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A MODIFIED COLUMN CHROMATOGRAPHIC METHOD FOR THE RECOVERY OF THE GLYCEROGALACTOLIPID FRACTION OF NERVE TISSUE

SOME OBSERVATIONS ON THE FRACTIONATION OF NERVE TISSUE GLYCOLIPIDS ON SILICIC ACID WITH CHLOROFORM AND ACETONE MIXTURES

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SUMMARY

The elution properties of some chloroform and acetone mixtures were investigated in relation to the recovery of minor glycolipids from total lipid extracts of nerve tissue chromatographed on silicic acid.

Initially, small columns were eluted first with chloroform alone to remove the neutral lipids and sterols and then with chloroform containing 5, 10, 20, 25, 33 and 50% acetone. Fractions were analysed qualitatively by thin-layer chromatography. Glycerogalactolipids were eluted with chloroform containing from 10–25% acetone while chloroform containing 33% acetone resolved kerasin from phrenosine.

Larger scale columns were investigated quantitatively with lipid extracts from bovine, sheep and human brain tissue. A minor fraction, which accounted for between 0.9 and 1.6% of the total lipid, was eluted with chloroform containing 25% acetone. The total glycerogalactolipid component was recovered in this fraction but other minor glycolipids were incompletely resolved.

Some variation in the properties of commercial preparations of silicic acid were detected with respect to the elution of minor and major glycolipids. Chromatography of total lipid extracts at two different lipid–adsorbent loading ratios had no significant effect on the resolution and recovery of minor glycolipids.

INTRODUCTION

A column chromatographic method has been described[1] as part of a procedure for the purification of the glycerogalactolipid fraction of nerve tissue. The method involved column chromatography of the acetone-soluble lipids of brain tissue on silicic acid and the recovery of a minor glycolipid fraction by elution with a 2:1, v/v,

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mixture of chloroform and acetone. The glycerogalactolipid component was subsequently purified from this fraction by thin-layer chromatography (TLC).

Chromatography of the acetone-soluble lipids, prepared from a total lipid extract of nerve tissue by precipitation of phosphatides with acetone, allowed the use of a high lipid loading ratio on silicic acid columns and it has been reported[2] that only traces of the glycerogalactolipid are lost during preparation of acetone-soluble lipid extracts by this method. However, more recent work in this laboratory has indicated that significant levels of minor glycolipids can be recovered from the acetone-insoluble lipid fraction. Therefore, to avoid any loss of minor glycolipids, total lipid extracts are now used in the chromatographic method for the preparation of glycerogalactolipids. This change necessitated a reinvestigation of the elution properties of the chloroform and acetone mixtures as it was found that elution of the total lipid extracts of nerve tissue on silicic acid with the chloroform–acetone (2:1) mixture used previously to recover minor glycolipids, now gave a fraction contaminated with cerebroside.

This report gives an account of the development of the method now in use. Fractions recovered by elution of total lipid extracts of brain tissue on silicic acid with chloroform, chloroform–acetone mixtures, acetone and methanol have been analysed quantitatively and these results are discussed in relation to the elution of minor and major glycolipid classes. The properties of three commercially available brands of silicic acid used in the method are compared and lipid extracts have been chromatographed at two different lipid: adsorbent loading ratios. A brief account of the modified method used to recover glycerogalactolipids from total lipid extracts of nerve tissue has already appeared[3].

EXPERIMENTAL

Preparation of lipid extracts

Whole fresh brain tissue from sheep and ox was kept on ice throughout. The tissue was cleaned of blood vessels, meninges, etc., and was weighed and then homogenised in a Waring Blendor with small volumes of distilled water. The homogenate was then lyophilised. Portions of cerebellum and hemispheres from human brain were treated similarly.

The freeze-dried brain tissue was then extracted with 50 ml/g chloroform–methanol (2:1) by homogenisation for 5 min at room temperature. The extract was filtered and the residue reextracted twice more with fresh solvent. Finally the clear extracts were pooled and solvent removed under vacuum below 50°. Proteolipid was disrupted by repeated addition and removal of the extraction solvent. Finally the lipid was dissolved in chloroform–methanol (2:1), filtered and partitioned against 0.1 *M* KCl. After standing overnight at 4° the lower chloroform layer was recovered, solvent removed and the total lipid extract dried to constant weight under vacuum at 4°.

