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DEAE-SILICA GEL AND DEAE-CONTROLLED POROUS GLASS AS ION EXCHANGERS FOR ISOLATION OF GLYCOLIPIDS

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SUMMARY

DEAE-silica gel and DEAE-controlled porous glass have been used for the quantitative isolation of gangliosides and neutral glycosphingolipids from animal tissues and cells. A direct comparative study between DEAE-silica gel, DEAE-controlled porous glass and DEAE-Sephadex was made; the results indicated that DEAE-silica gel is preferable to the other two ion exchangers. DEAE-silica gel has also been found to be suitable for the fractionation of ganglioside mixtures.

INTRODUCTION

A variety of methods has been reported for the isolation and purification of gangliosides and neutral glycosphingolipids (GSL)[1–3]. In many cases, column chromatographic procedures utilizing alumina[4,5], silicic acid[6–8], Florisil[9–13], Unisil[14,15], Anasil S[16–18] and Iatrobeads[19–21] were used for preparative separation of glycolipids. The application of DEAE-cellulose and TEAE-cellulose have also been described[1,22,23]. More recently, DEAE-Sephadex has been routinely used as an ion exchanger for the quantitative separation of gangliosides and neutral GSL from animal tissues and cells[1,15,24–26]. This procedure gives nearly quantitative (*ca.* 94%) yields of total gangliosides without selective loss of the less polar constituents which is often a problem in the classical partitioning method[27,28]. In recent communications[29,30] we described the preparation of a new ion exchanger "DEAE-silica gel" from commercially available materials and its application to the quantitative isolation of total gangliosides and neutral GSL from beef brain and human erythrocytes.

In this paper we report the utilization of DEAE-silica gel for the fractionation of total ganglioside mixtures. We have also used DEAE-controlled porous glass (CPG) for the isolation of gangliosides and neutral GSL. A comparative study of DEAE-silica gel, DEAE-CPG and DEAE-Sephadex was made; the results indicated that DEAE-silica gel is preferable to the other two ion exchangers.

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MATERIALS AND METHODS

DEAE-silica gel (total capacity, 140 μmol/g dry weight) was prepared by treating porous silica gel (pore diameter, 200 nm; 120–200 mesh; surface area, 150 m^2/g; pore volume, 1.68 cm^3/g) with γ-glycidoxypropyltrimethoxysilane and N,N-diethylethanolamine as described previously[30]. DEAE-Sephadex A-25 was purchased from Pharmacia (Piscataway, N.J., U.S.A.). DEAE-CPG (pore diameter, 170 nm; total capacity, 200 μmol/g dry weight; surface area, 140 m^2/g; pore volume, 0.80 cm^3/g) and DEAE-CPG (pore diameter, 500 nm; total capacity, 60 μmol/g dry weight; surface area, 50 m^2/g; pore volume, 1.00 cm^3/g) were obtained from Electronucleonics (Fairfield, N.J., U.S.A.). DEAE-silica gel, DEAE-CPG and DEAE-Sephadex were converted into the acetate forms by shaking with chloroform–methanol–0.8 *M* sodium acetate in methanol (30:60:8) or 0.2 *M* sodium acetate in methanol as described[30]. The purified gangliosides and neutral GSL used as standard compounds have been described previously[26,31]. The lipid-bound sialic acid was determined by gas–liquid chromatography (GLC)[32] and total hexose by phenol–sulfuric acid reaction[33]. Quantification of individual GSL was carried out as described previously[26,34]. α-Naphthol and resorcinol reagents[22,35] were utilized to detect neutral GSL and gangliosides, respectively.

Isolation of gangliosides and neutral GSL from beef brain and human erythrocytes

The gangliosides and neutral GSL from beef brain and human erythrocytes were isolated by use of DEAE-silica gel, DEAE-CPG and DEAE-Sephadex according to the procedure already described[30].

Fractionation of beef brain and human erythrocyte ganglioside mixtures using gradient chromatography

The beef brain and human erythrocyte ganglioside mixtures were fractionated by DEAE-silica gel and DEAE-Sephadex into individual ganglioside species using a linear gradient of ammonium acetate in methanol[20].

