For further identification the unknown hydroxy ester should be converted to the acetoxy ester. Using the polyester column and 12-acetoxystearate as standard it can be seen from Figure 1B that 14-acetoxy ester can be partly resolved from 12-ester if the relative amt in the mixture are suitable and that 2-,3-,4-, 15-,16-,17- and 18-acetoxy esters are partly or completely separated. If the unknown hydroxy ester is converted to the oxo ester (2,11) further information can be obtained by analysis on the QF-1 column, particularly about isomers with the substituent at positions 4–8. For this purpose it was found best to use a 12 ft x $\frac{3}{16}$ in. column, as was used to obtain Figure 1C, instead of the 6 ft x $\frac{3}{16}$ column used to obtain the carbon numbers. Figure 1C shows that the 2-,4-, 5-,16- and 17-oxo esters all are separated from each other and that 6-oxo is partly separated from 8-oxo. Using this column the 6- and 12-oxo esters are completely separated, 7- and 12-oxo esters are partly separated and 8-oxo ester forms a pronounced shoulder on the 12-oxo ester peak. Thus by using a combination of the three types of oxygenated ester and the three columns 2-,3-,4-,5-,6-,7-,8-,14-,15-,16-,17- and 18-isomers can be identified using the 12-isomer as a known standard but the 9-,10-,11- and 13-isomers cannot be characterized in this way.

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Analytical Fractionation of Complex Lipid Mixtures: DEAE Cellulose Column Chromatography Combined With Quantitative Thin Layer Chromatography

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Abstract

A quantitative chromatographic procedure for the fractionation of complex lipid mixtures is described. The method utilizes diethylaminoethyl (DEAE) cellulose column chromatography followed by thin layer chromatography (TLC). Spots produced in TLC are charred with sulfuric acid-potassium dichromate and heat and are then measured by quantitative densitometry. Results obtained with beef brain and beef heart mitochondrial lipids are presented, and the close correspondence between column isolation procedures and the new procedure is demonstrated. Methods utilizing only column chromatography, column chromatography and TLC, and one- and two-dimensional TLC without column chromatography are compared.

Introduction

CHROMATOGRAPHIC PROCEDURES are widely used for the determination of lipid class composition. Silicic acid column chromatography has been the most commonly utilized approach based on the initial observations of Borgström (1), Fillerup and Mead (2), Barron and Hanahan (3) and Lea et al. (4). Diethylaminoethyl (DEAE) cellulose column chromatography was introduced by Rouser et al. (5) to eliminate the problem of the elution of acidic lipids with other lipid classes encountered with silicic acid column chromatography. DEAE column chromatography combined with silicic acid column chromatography and with silicic acid-silicate column chromatography was used to obtain separation of most lipid classes of brain (5,6) and other mixtures (6). Recently, Privett and Blank (7) and Blank et al. (8) have greatly extended the possibilities of TLC for quantitative applications. These investigators demonstrated that a spray reagent composed of H_2SO_4 and potassium dichromate can be used to char spots obtained by TLC to a reproducible optical density. Under proper conditions the extent of charring is independent of the degree of unsaturation of lipid classes such as lecithin and sphingomyelin, can be made to give a linear response over a fairly wide concentration range, and is readily determined by transmission densitometry.

The present report describes a new approach to

TABLE I

DEAE Column Chromatography

Solvent	Vol (ml)	Components of fraction
C/M 9/1	440	cholesterol, cerebroside, lecithin, sphingomyelin
C/M 7/3	550	phosphatidyl ethanolamine
CH_3OH	350	water soluble nonlipid
HAc/$CHCl_3$ 6/1	500	phosphatidyl serine
HAc	500	gangliosides
CH_3OH	400	none (used to remove HAc)
C/M 4/1 containing 20 ml 28% aqueous ammonia per liter and 0.01 M NH_4Ac	750	sulfatides, phosphatidyl inositol, cardiolipin, phosphatidic acid, triphosphoinositide, and uncharacterized components

C = chloroform; M = methanol; HAc = glacial acetic acid. Vol of solvent are for a column 20 x 2.5 (i.d.) cm prepared with 15 g DEAE and 150 mg of whole beef brain lipid applied as sample.

quantitative analysis of lipid class composition using DEAE cellulose column chromatography combined with quantitative TLC. Useful new one- and two-dimensional TLC methods are also described.