Solvents

Chloroform was reagent grade (Merck) and contained 0.5–1.0% ethanol as stabiliser. Acetone and methanol were analytical grade (Merck) and contained 0.2 and 0.05% water, respectively. All solvent mixtures are v/v.

Column chromatography

For routine column chromatography Mallinckrodt silicic acid (100 mesh, A.R.), activated at 110° for 15 h, was used. Unisil silicic acid (100–200 mesh, Clarkson Chemical Co.) and Bio-Rad silicic acid (200–325 mesh, CalBiochem), without additional activation, were also used under similar elution conditions.

A loading factor of 1 g lipid to 20 g adsorbent was used previously in the chromatography of acetone-soluble lipid extracts of brain on silicic acid[1]. However, for the chromatography of total lipid extracts a lower ratio of 1:40, as suggested by HANAHAN[4], was used because of the greater concentrations of polar lipids present. Lipid extracts have also been chromatographed at the lower ratio of 1 g lipid to 80 g adsorbent, as described by ROUSER *et al.*[5].

Initially the elution properties of several chloroform and acetone mixtures were investigated qualitatively and, for this purpose, small columns of Mallinckrodt silicic acid (10 g), packed in chloroform and giving an adsorbent bed about 6 × 2 cm diameter, were prepared. Total lipid extract (200 mg) from beef or sheep brain was applied in chloroform and the column was then eluted with chloroform (25 column volumes) followed by chloroform–acetone (19:1 and 9:1) (10 column volumes), chloroform–acetone (4:1, 3:1, 2:1 and 1:1) (20 column volumes). Fractions were collected in bulk, solvent removed and the lipid analysed by TLC on Silica Gel H in a solvent system of chloroform–methanol (185:15)[6]. Lipids were visualised by sulphuric acid charring and were identified by reference to standards. Columns prepared with Unisil and Bio-Rad silicic acids were eluted in the same way.

Larger scale columns, supporting 1 g of total lipid, were investigated quantitatively. For such separations 40 g silicic acid was slurried in chloroform and packed into a chromatography tube 60 × 4 cm I.D. fitted with a sintered glass disc and teflon stop-cock. The resulting adsorbent bed (approx. 6 × 4 cm) was then washed with two column volumes of chloroform and 1 g of total brain lipid, in chloroform, was carefully applied and allowed to run slowly into the adsorbent. The lipid was washed into the silicic acid with further small volumes of chloroform and the column then eluted in a discontinuous gradient, at room temperature, with the solvents and their respective volumes shown in Table I. A flow rate of about 3–4 ml/min was maintained.

TABLE I

ELUTING SOLVENTS AND SOLVENT VOLUMES FOR THE FRACTIONATION OF THE TOTAL LIPID EXTRACTS OF BRAIN TISSUE (1 g) ON SILICIC ACID COLUMNS (40 g)

Fraction	*Eluting solvent*[a]	*Column volumes*[b]
1	Chloroform	25
2	Chloroform–acetone (19:1)	5
3	Chloroform–acetone (3:1)	15
4	Chloroform–acetone (1:1)	25
5	Acetone	25
6	Methanol	25

[a] Solvent mixtures v/v.
[b] Column volume approx. 40 ml.

Analysis of fractions

Fractions were collected in bulk, solvent removed in a rotary evaporator below 50° and the lipid weighed after storage under vacuum at 4° over KOH pellets for at least 12 h. To remove any suspended silicic acid eluted from the columns, fractions 4, 5 and 6 were always filtered prior to gravimetric analysis. After weighing the fractions were then dissolved in chloroform or chloroform–methanol (4:1) to known volumes and aliquots analysed for lipid phosphorus[7] and sterol[8].

Suitable aliquots were also analysed for total lipid hexose after the hydrolysis of the glycolipids with methanol (0.5 ml) and 3 N H_2SO_4 (2 ml) at 100° for 2½ h in sealed tubes. Inconsistent results have been reported[9] in connection with the hydrolysis of glycolipids with aqueous acid. The method used in this study was essentially that described by SVENNERHOLM[10] with initial solubilisation of the lipid in methanol by warming followed by addition of acid. Hydrolyses were always made in duplicate and good agreement obtained. Blanks of methanol (0.5 ml) and acid (2 ml), in sealed tubes, were always taken through the complete procedure. Absolutely clean glassware is essential for reproducible results and hydrolysis tubes were cleaned by soaking in chromic acid for 24 h followed by thorough washing and rinsing with distilled water.