RESULTS AND DISCUSSION

The yields of the lipid-bound sialic acid from beef brain and human erythrocytes by use of DEAE-CPG, DEAE-silica gel and DEAE-Sephadex were quite similar and the results agree very well with our previous data[30]. The thin-layer chromatographic (TLC) patterns of gangliosides from beef brain and human erythrocytes were also identical as shown in Figs. 1 and 2, respectively. The total hexose analysis of the neutral GSL fractions from beef brain and human erythrocytes by the three ion exchangers were also in excellent agreement with our previous results[30,36]. The TLC of the neutral GSL from beef brain showed mostly cerebrosides[20,37] (not shown here), whereas the neutral GSL from human erythrocytes showed increased amounts of higher oligoglycosyl ceramides as expected (Fig. 3)[3,30]. Analysis of individual GSL, isolated as their acetates by preparative TLC of the acetylated erythrocyte GSL fractions, showed similar patterns of distribution (Table I) as previously described by us[26,30,38].

The recovery of gangliosides from DEAE-CPG, DEAE-silica gel and DEAE-

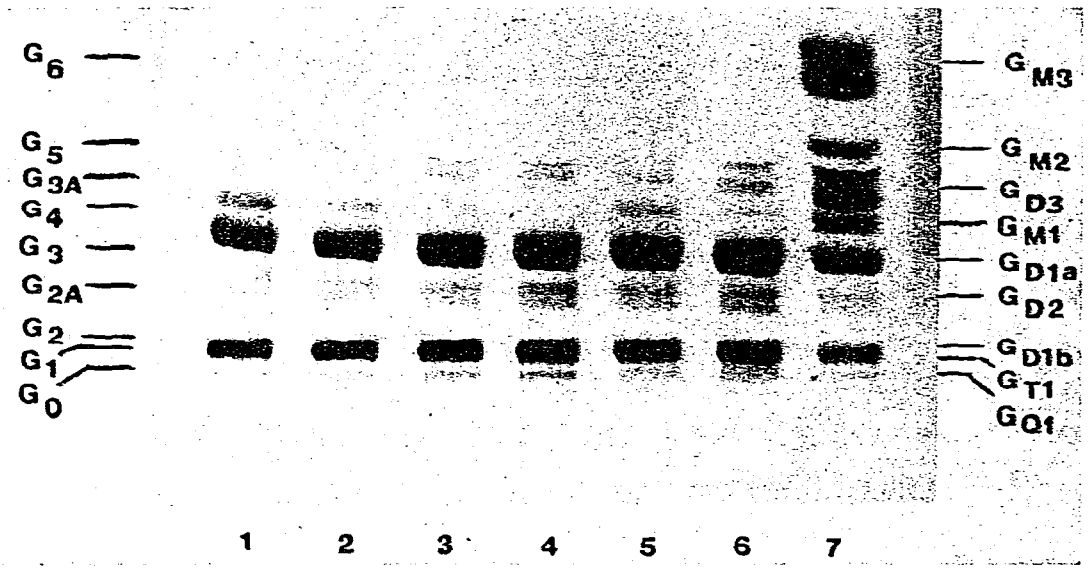

Fig. 1. TLC of BBG. (1) and (2) standard BBG; (3) by DEAE-CPG, 170 nm; (4) by DEAE-CPG, 500 nm; (5) by DEAE-silica gel; (6) by DEAE-Sephadex; (7) standards containing BBG, G_{M3} (= G_6) and G_{M2} (= G_5). Each of the lanes 2–6 contained 10–11 μg sialic acid. A precoated plate of silica gel 60 (E. Merck), 250 μm thick, was activated at 110° for 45 min before spotting. Solvent system: chloroform–methanol–2.5 *M* ammonium hydroxide (60:40:9). All bands were purple after detection by resorcinol spray[35]. Ganglioside nomenclatures of Korey and Gonatas[40] and Svennerholm[41] are depicted on left and right of this figure, respectively.