Materials and Methods

Extraction of Lipids and Column Chromatography. Chloroform/methanol 2/1 extracts of whole beef brain were prepared and the lipid class composition determined by chromatography on magnesium silicate, DEAE cellulose, silicic acid, and silicic acid-silicate columns (6). The values obtained for lipid class composition by these column chromatographic procedures were obtained for comparison with the new DEAE-TLC procedure and the pure lipid classes isolated by column chromatography were used as standards for quantitative TLC.

Beef heart mitochondrial lipids were extracted with chloroform/methanol 2/1 (6) by Sidney Fleischer. The mitochondria were isolated and characterized by electron microscopy and enzymatic assays at The Institute for Enzyme Research, University of Wisconsin. The mitochondrial lipids were analyzed previously by column chromatography (9) and the values for lipid class composition from these investigations were compared with values obtained by the new DEAE-TLC procedure.

DEAE cellulose column chromatography was carried out with columns 2.5 (i.d.) x 20 cm packed with 15 g of DEAE (6). The details of the elution scheme employed for brain lipids are shown in Table I.

Thin Layer Chromatography. Several adsorbents were used for TLC. Silica Gel G (Research Specialties Co., Richmond, Calif.) was used in preliminary studies, but this adsorbent was inferior to an adsorbent prepared by mixing nine parts of Silica Gel Plain (Research Specialties Co.) with one part of reagent-grade, anhydrous $CaSO_4$. The best adsorbent for most purposes, however, was prepared by mixing nine parts of Silica Gel Plain with one part of finely powdered magnesium silicate (Allegheny Industrial Chemical Corp., Butler, N.J.).

Commercial Silica Gel G preparations contained impurities which could be removed by washing with chloroform/methanol 1/1 (3–4 bed volumes) followed by drying under nitrogen as described by Rouser et al. (10) for the preparation of silicic acid for column chromatography.

To obtain very even layers adsorbents are spread over plates using a relatively dilute slurry in water or 0.01 M aqueous NaCl. Thus 20–30 g of adsorbent with 60–75 ml of water or 0.01 M NaCl will spread five 20 x 20 cm plates. Different batches of adsorbent may require different amt of water, although a 3/1 water/adsorbent ratio is generally applicable. Both fixed distance and adjustable spreaders have been used and the fixed distance (ca. 250 μ) spreader in common use is preferred. After spreading, plates are stored in an air-tight box. Heat activation is carried out just before TLC and accomplished at 120–130C for 20 min. After heating, the plates are cooled for 30 min, spotted with a microsyringe, and placed immediately in the developing chamber.

TABLE II

Comparison of Column and DEAE-TLC Values for Brain Lipids[a]

Substance	Column[b]	DEAE-TLC	Direct TLC
Cholesterol	20.3	20.4	19.0
"Ceramide"[c]	0.31	0.43	0.43
Cerebroside	13.1	13.5	13.7
Sulfatide	4.0	4.0	4.0
Lecithin	.....	11.3	.....
Sphingomyelin	.....	7.9	.....
Phosphatidyl ethanolamine	14.0	14.0[d]	14.7
Phosphatidyl serine	7.5	7.5[d]	.....
Gangliosides	2.5	2.5[d]	.....
Phosphatidyl inositol	2.0	2.0	.....

[a] Analyses carried out on a beef brain with a lipid composition more like beef brain white matter than the usual whole brain.
[b] A magnesium silicate column eluted with chloroform, chloroform/methanol 95/5, and chloroform/methanol 2/1 (all solvents containing 5% 2,2-dimethoxypropane) to give cholesterol, "ceramide," and cerebroside plus sulfatide fractions respectively as described by Rouser et al. (5) was used. The mixture of cerebroside and sulfatide was separated on a DEAE column by eluting with chloroform/methanol 9/1 for cerebroside followed by seven column volumes each of methanol, chloroform/acetic acid 3/1 containing 0.01 M ammonium acetate, methanol, and glacial acetic acid to remove impurities, and pure sulfatide was then eluted with chloroform/methanol 4/1 containing 20 ml/liter of 28% aqueous ammonia and made 0.01 M with ammonium acetate.
[c] The "ceramide" fraction contains other lipids.
[d] These lipid classes determined directly by DEAE column chromatography without TLC.

