Successive Isolation and Separation of the Major Lipid Fractions Including Gangliosides from Single Biological Samples

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Currently available techniques concerning extraction and characterization of the different lipids from biological specimens are designed for particular families and do not address consecutive isolation of lipid constituents in their globality. We describe here a simple, nondestructive chromatographic procedure that allows efficient elution and further analysis of the major lipid classes (neutral lipids, phospholipids, nonsialylated sphingolipids, and gangliosides) in their natural states from the same starting material. The procedure describes the use of solvent mixtures adapted to silicic acid column chromatography and permits 90–97% recovery of each of the above lipid groups. We have particularly concentrated on optimizing the efficient recovery of the diverse minor forms of gangliosides, free of other contaminants, from relatively small amounts of neural tissue. As model systems we have used in vivo and in vitro preparations of mammalian retina for which only fragmentary data are available on lipid composition. We show that relative to brain, retina contains, for example, twofold more sphingomyelin and sixfold more GD3 ganglioside. In turn, cultured retinal glial cells contain twofold higher levels of globoside and eightfold higher amounts of GM3 ganglioside with respect to intact retina. Compared to previously published techniques, we obtain improved total ganglioside recovery, with enrichment of poly-sialogangliosides. The technique presented here should be widely applicable to analyze global lipid composition of diverse biological samples.

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The involvement of different lipid components in cell structure and function [including cells in the central nervous system (CNS)] is still incompletely understood. It is known that they participate in a wide variety of vital cellular functions: for example, free fatty acids (FFA) regulate protein kinase C activation (1), phospholipids (PL) are reservoirs of key second messengers involved in numerous metabolic pathways (2), and sphingolipids (SL) are intimately involved in cell signaling (3, 4). The sialylated SL or gangliosides (GG) have raised a good deal of interest in the CNS due to their abundance and complexity in this tissue (5). Various roles have been proposed for GG, including neurotrophic actions (6), involvement in cell–cell interactions, recognition and signaling (7–10), and cellular immunity (11, 12).

As many of the lipid precursors and components undergo continual turnover and conversion into alternative forms, it is important to be able to analyze the diverse forms in their entirety. Published techniques deal mostly with recovery of only certain lipid groups such as PL and rely on methodologies that destroy or lose other species. Furthermore, these techniques in-

2 Abbreviations used: Ac, acetic acid; A, acetone; CA, cardiolipin; CNS, central nervous system; CER, ceramide; CMH, cerebroside/monohexosyl ceramide; CDH, dihexosyl ceramide; CTH, trihexosyl ceramide; C, chloroform; CHOL, cholesterol; DG 1-2, 1,2-diglyceride; DG 1-3, 1,3-diglyceride; DMEM, Dulbecco’s modified Eagle’s medium; Et, ethanol; FCS, fetal calf serum; FFA, free fatty acids; GG, gangliosides; GLOB, globoside; HPTLC, high-performance thin-layer chromatography; M, methanol; MG, monoglycerides; NL, neutral lipids; NeuAc, N-acetyl neuraminic acid; NSL, nonsialylated sphingolipids; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PL, phospholipids; P, phosphorus; RMG, retinal Müller glia; SL, sphingolipids; SM, sphingomyelin; SPH, sphingosine; SULF, sulfatide; TG, triglyceride.
volve different chromatographic steps combined with biochemical treatments (such as alkaline hydrolysis) to improve resolution of the final product (13–17). Such approaches are suitable for situations in which large amounts of starting material, such as tumors or brain tissue, are available. However, the use of tissues or cells available only in limited amounts necessitates more sensitive and economic techniques for accurate isolation and quantification. An example of such a case is the mammalian retina, particularly from immature animals or using primary cultured cells.

We report here the use of a modified, sensitive assay method enabling isolation of each lipid class in its natural state. As an example of its application we have separated the lipid families [neutral lipids (NL), PL, nonsialylated SL (NSL), and GG] and isolated and quantitated the majority of the different lipid components of total retina (from pig and rat) and specific cells (cultured Müller glia from rat retina). We show that the described technique permits the isolation of successive lipid classes, that there is negligible loss of material, that there is good yield of particularly labile forms of GG, and that this improved resolution will permit a detailed examination of the lipid composition of CNS tissues and cells.