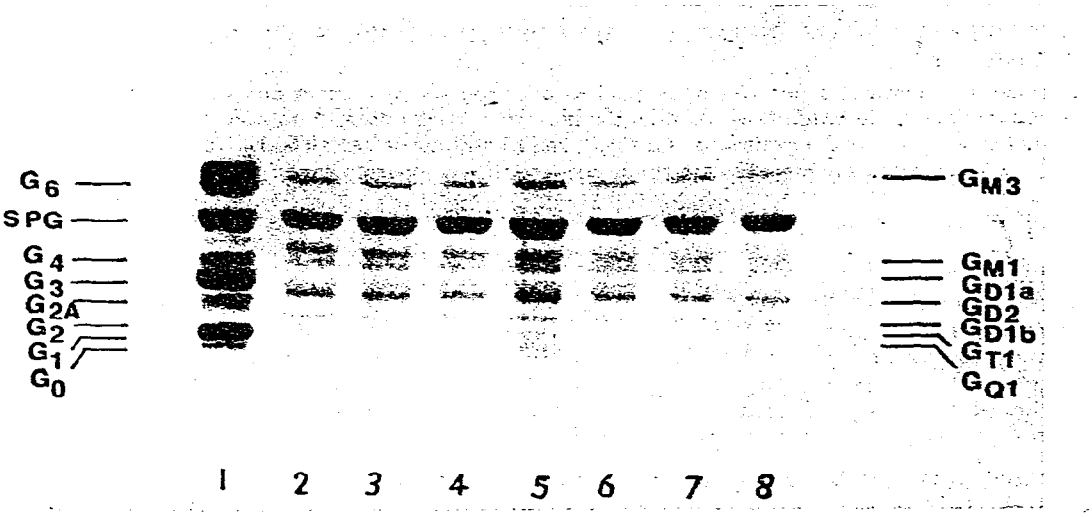

Fig. 2. TLC of gangliosides from human erythrocytes. (1) Standards containing BBG, G_{M3} (= G_6) and sialosylparagloboside (SPG); (2) and (6) by DEAE-silica gel; (3) and (7) by DEAE-CPG, 170 nm; (4) and (8) by DEAE-CPG, 500 nm; (5) by DEAE-Sephadex. Each of the lanes 2–8 contained 9–10 μg sialic acid. Other details as described in Fig. 1.

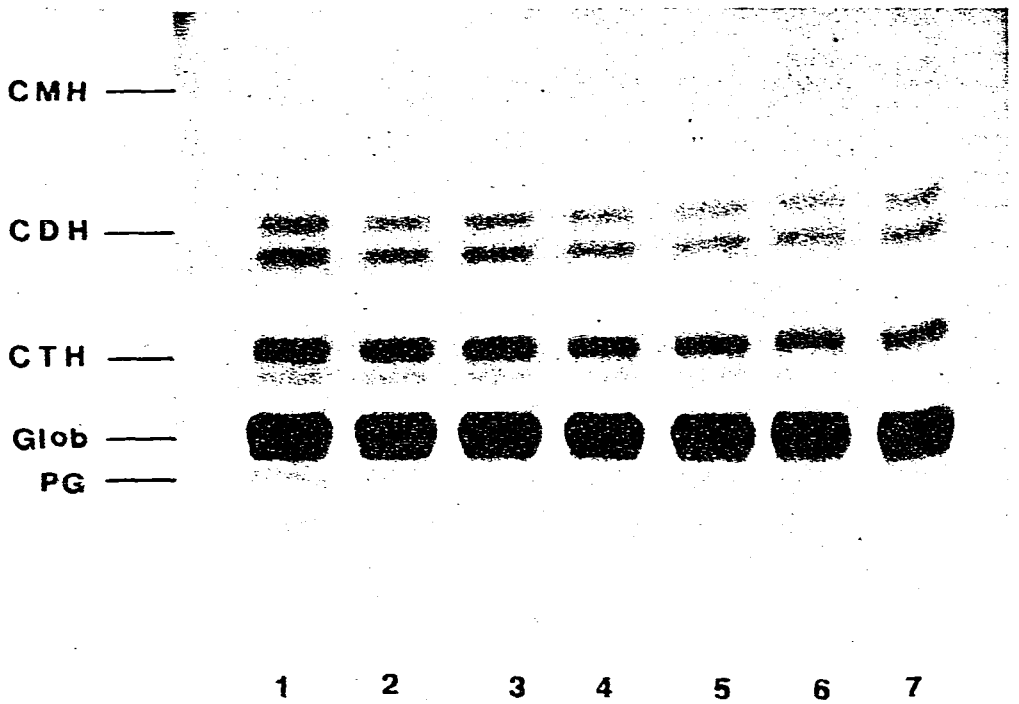

Fig. 3. TLC of GSL from human erythrocytes. (1) By DEAE-Sephadex; (2) and (3) by DEAE-silica gel; (4) and (5) by DEAE-CPG, 170 nm; 6 and (7) by DEAE-CPG, 500 nm. Each lane contained 100–110 μg GSL. Solvent system: chloroform–methanol–water (60:30:5). All bands were purple after detection by α-naphthol spray[22]. GSL nomenclature: CMH = glucosyl ceramide; CDH = lactosyl ceramide; CTH = ceramide trihexoside; Glob = globoside; PG = paragloboside.