The following neutral solvents are used for ascending chromatography: 1) chloroform (determination of cholesterol); 2) chloroform/methanol 98/2 (determination of cholesterol, ceramide, monoglyceride and diglyceride); and 3) chloroform/methanol/water mixtures (65/25/4, 65/30/5, 65/35/5, 60/35/3, 60/40/5, and 60/40/8) for the determination of phospholipids, cerebrosides and sulfatides. The choice of solvents and solvent ratios depends upon the substances to be determined and the characteristics of each batch of adsorbent. Useful acidic solvents are *n*-butanol/acetic acid/water (60/20/20) and *n*-propanol/acetic acid/water (60/20/20 and 80/10/10). Two-dimensional TLC is carried out generally using chloroform/methanol/water 65/25/4 in the first dimension followed by drying in air for 10 min and development with *n*-butanol/acetic acid/water 60/20/20 in the second dimension. The same type and size of chamber, paper liner, and method of saturation of the liner is used as described for silicic acid impregnated paper chromatography (11). Chambers are lined on all sides with paper (Whatman No. 1 or 3 MM) saturated with solvent just before use in order to obtain reproducible results with straight solvent fronts. Fresh solvent (ca. 200 ml) and chamber liners are used for each run.

Chromatograms developed with neutral solvents are air dried for several min while chromatograms developed with acidic solvents are dried for several hr before spraying. Chromatograms are sprayed with a reagent, similar to that of Privett and Blank (7), prepared by dissolving 1.2 g of $K_2Cr_2O_7$ in 200 ml of 55% reagent grade H_2SO_4. Spraying is performed with a very fine mist that is allowed to cover the plate completely and uniformly but without the plate becoming visibly wet. Spots are developed in an oven at 180C for 30–60 min (or until fumes cease to appear from the plates). Each plate is cooled, the back side cleaned by wiping with paper moistened first with chloroform and then with water, placed on the stage of the densitometer, and densities determined either manually or automatically. A Model 530 Photovolt densitometer equipped with a stage for 20 x 20 cm glass plates, a Varicord recorder (Model 42B) and integrator (Model 49) were used.