MATERIALS AND METHODS

Materials

Lipid standards were procured from the following companies: NL, oleic acid (used as standard for FFA), 1-monoleoyl-rac glycerol (used as standard for mono-glycerides, MG), 1,2-dipalmitoyl-sn-glycerol (DG 1-2), 1,3-dilinolenyl glycerol (DG 1-3), triglyceride (TG), and cholesterol (used as standard for cholesterol, CHOL) were all obtained from Sigma–Aldrich (St. Louis, MO); PL, cardiolipin (CA), phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylycerine (PS), were also obtained from Sigma–Aldrich; NSL, “Qualmix” containing 29% globoside (GLOB), 42% cerebrosides/monohexosyl ceramide (CMH), 14.5% dihexosyl ceramide (CDH), and 14.5% trihexosyl ceramide (CTH) (each present in approximately 50% hydroxylated and nonhydroxylated forms) was purchased from Matreya Inc. (Pleasant Gap, PA); sulfatide (SULF) and sphingomyelin (SM) were also purchased from Matreya Inc.; ceramide (CER) and d-sphingosine (SPH) were from Sigma–Aldrich; GG, standard mixture from bovine brain type II, was from Sigma–Aldrich; additional GM3, GM2, GD3, and GQ1b were from Matreya Inc. (Pleasant Gap, PA).

Chromatographic supplies were as follows: Sephadex G25 superfine beads were from Pharmacia (Uppsala, Sweden); silicic acid (Kieselgel 60, 230–400 mesh) and high-performance thin-layer chromatography (HPTLC) plates 10 × 10 cm for NL, NSL, and GG analysis (reference 1.05628) were all from Merck (Darmstadt, Germany); LK5 concentrating zone TLC plates 20 × 20 cm were from Whatman Laboratories (Clinton, NJ). Tissue culture supplies (media, sera, and dishes) were from Gibco BRL Life Technologies (Strasbourg, France). All other chemicals and solvents were of analytical grade and were purchased from Sigma–Aldrich or Carlo Erba (Milan, Italy).

Tissue and Cell Collection

For analyses of whole retina, tissue was isolated from adult pig eyes obtained from the local slaughterhouse. Retinas were dissected free from other ocular tissue using previously published methods (17, 18) and transferred to glass Potter tissue homogenizers (2 ml) in 0.5 ml H2O. For comparative purposes, whole retinal and brain tissue was also removed from adult Wistar rats and treated similarly to pig retina.

Retinal Müller glia (RMG) cells from Wistar rat retinas were cultured according to previously published methods (18). Briefly, retinas from Postnatal Day 8 rats were carefully dissected following anesthesia and decerebration, chopped into small fragments, plated in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (DMEM/10% FCS) in 100-mm culture dishes (8 retinas per dish), and maintained in a humidified incubator at 37°C in an atmosphere of 95% air/5% CO2. After 4–5 days in vitro, the retinal fragments were removed by vigorous washing with DMEM, leaving a monolayer of flat cells. Cells were then detached with fresh DMEM/10% FCS and grown to confluence. The RMG, identified by immunocytochemical criteria (18), were used at confluence after the second passage. Before lipid extraction, cells were washed with 5 ml cold 0.9% NaCl, then scraped off the plates and rinsed with 0.5 ml double-distilled H2O before homogenization and dispersion in a sonication bath.

One pig retina, six rat retinas, 5 to 10 100-mm-diameter RMG dishes, and one rat brain were used per experiment. Protein levels in homogenates of retinas, brain, and RMG were determined by the Lowry method (19).

Extraction and Purification of Lipids

Lipids of whole retinas or brain and of cultured RMG cells were extracted as described by Folch–Pi et al. (20) with the modifications reported by Suzuki (21). Tissue and cellular homogenates representing 2–10 mg protein in 0.5 ml H2O were mixed with 5 ml chloroform (C):methanol (M) (1:1, v/v) [i.e., final composition C:M:H2O (5:5:1, v/v/v)] for 2 h to extract total lipids. Three further extractions were performed on the resid-
### TABLE 1

Quantitative Estimates of Recovery for Different Lipid Classes Separated by Column Chromatography

<table>
<thead>
<tr>
<th>Lipid (μg)</th>
<th>Amount loaded on Sephadex</th>
<th>Recovery after Sephadex*</th>
<th>% recovery</th>
<th>Amount loaded on silicic acid</th>
<th>Recovery after silicic acid*</th>
<th>% recovery</th>
<th>% average total recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>88.0</td>
<td>85.8</td>
<td>97.5</td>
<td>96.0</td>
<td>95.7</td>
<td>99.7</td>
<td>97.0</td>
</tr>
<tr>
<td>PL</td>
<td>450.0</td>
<td>425.0</td>
<td>94.4</td>
<td>507.5</td>
<td>500.0</td>
<td>98.5</td>
<td>90.2</td>
</tr>
<tr>
<td>GG-NeuAc</td>
<td>18.9</td>
<td>18.6</td>
<td>98.4</td>
<td>20.7</td>
<td>18.3</td>
<td>88.4</td>
<td>89.5</td>
</tr>
</tbody>
</table>

Note. Estimates of recovery of different lipid components separated by successive column chromatography. Purified lipids were loaded onto either Sephadex G-25 or silicic acid columns and their recovery was determined after each column passage. For each lipid, two independent runs were performed (*indicates values obtained in both cases). Elution and quantification were achieved according to the protocols described under Materials and Methods.