TABLE I

CONCENTRATION OF NEUTRAL GLYCOSPHINGOLIPIDS IN NORMAL HUMAN ERYTHROCYTES

Expressed as micromoles per 100 ml of packed erythrocytes of a male donor, age 25 years. The concentration of α-naphthol-positive minor bands more polar than paragloboside was not determined. The values were determined by GLC[26,34] and quantified as described by Vance and Sweeley[14]. The values presented in this table are the means from two separate experiments.

Glycolipid	*DEAE-Silica gel*	*DEAE-Controlled porous glass*		*DEAE-Sephadex*
		170 nm	*500 nm*	
Glugosyl ceramide	0.42	0.40	0.34	0.34
Lactosyl ceramide	2.40	2.44	2.34	2.39
Ceramide trihexoside	6.34	6.14	6.02	6.16
Globoside	18.16	17.95	17.94	17.84
Paragloboside	1.21	0.92	0.88	0.86

Sephadex was ascertained by adding known amounts of total ganglioside mixtures to the total lipid extracts prior to DEAE-chromatography[30]. For the beaf brain ganglioside mixture (BBG), a recovery of 96–97% was achieved, whereas for human erythrocyte gangliosides, the recovery was 92–94%. These results agree well with the previous data[15,30].

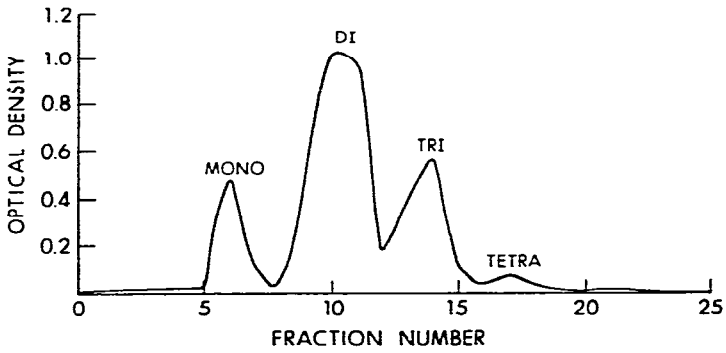

Fig. 4. Elution profile of total gangliosides from beef brain on DEAE-silica gel (acetate form). Bed dimensions, 2 × 60 cm. Sample applied, 40 mg ganglioside mixture. Flow-rate, 1.5 ml/min. Eluents: methanol, 0.2 *M* ammonium acetate in methanol and 0.5 *M* ammonium acetate in methanol (200 ml of each in series connected to each other through a gradient mixer). Fractions of 15 ml of effluent were collected and aliquots of 500 μl were used for sialic acid assay by the resorcinol method[42]. Mono, di, tri and tetra denote the numbers of sialic acid residues in the ganglioside fractions. The gangliosides were isolated after removal of ammonium acetate by dialysis against cold water.

In the present study we have utilized DEAE-silica gel for the fractionation of BBG using a linear gradient of ammonium acetate in methanol. The elution profile of the ganglioside mixtures is shown in Fig. 4. Mono-, di- and trisialo-species were well separated but, for the tetrasialo-species, some tailing of the trisialo peak was visible. Using DEAE-Sephadex as an ion-exchange medium and similar gradient mixtures to those employed by Momoi *et al.*[20], we obtained a similar pattern of separation (not shown here). The percentages of the lipid-bound sialic acid of mono-, di-, tri- and tetrasialo-species obtained by the fractionation of BBG by DEAE-silica gel and DEAE-Sephadex showed good agreement (Table II).

TABLE II

LIPID-BOUND SIALIC ACID ANALYSIS OF INDIVIDUAL GANGLIOSIDES PECIES FROM BEEF BRAIN AND HUMAN ERYTHROCYTES

Expressed as percentages of the total lipid-bound sialic acid determined by GLC[32]. The values presented in this table are the means from two separate experiments.