After hydrolysis the tubes were opened and the contents filtered through pre-washed glass-fibre paper. The tubes were rinsed several times with distilled water and the washings filtered and added to the hydrolysates which were then made up to volume. Aliquots were taken in triplicate for hexose determination by the phenol–sulphuric acid method[11]. Samples were read against distilled water blanks as hydrolysis blanks (methanol and acid) gave a slight but consistent colour. This latter value was measured and deducted where appropriate. Galactose (B.D.H.) in 3 N H_2SO_4 was used as standard. It has been claimed[12] that glycerol interferes in the determination of hexose by the phenol–sulphuric acid method. However, ROUGHAN AND BATT[13] reported that glycerol (200 μg) gave no detectable response by this method but that some interference can arise from the reaction of unsaturated fatty acids with phenol and sulphuric acid.

Lipid components in the fractions were resolved by TLC on Silica Gel H in solvent systems of C/M (185:15)[6] which separated neutral lipids, sterols, minor glycolipids, and kerasin and phrenosine; and in chloroform–methanol–water (65:25:4) which resolved the more polar lipids. Charring with 20% sulphuric acid was again used to detect the lipids which were identified by reference to standards chromatographed under similar conditions.

RESULTS AND DISCUSSION

Until recently the use of acetone in the chromatographic fractionation of glycolipids on silicic acid had not been investigated in any detail. Earlier, SMITH AND FREEMAN[14] had recovered a cerebroside fraction from milk phosphatides on silicic acid by elution with acetone while, more recently, the fractionation of bacterial lipids on silicic acid and recovery of glycosyl diglycerides by elution with chloroform–acetone (1:1) and acetone has been described[15,23]. The quantitative recovery of plant galactolipids and sulpholipids and brain cerebrosides and sulphatides with acetone from silicic acid has been discussed[16]. A minor glycolipid fraction, free of cerebroside, has also been recovered from acetone-soluble lipid extracts of brain tissue on silicic

acid by elution with chloroform containing 33% acetone[1]. Recently experimental details for the chromatagraphy of lipids on silicic acid, including elution with acetone, have been presented[5].

In connection with further studies on the glycerogalactolipid fraction of nerve tissue it became necessary to investigate more thoroughly the elution properties of a range of chloroform–acetone mixtures in relation to the recovery of minor glycolipids from the total lipid extracts of nerve tissue. Silicic acid was chosen as the adsorbent as it had been used previously[1] and small columns of this adsorbent supporting 200 mg of total lipid were used initially to investigate the behaviour of a range of chloroform–acetone mixtures and elution with the preliminary solvent sequence described resulted in the lipid distribution shown in Fig. 1. Corresponding fractions from lipid extracts chromatographed on the different silicic acid preparations had the same qualitative lipid composition as revealed by TLC.

Initially chloroform was used to remove most of the sterol and neutral lipid and this fraction was discarded. A minor fraction was then recovered, by elution

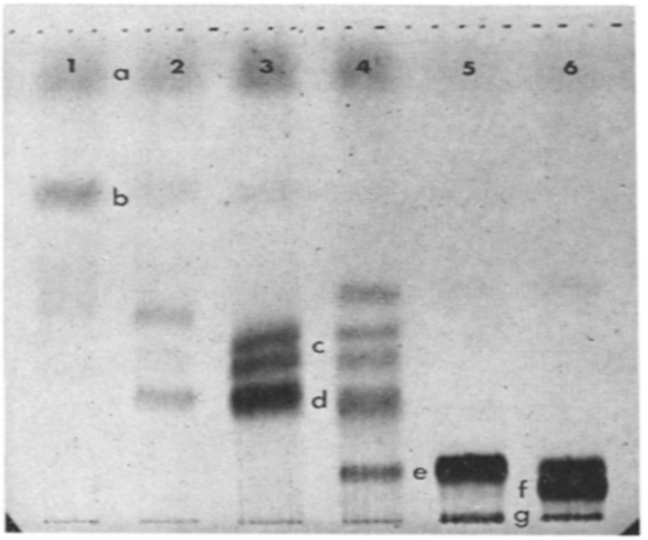

Fig. 1. TLC analysis of fractions recovered from total lipid extracts of sheep brain (200 mg) on Mallinckrodt silicic acid (10 g) by elution with: 1 = chloroform–acetone (19:1); 2 = chloroform–acetone (9:1); 3 = chloroform–acetone (4:1); 4 = chloroform–acetone (3:1); 5 = chloroform–acetone (2:1); 6 = chloroform–acetone (1:1). Lipids identified as: a = neutral lipids; b = sterol; c = minor glycolipids; d = glycerogalactolipids; e = kerasin; f = phrenosine; g = sulphatide at origin. Adsorbent: Silica Gel H. Solvent system, chloroform–methanol (185:15). Sufficient lipid applied to allow detection of minor components.