Separation and Isolation of the Different Lipid Classes

These were achieved by column chromatography (column diameter 0.6 cm, height 2.5 cm) on silicic acid by adapting and modifying a combination of solvents used separately for different purposes (15-17). The purified lipids (0.5-5.0 mg) were dissolved in 0.5 ml C and loaded onto the silicic acid column preequilibrated with the same solvent. The different chromatographic steps were as follows:

(i) Recovery of neutral lipids (NL). NL were eluted with 12 ml C. Aliquots of about 2 ml were used for CHOL determination (22) and were compared with CHOL levels measured prior to column chromatography to assess losses. The remaining fraction was further used for NL separation by HPTLC.

(ii) Recovery of PL and NSL. The second elution system consisted of 1.5 ml C:acetone (A):acetic acid (Ac):H₂O (52:8:8:18:4, by volume) followed by 8 ml C:M (4:1, v/v). The eluate contained all the PL and NSL. Aliquots were used for total lipid P determination (23). The remaining extract was used for PL and NSL analysis. An aliquot containing 200 μg PL was dried under nitrogen and dissolved in 3.8 ml C:M:H₂O (1:2:0.8, v/v/v). Then 1 ml C and 1 ml 0.88% KCl were added to break phases and the phases separated by brief centrifugation. The upper phase was discarded and the lower phase washed three times with the Folch upper phase C:M:0.88% KCl (3:48:47, v/v/v). The C phase was dried and used for PL separation by TLC. The loss of PL obtained from retina was estimated by loading known amounts onto Sephadex and silicic acid columns.
TABLE 2
Quantitative Estimates of Lipid Contents of Different Retinal and Brain Fractions

<table>
<thead>
<tr>
<th>Lipid (nmol/mg protein)</th>
<th>Rat RMG (7)</th>
<th>Pig retina (4)</th>
<th>Rat brain (5)</th>
<th>Rat retina (4)*</th>
<th>Rat retina (1)#</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOL</td>
<td>96.6 ± 10.0</td>
<td>102.0 ± 11.0</td>
<td>378.0 ± 49.0</td>
<td>72.4 ± 8.0</td>
<td>ND</td>
</tr>
<tr>
<td>PL</td>
<td>268.0 ± 36.0</td>
<td>252.0 ± 39.0</td>
<td>423.0 ± 64.0</td>
<td>270.0 ± 30.0</td>
<td>ND</td>
</tr>
<tr>
<td>SM</td>
<td>24.1 ± 2.9</td>
<td>12.6 ± 1.7</td>
<td>33.8 ± 4.2</td>
<td>10.8 ± 1.5</td>
<td>ND</td>
</tr>
<tr>
<td>GG-NeuAc</td>
<td>7.4 ± 1.0</td>
<td>9.7 ± 1.3</td>
<td>17.8 ± 1.9</td>
<td>9.1 ± 1.0</td>
<td>4.3*/12.0*</td>
</tr>
<tr>
<td>GG</td>
<td>6.3 ± 0.8</td>
<td>6.1 ± 0.8</td>
<td>9.7 ± 1.1</td>
<td>4.8 ± 0.6</td>
<td>5.0c</td>
</tr>
</tbody>
</table>

Note. Quantitative estimates of the lipid content for each sample group. Values are given as means ± SD for rat RMG, pig retina, and rat brain (number of experiments in parentheses). In addition, quantitative data for adult rat retina both obtained in the present study (*) and derived from published data (#) (17, 43) are given. The different measures were performed as described under Materials and Methods. Abbreviations as in text. ND, not determined.

a Data from (17).
b Data from (43).
c Determined by us using the method described in (17).