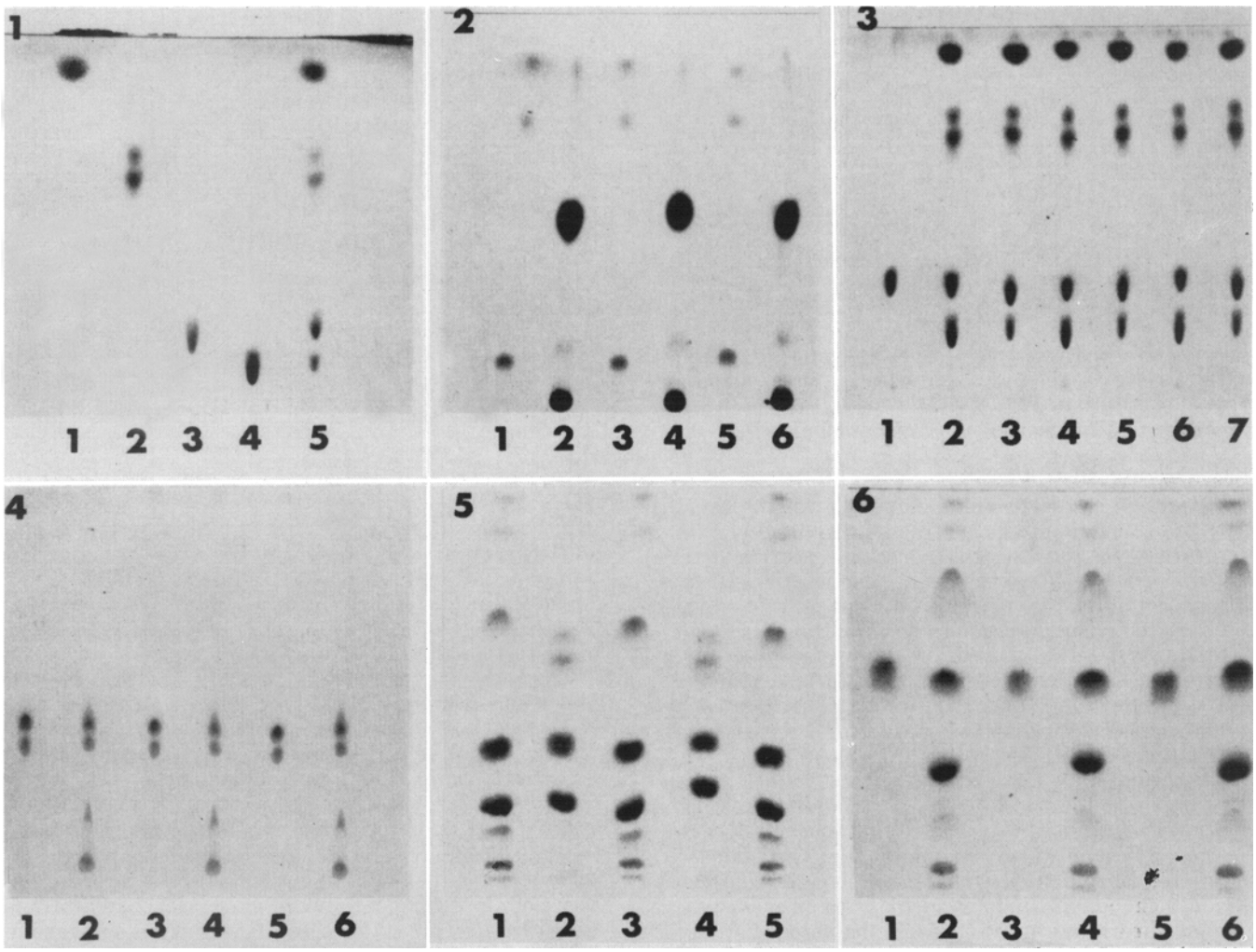

FIG. 1. Chromatogram prepared using Silica Gel G and chloroform/methanol/water (65/25/4) to show the purity of several standard substances utilized for quantitative TLC. Spottings are 10 μg each of 1) cholesterol, 2) cerebroside (three distinct spots produced), 3) lecithin, and 4) sphingomyelin followed by 5) the mixture of cholesterol, cerebroside, lecithin, and sphingomyelin of brain lipids eluted from DEAE with choloroform/methanol 9/1.

FIG. 2. Chromatogram prepared using Silica Gel G and chloroform/methanol 98/2 as solvent for the quantitative determination of cholesterol and "ceramide." Alternate spottings are from a standard mixture and from a chloroform/methanol 9/1 eluate from a DEAE column. The standard mixture contained 5 μg each of from above downward tripalmitin, dipalmitin and monopalmitin. The spots visible on the sample from brain lipids are a trace hydrocarbon and other components migrating to and nearly to the solvent front, a very large spot of cholesterol, a clear spot in the ceramide region of the chromatogram, and a large amt of material that failed to migrate from the origin (lecithin + sphingomyelin). The plate was used for the estimation of the amt of "ceramide" in beef brain (see value in Table II). In this determination monopalmitin was used as a standard for ceramide.

FIG. 3. Chromatogram prepared using Silica Gel G and chloroform/methanol/water 65/35/5 for the determination of cerebroside, lecithin and sphingomyelin in the chloroform/methanol 9/1 eluate from a DEAE column with brain lipids applied. Spotting is 1) 10 μg of lecithin standard; 2) a mixture of standards (cholesterol, cerebroside, lecithin, and sphingomyelin, 12.5 μg each); and 3) the chloroform/methanol 9/1 eluate from a DEAE column with brain lipids applied showing cholesterol, cerebroside, lecithin and sphingomyelin (total wt of sample 50 μg). The other 4 spottings are alternately a mixture of standards and the chloroform/methanol 9/1 eluate so that an average value could be obtained for the three lipids being determined.

FIG. 4. A chromatogram prepared using Silica Gel G and chloroform/methanol/water 65/35/5 as solvent. The plate was used for the quantitative determination of the amt of sulfatide present in a chloroform/methanol/ammonia/ammonium acetate eluate from a DEAE column (see value in Table II). Spottings 1, 3 and 5 from 10 μg of sulfatide (giving 2 distinct spots) and spottings 2,4 and 6 from 25 μg of a mixture of lipids eluted from DEAE with chloroform/methanol 4/1 containing 20 ml/liter of 28% by wt aqueous ammonia and made 0.01 M with respect to ammonium acetate. The mixture contained sulfatide, phosphatidyl inositol, and material failing to migrate from the origin.