with chloroform containing 5% acetone, which consisted chiefly of residual cholesterol and neutral lipids (Fig. 1). Although much of the remaining sterol was eluted with this solvent both cholesterol and neutral lipids (di- and triglycerides) were detected in later fractions.

Minor glycolipids of nerve tissue were mainly eluted in three fractions with chloroform containing from 10 to 25% acetone. The major proportion of the glycerogalactolipid component was recovered by elution with chloroform–acetone (4:1) (Fig. 1). However, minor glycolipids were always detected in the chloroform–acetone 3:1 fraction (Fig. 1) and this solvent appeared to effect the complete recovery of

glycerogalactolipids from the adsorbent as this component was not detected subsequently. Low concentrations of other minor glycolipids were eluted with chloroform–acetone (2:1 and 1:1). Significant levels of kerasin were also detected in the chloroform–acetone (3:1) fraction under the elution conditions described.

Elution with larger volumes of chloroform–acetone (9:1) did not increase the recovery of glycerogalactolipid fraction and, in view of the elution of this lipid fraction over such a wide concentration range, it is possible that the less polar alkyl acyl glycerogalactoside component of the fraction[1,3,17] is resolved from the diacyl analogue and is eluted with chloroform containing a lower concentration of acetone. This possibility is being investigated further.

Chloroform–acetone (2:1) has been used previously to recover a cerebroside-free minor glycolipid fraction from acetone-soluble lipid extracts of sheep brain on silicic acid[1]. However, elution of total lipid extracts with this solvent mixture resulted in the effective resolution of the two cerebroside components observed on TLC and a fraction containing the faster migrating cerebroside component, identified as kerasin by reference to standards, was recovered (Fig. 1). The resolution of kerasin and phrenosine by chromatography of crude glycolipid[18] or total lipid[19] extracts on silicic acid has been described using gradient elution with increasing concentrations of methanol in chloroform. Such separations have not been described previously with chloroform and acetone mixtures. However, recovery of kerasin was not quantitative under the conditions described and this cerebroside was detected in later fractions in low concentration (Fig. 1). Nevertheless, these findings provide the basis for a

TABLE II

DISTRIBUTION OF LIPID[a] IN FRACTIONS FROM TOTAL LIPID EXTRACTS OF BRAIN (1 g) CHROMATOGRAPHED ON MALLINCKRODT SILICIC ACID (40 g) AND ELUTED WITH THE SOLVENTS AND VOLUMES GIVEN IN TABLE I

Fraction	*Sheep*		*Bovine*		*Human*	
	1	*2*	*1*	*2*	*1*	*2*
1	24.4	24.5	23.9	25.4	28.0	25.8
2	0.8	0.5	0.9	0.5	0.3	0.3
3	1.0	1.6	1.5	1.1	1.5	1.4
4	11.8	12.9	9.8	8.5	11.4	11.6
5	11.0	9.5	9.3	8.8	10.6	9.7
6	51.9	51.8	54.6	55.7	48.7	51.1

[a] As % of total lipid applied.

preparative technique for the recovery of kerasin from total lipid extracts on silicic acid and the separation is achieved without the need for gradient elution apparatus.

Chloroform containing 50% acetone eluted a fraction which consisted mainly of cerebroside (Fig. 1). Phrenosine was the main component detected and low concentrations of kerasin, eluted chiefly in the previous fraction, were also present.

