H. A. Lardy, John Wiley and Sons, London, New York Publishers.
38. Wren, J. J., Chromatog. Rev. *3*, 111–133 (1961).
39. Privett, O. S., and M. L. Blank, JAOCS *39*, 520 (1962).
40. Smith, L. M., and N. K. Freeman, J. Dairy Sci., *42*, 1450 (1959).

[Received August 5, 1963—Accepted January 21, 1964]