FIG. 5. A chromatogram prepared for the quantitative determination of cardiolipin, phosphatidyl ethanolamine, and lecithin in beef heart mitochondria using an adsorbent composed of nine parts of Silica Gel Plain (silicic acid) and one part of magnesium silicate as adsorbent with chloroform/methanol/water (65/25/4) as solvent. The sample was spotted as a row of small spots. Spottings 1, 3 and 5 are 100 μg of mitochondrial lipid and 2 and 4 are a mixture of cerebroside (10 μg), phosphatidyl ethanolamine (20 μg) and lecithin (25 μg). Note the discrete and well formed spots using this absorbent and in particular the much more discrete nature of the cardiolipin spot with this adsorbent as compared to adsorbents containing calcium sulfate (see Fig. 6).

FIG. 6. A chromatogram prepared using an adsorbent composed of nine parts of Silica Gel Plain and one part of anhydrous reagent grade calcium sulfate spread using 0.01 M sodium chloride solution instead of water. The chromatogram was developed with chloroform/methanol/water 65/25/4. The plate was used for the quantitative determination of phosphatidyl ethanolamine in beef heart mitochondrial lipids. Spottings 1, 3 and 5 are 25 μg of phosphatidyl ethanolamine standard (isolated from beef brain lipid by elution from a DEAE column with chloroform/methanol 7/3) while spottings 2, 4 and 6 are 100 μg of chloroform/methanol 2/1 extract of beef heart mitochondria. The components in the mitochondrial lipid mixture are from above downward; lipids less polar than cholesterol mixed with the impurities in the adsorbent that are pushed to the solvent front, cholesterol immediately behind the solvent front, cardiolipin (giving a diffuse spot), phosphatidyl ethanolamine, lecithin, a trace of sphingomyelin, phosphatidyl inositol, sucrose (introduced during isolation of mitochondria in 0.25 M sucrose), and a trace of more polar material failing to migrate from the origin. Note the rather diffuse spot of cardiolipin and compare this with the more discrete spot obtained with silicic acid plus magnesium silicate as adsorbent (Fig. 5).

Results

Beef Brain and Mitochondrial Lipid Determinations. Table II lists the amt of the different lipid classes occurring in beef brain as determined by column chromatography alone, the DEAE-TLC procedure, and direct TLC when applicable. The relatively close correspondence of values by the multicolumn procedure and the DEAE-TLC method is clear. With the elution scheme chosen for the DEAE column, phosphatidyl ethanolamine, phosphatidyl serine, and the gangliosides are separated from the other lipid classes (6). Similarly, the water soluble nonlipid components extracted with lipids are eluted from the column with methanol and do not require further consideration. Two fractions from the column require further fractionation and quantitative determination of the components: the chloroform/methanol 9/1 fraction (containing cholesterol, cerebroside, lecithin, and sphingomyelin) and the chloroform/methanol/ammonia/ammonium acetate fraction (containing sulfatide and phosphatidyl inositol as principal components and a number of minor acidic lipid components).

The purity of some of the standards used for quantitation is illustrated in Figure 1. Figures 2–6 show plates used for the quantitative determination of brain and mitochondrial lipids.

The results obtained for the major lipid classes of mitochondria by the all column and DEAE-TLC procedures were the same: cardiolipin, 12.8%; phosphatidyl ethanolamine, 23.7%; and lecithin, 29.6%. This relatively simple mixture was examined by direct one-dimensional TLC and values of 12.8, 30.6 and 30.0% were obtained for cardiolipin, phosphatidyl ethanolamine and lecithin, respectively.

Figures 7 and 8 illustrate two-dimensional TLC of beef brain and beef heart mitochondrial lipid mixtures. Good resolution of all major lipid classes in both mixtures is obtained.

Spotting Procedures. Several spotting techniques were studied. The application of a single spot in the usual manner gives results illustrated in Figures 1–4 and is generally useful. When a number of very small spots are applied in a row to give more bar-shaped spots on the final chromatogram (6) results similar to those illustrated in Figures 5 and 6 are obtained. Resolution is improved and more sample can be applied, but care must be taken with the present equipment that the width of the final spot is no more than 0.90 cm since this is the diam of the aperture of the probe. A linear response between concn and integrated area is obtained when spots are uniform in shape (symmetrical and streaking forward or backward absent) and 0.9 cm or less in width.