Ion exchanger	*Ganglioside mixture*	*Ganglioside species*			
		Monosialo	*Disialo*	*Trisialo*	*Tetrasialo*
DEAE-silica gel	Beef brain	20.50	53.53	21.41	4.55
DEAE-Sephadex	Beef brain	20.12	53.46	21.91	4.46
DEAE-silica gel	Human erythrocytes	94.95	5.05	N.D.	N.D.
DEAE-Sephadex	Human erythrocytes	95.24	4.76	N.D	N.D.

A comparative TLC pattern of the ganglioside species shown in Fig. 5 indicated similar patterns for mono- (lanes 2 and 9), di- (lanes 3 and 8) and trisialo (lanes 4 and 7) species, but for the tetrasialo-species some contamination by the trisialo-species was noted.

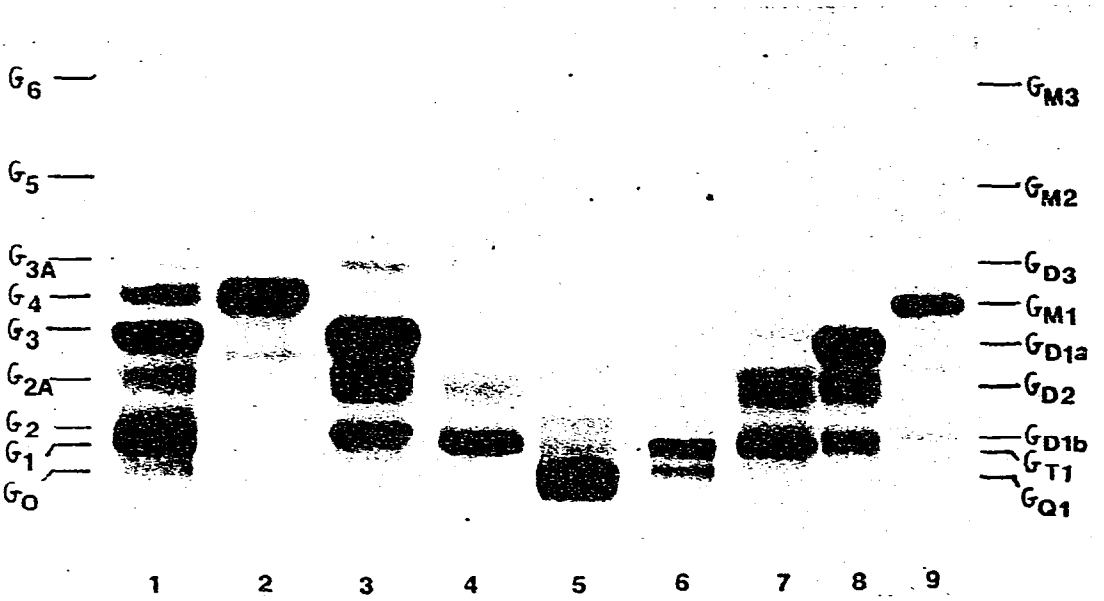

Fig. 5. TLC of individual ganglioside species of beef brain by, DEAE-gradient chromatography. (1) Standard BBG; (2) and (9) monosialo; (3) and (8) disialo; (4) and (7) trisialo; (5) and (6) tetrasialo-species. The gangliosides in lanes 2–5 were isolated by DEAE-Sephadex and those in 6–9 by DEAE-silica gel. Other details as described in Fig. 1.

Human erythrocyte ganglioside mixtures were also separated into the major mono- and the minor disialo-species (which has not yet been described in the literature) using analogous gradient mixtures to those employed for the fractionation of BBG. The elution profile obtained by use of DEAE-silica gel is shown in Fig. 6. A similar separation pattern was obtained (not shown here) with DEAE-Sephadex as ion-exchange medium. The percentages of lipid-bound sialic acid of mono- and di-

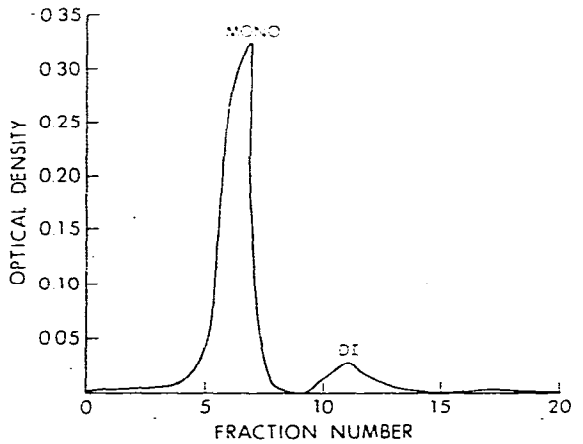

Fig. 6. Elution profile of total gangliosides from human erythrocytes on DEAE-silica gel (acetat form). Sample applied: 21 mg ganglioside mixture. Other details as described in Fig. 4.

sialo-species obtained from the fractionation of human erythrocyte ganglioside mixture by both ion exchangers were similar (Table II). A comparative TLC pattern shown in Fig. 7 indicated similar patterns for mono- (lanes 3 and 5) and disialo- (lanes 4 and 6) species.