These preliminary observations suggested that recovery of glycerogalactolipids was incomplete following elution with chloroform–acetone (9:1 and 4:1), but that the bulk of this minor glycolipid component could probably be recovered, to-

BLE III

D HEXOSE[a], STEROL[b], LIPID PHOSPHORUS[c] CONTENT OF THE FRACTIONS DETAILED IN TABLE II

Sheep						Human						Bovine					
Lipid hexose		*Sterol*		*Lipid phosphorus*		*Lipid hexose*		*Sterol*		*Lipid phosphorus*		*Lipid hexose*		*Sterol*		*Lipid phosphorus*	
1	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
—[d]	—	99.1	99.8	—	—	—	—	99.6	99.5	—	—	—	—	98.9	97.92	—	—
0.1	0.2	0.7	0.1	0.3	0.2	—	—	0.1	0.1	nd[e]	nd	—	0.2	0.9	2.06	0.1	0.1
2.2	2.1	0.2	0.1	0.4	0.5	1.4	1.0	0.3	0.4	nd	0.3	2.1	1.6	0.2	0.02	0.9	0.4
42.5	45.2	—	—	1.2	1.1	49.7	49.2	—	—	0.8	0.65	33.2	29.8	—	—	0.6	1.6
29.2	29.5	—	—	0.2	0.4	23.2	25.6	—	—	0.6	0.55	27.9	31.8	—	—	0.3	0.2
26.0	23.0	—	—	97.9	97.8	25.7	24.2	—	—	98.6	98.5	36.8	36.6	—	—	98.1	97.7

[a] As % total lipid hexose recovered.
[b] As % total sterol recovered.
[c] As % total lipid phosphorus recovered.
[d] — not detected.
[e] nd = not determined.

gether with other minor glycolipids and some cerebroside, by elution with chloroform containing 25% acetone. None of the mixtures of chloroform and acetone used initially resolved glycerogalactolipids free from other glycolipids. Accordingly chloroform–acetone (3:1) was included in an overall elution scheme in order to study the resolution of the minor glycolipids from total lipid extracts of nerve tissue on silicic acid. Chloroform has been widely used to resolve neutral and polar lipid classes on silicic acid and, therefore, elution with this solvent and with chloroform containing 5% acetone, was used initially to remove neutral lipid and cholesterol prior to recovery of minor glycolipids. The elution of cerebrosides and sulphatides from three commercial preparations of silicic acid was also studied using chloroform–acetone (1:1) and acetone, as described by ROUSER *et al.*[5] at two lipid–adsorbent loading ratios. Methanol was used finally to clear the column of polar lipids. The overall elution scheme is shown in Table I and details of lipid recovery following chromatography of lipid extracts on Mallinckrodt silicic acid with this elution scheme are recorded in Table II. Results of the lipid hexose, lipid phosphorus and sterol content of these fractions are summarised in Table III. Lipid extracts of sheep brain were also fractionated on Unisil and Bio-Rad silicic acid under similar elution conditions and the lipid distribution and lipid hexose content of the fractions is given in Table IV. Analysis of the fractions from one column by thin-layer chromatography is shown in Fig. 2 and corresponding fractions from the different columns all had similar lipid patterns.

Chloroform eluted between 97 and 99% of the total sterols from the lipid extracts of nerve tissue chromatographed on silicic acid (Table III). The other main components of this fraction were tri- and diglycerides and free fatty acid (Fig. 2). Elution with larger volumes of chloroform did not significantly increase the recovery of sterol or neutral lipids and low concentrations of these lipids remained on the columns and were largely recovered by elution with chloroform containing 5%

TABLE IV

DISTRIBUTION OF LIPID AND LIPID HEXOSE[a] IN FRACTIONS FROM TOTAL LIPID EXTRACTS OF SHEEP BRAIN (1 g) CHROMATOGRAPHED ON UNISIL AND BIO-RAD SILICIC ACID (40 g) AND ELUTED WITH THE SOLVENTS AND VOLUMES GIVEN IN TABLE I

Fraction	*Unisil*		*Bio-Rad*	
	% of total lipid applied	*Lipid hexose*	*% of total lipid applied*	*Lipid hexose*
1	23.1	—[b]	24.0	—
2	0.2	—	0.1	—
3	0.9	2.1	1.1	2.5
4	8.8	34.3	9.2	32.8
5	14.0	34.0	12.6	29.0
6	53.0	29.6	53.0	35.7

[a] As % total lipid hexose recovered.
[b] — not detected.

acetone (Table III). Lipid hexose was detected in low concentration in fraction 2 eluted from Mallinckrodt silicic acid (Table III) but was absent in corresponding fractions recovered from Unisil and Bio-Rad columns (Table IV).