The most precise results are obtained by alternately spotting the sample to be analyzed and standard lipid preparations so that at least three identical aliquots of the same standard mixture and the same sample appear on each plate. A minimum of two plates is used for quantitative evaluation and thus at least six independent determinations of both standards and samples are averaged to give the percentage of the individual components in the samples. This procedure minimizes variations related to differences in the uniformity of adsorbent layers, spotting, spraying and charring. Initially when a mixture of standards was desired, pure standard preparations were mixed together prior to spotting. This procedure has many short-comings and is wasteful of standard preparations. Different proportions of the same lipid classes and different dilutions are required so that many solutions of standard mixtures must be available if the different lipid classes are mixed together. If evaporation or oxidation takes place, one lipid class may be altered more than the others in a mixture and the entire mixture may have to be discarded. These problems can be avoided in part by preparing standard solutions of each lipid class separately in specially ground, glass-stoppered, graduated, centrifuge tubes (courtesy of Robert Cheatley, Lab Glass of California, Costa Mesa, Calif). No significant evaporation of solvents occurs from these tubes even after solutions are stored in them for several weeks at room temp. Instead of mixing lipid classes prior to spotting, each lipid class is spotted separately on the adsorbent. Uneven application can be avoided by spotting solutions of different lipids just above each other. The class with the lowest R_f value is spotted along the line used for the samples and the other standards desired are spotted in order of increasing R_f immediately above the preceding spots. Little or no change in R_f is observed when this procedure is compared to the usual technique of spotting all standards as a mixture to a single position.

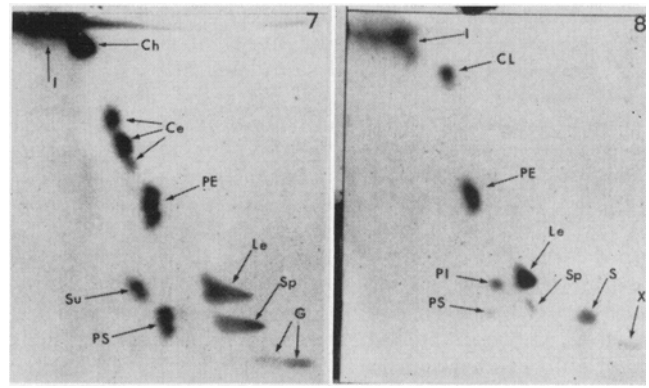

FIG. 7. Two-dimensional TLC of whole beef brain lipid (200 μg). Spotting applied at lower right corner onto a plate spread with a mixture of silicic acid and magnesium silicate (9/1). Developed with chloroform/methanol/water (65/25/4) in the first dimension (vertical), dried 10 min in air and developed with 1-butanol/acetic acid/water (60/20/20) in the second dimension. The plate was dried in air for several hr, sprayed with the sulfuric acid-potassium diehromate reagent and heated (180C). Ce, cerebrosides; Ch, cholesterol; G, gangliosides; I, impurities in adsorbent pushed to solvent front; Le, lecithin; PE, phosphatidyl ethanolamine; PS, phosphatidyl serine; Sp, sphingomyelin; and Su, sulfatides.

FIG. 8. Two-dimensional TLC of beef heart mitochondrial lipids (100 μg). Prepared as described in legend for Figure 7. CL, cardiolipin; PI, phosphatidyl inositol; S, sucrose (introduced in isolation of mitochondria); X, uncharacterized substances remaining at the point of application of the sample. Other designations as for Figure 7.

Lipid Class Standards for Quantitative TLC. The basic standard to determine a given class of lipid is a preparation of that class of lipid, but a lipid class moving in the same general area of the chromatogram can be substituted for determination of another lipid class. Thus, we have found it convenient to use monopalmitin as a substitute for a ceramide standard when determining ceramide, cerebroside as a substitute for cardiolipin, and lecithin as a substitute for either sphingomyelin, phosphatidyl inositol, phosphatidyl serine or sulfatide. These substitutions have been shown to be appropriate by direct comparison of results with both types of standards. Substitutions can be made when a chromatographic system is used in which the lipid being substituted migrates with or very near the lipid to be determined. Since the charring reaction is dependent upon carbon content,

the carbon content of the standards must be known and correction factors used where carbon content is different for standards and samples.