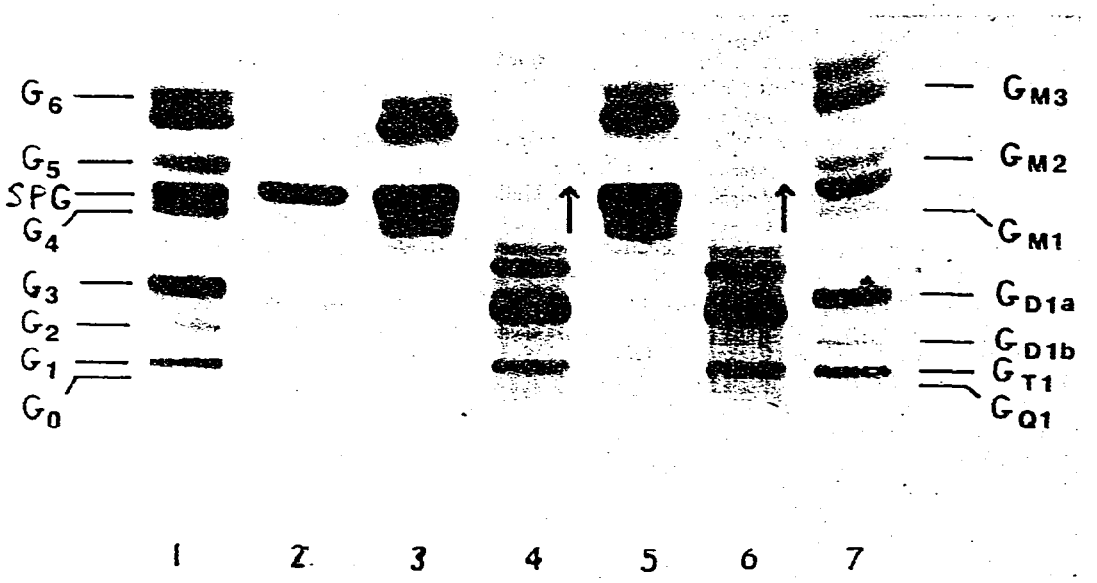

Fig. 7. TLC of individual ganglioside species of human erythrocytes by DEAE-gradient chromatography. (1) and (7) standards containing BBG, G_{M3} (= G_6), G_{M2} (= G_5) and sialosylparagloboside (SPG); (2) erythrocyte ganglioside mixture; (3) monosilao; (4) disialo-species. The gangliosides in 3 and 4 were isolated by DEAE-silica gel and those in 5 and 6 were by DEAE-Sephadex. Solvent system: chloroform–methanol–water (60:40:9) containing 0.02% calcium chloride (w/v). All bands were purple after detection by resorcinol spray[35], except lanes 4 and 6. The faint bands in these two lanes starting from the arrows are yellow in color. Other details as in Fig. 1.

Attempts to fractionate BBG or human erythrocyte ganglioside mixture by DEAE-CPG and similar gradient mixtures to those used for DEAE-silica gel and DEAE-Sephadex were unsuccessful. In all cases, the whole ganglioside mixture emerged as a single peak.

In a previous communication[30], we showed that DEAE-silica gel is an improvement over DEAE-Sephadex for the quantitative separation of acidic and neutral lipids for a number of reasons. Although the present study showed that DEAE-CPG can also be used for the same purpose, its high cost, non-availability from commercial sources and its unsuitability for use in gradient chromatography limit its application. We have also shown in this report that DEAE-silica gel can be used for the fractionation of complex ganglioside mixtures. Because of the superiority of DEAE-silica gel over DEAE-Sephadex and also over DEAE-CPG, we believe that DEAE-silica gel, will find wide applications for the separation and purification of gangliosides and neutral GSL from animal cells and tissues of biological interest. A report on some aspects of this work was presented as a preliminary communication[39].

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