A minor fraction which accounted for only 0.9–1.6% of the total lipids was recovered by elution with chloroform containing 25% acetone (Tables II, IV). The glycerogalactolipid component of nerve tissue was completely recovered in this fraction from lipid extracts chromatographed on the three different silicic acid preparations (Fig. 2). Elution with this solvent mixture therefore provides a useful

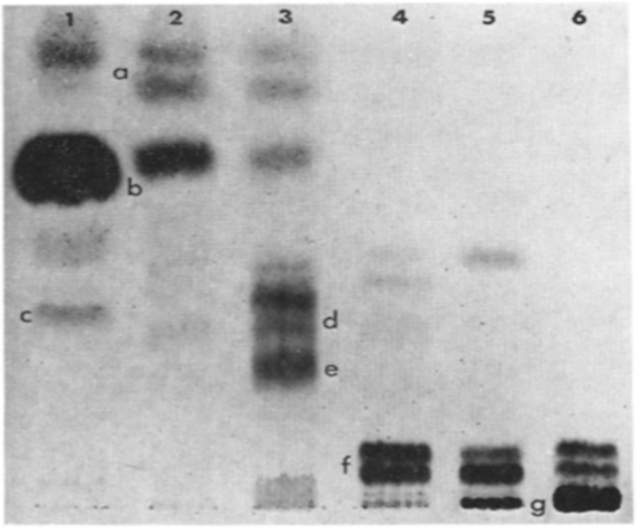

Fig. 2. TLC analysis of fractions recovered from total lipid extracts of sheep brain (1 g) on Mallinckrodt silicic acid (40 g) by elution with: 1 = chloroform; 2 = chloroform–acetone (19:1); 3 = chloroform–acetone (3:1); 4 = chloroform–acetone (1:1); 5 = acetone; 6 = methanol. Lipids identified as: a = neutral lipids; b = cholesterol; c = free fatty acid; d = minor glycolipids; e = glycerogalactolipids; f = cerebrosides; g = sulphatide and phospholipids at origin. Adsorbent: Silica Gel H. Solvent system, chloroform–methanol (185:15). Sufficient lipid applied to allow detection of minor components.

TABLE V

LIPID HEXOSE[a] CONTENT OF FRACTIONS FROM TOTAL LIPID EXTRACTS CHROMATOGRAPHED ON SILICIC ACID AT A LOADING RATIO OF 1 g LIPID: 80 g ADSORBENT, AND ELUTED WITH THE SOLVENTS DESCRIBED IN TABLE I PLUS AN ADDITIONAL 25 COLUMN VOLUMES OF ACETONE

Fraction	*Mallinckrodt*	*Unisil*	*Bio-Rad*
	Lipid hexose[b]	*Lipid hexose*[c]	*Lipid hexose*[b]
1	—[d]	—	—
2	—	—	—
3	2.1	2.0	2.3
4	41.0	28.9	27.3
5a	33.4	33.2	28.5
5b	7.6	7.7	8.7
6	15.9	28.2	33.2

[a] As % total lipid hexose.
[b] Bovine brain lipid.
[c] Sheep brain lipid.
[d] — not detected.

system for the recovery of a minor fraction from which the total glycerogalactolipid component can be isolated for further study[3].

Minor glycolipids, other than glycerogalactolipids, were incompletely eluted with chloroform–acetone (3:1) from Mallinckrodt silicic acid and were detected in low concentrations in later fractions (Fig. 2). Thus, with this adsorbent, for the recovery of total minor glycolipids, elution with acetone followed by separation from major glycolipids by rechromatography on silicic acid or TLC is advised. A more complete recovery of the total of minor glycolipids in fraction 3 was obtained from Unisil and Bio-Rad columns, as judged by the very slight and occasional traces of these components detected in subsequent fractions; and, as lipid hexose was not found in fraction 2 (Table IV), chromatography of the total lipid extracts on either of these adsorbent preparations is more suited to the recovery of the complete minor glycolipid fraction by elution with chloroform–acetone (3:1). Contrary to preliminary observations cerebroside was not detected in fraction 3 eluted from the larger scale columns under the conditions described but fractionation with increased volumes of chloroform–acetone (3:1) might result in the elution of kerasin.