Adsorbents. Although, as indicated above, commercial Silica Gel G preparations are not the most useful adsorbents, they can be used for determinations of nonionic lipids (sterols, glycerides, cerebrosides) and nonacidic lipids (lecithin, sphingomyelin, phosphatidyl ethanolamine) as shown in Figures 1–4. The chief defect with such adsorbents is that spots of acidic lipids spread. Spreading of acidic lipids increases with increase in R_f and is very pronounced with cardiolipin that moves near the solvent front with chloroform/methanol/water 65/25/4 and similar solvent mixtures. Spreading is reduced when no more than 10% (by wt) $CaSO_4$ is added to silicic acid. Maximum reduction of spreading with preparations containing $CaSO_4$ is obtained using slurries of adsorbent in 0.01 M NaCl rather than water for spreading. The spreading of acidic lipid spots is almost completely eliminated when magnesium silicate is used as binder instead of $CaSO_4$ (compare Figs. 5,6). The adsorbent composed of silicic acid mixed with magnesium silicate is used routinely for this reason.

Discussion

The results presented above demonstrate clearly the value of the quantitative DEAE-TLC procedure for the determination of the lipid class composition of whole beef brain and beef heart mitochondria. The advantages and limitations of the DEAE-TLC procedure must be appreciated.

The column chromatographic methods presented previously for the quantitative determination of beef brain lipids (5,6) are primary procedures that are useful for quantitative determination, the isolation of enough lipid to be used for characterization by IR spectroscopy, hydrolysis, etc., and for the determination of fatty acid composition. These all-column procedures also provide the standards necessary for quantitative TLC. Column chromatography is easily performed in a nitrogen atmosphere to prevent changes brought about by autoxidation. This feature is particularly important in fatty acid composition studies. A distinct advantage of column chromatography lies in the ease with which the investigator can become aware of minor components of mixtures since the minor components can be concentrated in a column fraction. Column chromatography can be scaled up for the isolation of adequate amt of minor components for characterization by acceptable procedures.

The DEAE-TLC procedure has some of the advantages of the multicolumn procedures. The number of overlapping spots with TLC is diminished by prior use of column chromatography, and column chromatography is utilized for concentrating some minor components for quantitative determination by TLC. Nonlipid components of column fractions (adsorbent, water soluble nonlipids in the sample) do not interfere with quantitative TLC. The DEAE-TLC procedure is more rapid and requires smaller samples than the usual multicolumn procedures, but the DEAE-TLC procedure is not as suitable as the multicolumn proceduces for characterization of lipid classes and determination of fatty acid composition since some lipid classes are obtained as mixtures from the columns.

The DEAE-TLC procedure is very flexible. A wide range of elution schemes for DEAE can be formulated (6) depending upon the composition of the sample and the information desired. The solvents for TLC can also be varied depending upon the composition of the fractions and the standards available for comparison with sample components.

Both two-dimensional TLC and the DEAE-TLC procedure are of value for determining when direct, quantitative, one-dimensional TLC can be used without column chromatography. When TLC of DEAE fractions shows that there is no overlap of spots, TLC alone can be used for the original lipid mixture. Cholesterol, phosphatidyl ethanolamine and sulfatide of beef brain have all been determined successfully by direct TLC with the proper chromatographic systems. Table II shows the results obtained for these three lipid classes by TLC alone and by column chromatographic methods. It is apparent that the results by both methods are in close agreement.

The determination of lecithin and cardiolipin of beef heart mitochondria by one-dimensional TLC without column chromatography gives values in close agreement with those obtained by the DEAE-TLC procedure. The values for phosphatidyl ethanolamine by the two methods are different, however. DEAE column chromatography gave a value of 23.7% (phosphatidyl ethanolamine eluted with chloroform/methanol 7/3) while direct TLC gave 30.6% for total phosphatidyl ethanolamine. This difference has been traced in part to the elution of "altered" forms of phosphatidyl ethanolamine with methanol and chloroform/acetic acid 3/1 from DEAE columns following the elution of the "native" lipid with chloroform/methanol 7/3. The altered forms are not separated from the unaltered lipid by TLC. These altered forms may be autoxidation products and have always been obtained from beef heart mitochondria and have been detected in lipid extracts of whole beef heart as well (12). Since the lipids were extracted in a nitrogen atmosphere and carefully protected from air, and since the lipid preparation has remained stable for over two years, it appears that these altered forms of phosphatidyl ethanolamine are probably naturally occurring in whole beef heart and in beef heart mitochondria.

Two-dimensional TLC has also been made quantitative (13). It lacks the precision of the all-column or DEAE-TLC procedures but is very useful for rapid determinations of lipid class composition with very small samples.

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