Cerebrosides were the main components recovered on elution with chloroform–acetone (1:1) and, in agreement with other reports[5], only faint traces of sulphatide were detected in this fraction (Fig. 2). Analysis (Tables II–IV) revealed that the levels of total lipid and lipid hexose recovered in this fraction from Unisil and Bio-Rad columns were lower than in the corresponding fractions from Mallinckrodt silicic acid and such results probably arise from differences in the adsorptive properties of the various silicic acid preparations caused by variations in particle size, purity and degree of activation. Fraction 4, recovered from lipid extracts of bovine brain chromatographed on Mallinckrodt silicic acid, also contained lower proportions of total lipid and lipid hexose than were found in the corresponding fractions from sheep and human brain extracts eluted from the same adsorbent (Tables II, III) and these

observations suggest that cerebrosides from various animal species have slightly different chromatographic properties on silicic acid which can probably be accounted for by differences in their structure and fatty acid composition.

Sulphatide was eluted with acetone as found by other workers[16] and cerebroside was also detected in this fraction (Fig. 2). Only very low concentrations of lipid phosphorus were eluted with acetone (Table III). Lipid remaining on the columns was then eluted with methanol and between 97.7 and 98.6% of the total lipid phosphorus was recovered. The methanol fraction also contained between 23 and 37% of the total lipid hexose (Tables III, IV) and the detection of cerebroside (Fig. 2) in this fraction was unexpected in view of reports on the quantitative elution of glycolipids from silicic acid with acetone[16]. However, rather different experimental conditions were used in the present study. Total lipid recovery from columns was of the order of 98–101%.

Fractionation of total lipid extracts at a lower lipid:adsorbent ratio of 1:80 did not improve the resolution of the minor glycolipids and elution with chloroform–acetone (3:1) resulted in the recovery of lipid hexose in proportions similar to those eluted at the higher lipid loading (Table V). The glycerogalactolipid component was again completely eluted in fraction 3 and the other minor glycolipids were recovered more completely in this fraction from the Unisil and Bio-Rad silicic acid columns than from the Mallinckrodt columns, as found before. Therefore, for the recovery of minor glycolipids, chromatography of lipid extracts at the higher loading ratio of 1 g lipid/40 g adsorbent is preferred and is used in current studies as this allows the fractionation of greater concentrations of lipid material.

Differences in the elution of major glycolipids from total lipid extracts chromatographed at the two different loading ratios were most apparent in fractions recovered from the Mallinckrodt silicic acid column (Table V) but, as this study was mainly concerned with the elution of minor glycolipids, these findings were not investigated further. However, it was clear from the results (Table V) that increased elution with acetone, even at the lower lipid loading, did not result in the complete recovery of glycolipids from silicic acid as has been reported[16] and, always, under the chromatographic conditions described, appreciable amounts of lipid hexose (from cerebroside) could be subsequently recovered from the columns by elution with methanol. In this connection the quantitative recovery of glycolipids from total lipid extracts on silicic acid by elution with a tetrahydrofuran–methylal–methanol–water solvent mixture has recently been reported[20]. However, minor glycolipids, and especially the glycerogalactolipid component recovered by elution with this solvent would be degraded during the mild alkaline saponification procedure used to remove phospholipid contamination from the major glycolipids. Chloroform containing 20% methanol has been widely used to elute major glycolipids from silicic acid[21], while KISHIMOTO AND RADIN[22] have reported that cerebrosides and sulphatides are eluted from Unisil silicic acid with chloroform–methanol (94:6 and 85:15), respectively.

Minor glycolipids have previously been recovered from lipid extracts of nerve tissue on silicic acid by elution with chloroform containing low concentrations of methanol[17] while resolution of the individual minor glycolipids on silicic acid with chloroform and methanol in a continuous elution system has also been described[24]. In the discontinuous gradient elution scheme described above mixtures of chloroform and acetone are preferred since, being less polar than methanol, larger volumes of

acetone are needed in the mixtures with chloroform to achieve the same elution properties. With this system the results described above indicate that, following elution of neutral lipid and sterol with chloroform and chloroform containing 5% acetone, the total glycerogalactolipid component together with the bulk of the other minor glycolipids can be recovered from the total lipid extracts of nerve tissue on silicic acid by elution with chloroform containing 25% acetone. This procedure is being used currently to study the distribution of the glycerogalactolipids and other minor glycolipids in membrane fractions isolated from nerve tissue.

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