A second aliquot containing the same amount of lipids as above was subjected to alkaline methanolysis in order to remove glycerophospholipids, thus permitting determination of NSL. After drying, the aliquot was incubated in a screw-cap tube containing 0.6 ml KOH (0.3 N in M) for 1 h at 37°C. The mixture was then neutralized by a few drops of 3 N HCl using phenolphthalein indicator (1 mg/ml) and 1.5 ml C and 0.5 ml

FIG. 2. Histogram showing relative percentages of NL in different retinal and brain fractions. Data (means ± SD) are from four to seven separate experiments, each performed in duplicate as indicated in Table 2. Each NL percentage was determined as described under Material and Methods, analysis of NL. Abbreviations as in text.
(i) Analysis of NL. Samples of about 30–40 μg CHOL were applied to HPTLC plates using a Camag automatic TLC sampler III (ATS3) (Muttenz, Switzerland). They were separated with C:A (96:4, v/v) as migration solvent during 25 min. The HPTLC plate was then submerged during 20 s in a copper sulfate/orthophosphoric acid mixture (25) and placed in an oven at 130°C during 10 min in order to visualize the NL species. The relative amount of each NL was determined by densitometric scanning at 577 nm using a Camag TLC Scanner II (Muttenz, Switzerland) equipped with a software analysis package (CATS 3 program, Camag).

(ii) Analysis of PL. Separation of PL was achieved by TLC on LK5 plates previously sprayed with H₃BO₃ [(2.3% in ethanol (Et)], dried, and activated at 110°C for 15 min. Aliquots of about 15 μg lipid P were spotted with the ATS3 and PL separated using C:Et:H₂O:triethylamine (30:35:7:35, by volume) as migration solvent during 3 h. PL were then visualized by primuline spray (26) and each spot was scraped off the plate for lipid P determination (23).

(iii) Analysis of NSL. Separation of NSL spotted can be seen. Abbreviations as in text.

(iv) Analysis of GG. Separation of GG was achieved by HPTLC. Aliquots of about 3.5–6.5 μg GG±NeuAc were spotted with the ATS3 on plates previously washed in C:M (1:1, v/v). Three successive runs were performed using three different migration solvents, respectively: (a) C:M (2:1, v/v) during 70 min; (b)
C:M:H₂O (65:25:4, v/v/v) during 40 min; and (c) C:M:0.25% CaCl₂ in H₂O (50:37:10, v/v/v) during 40 min, with plates being thoroughly dried for 10 min between each run. GG were then visualized using resorcinol-HCl reagent (24). The GG patterns were determined by densitometric scanning at 577 nm as before.

RESULTS

1. Efficiency of Lipid Recovery

By comparing quantitative measures of different lipid constituents obtained at different stages of the isolation process we were able to monitor recovery following Sephadex and silicic acid column chromatography, respectively. Known concentrations of lipids were loaded onto the columns and eluted as described. As shown in Table 1, CHOL recovery following passage on the two columns was excellent (97%). PL recovery was estimated at 90% for Sephadex and silicic acid together. In addition, comparison of PL distributions from both samples by TLC revealed that no preferential losses in any single species occurred (data not shown). Finally, GG-NeuAc recovery was of the same order (90%), and examination of HPTLC plates comparing GG mixtures before and after passage through the columns again showed that the technique did not lead to removal of specific forms (data not shown). The separation procedure was valid between 0.5 and 5.0 mg total lipids, which between these values represented (in mg) for NL, 105; for PL, 200; for NSL (SM) 28; and for GG, 10. Use of lower amounts resulted in minor species remaining below detection limits, and use of higher amounts led to increasingly incomplete elution of the different classes, leading to contamination of fractions eluted at later stages (data not shown).

2. Neutral Lipids

Examination of typical HPTLC plates (Fig. 1) showed that MG, FFA, CHOL, DG, and TG were detected in all the retinal and brain samples. CHOL levels expressed on a unit protein basis were similar for whole pig retina and cultured rat RMG, but much higher for brain (Table 2). As revealed by HPTLC, CHOL was the major NL species in all samples (41–48%), followed by TG (30–35%) and FFA (12–15%) (Fig. 2). The minor
species were represented by MG and DG, with DG being more abundant in RMG cells (Fig. 1).

3. Phospholipids

The total PL amounts expressed per unit protein were similar for retinas and RMG cells (260 nmol), which represented about 60% of the value for brain (Table 2). Examination of individual PL species (Figs. 3 and 4) showed that retinal and brain samples contained a majority of PC (39–53%) and PE (22–30%). Retinas contained somewhat higher relative amounts of PC and brain slightly higher relative amounts of PE. The minor PL species were represented by CA and PA. Overall, the distribution of individual PL species was largely similar between whole retinas, RMG cells, and brain. The quantitative and relative amounts of SM, which belongs to both PL and NSL families, were the highest for brain and RMG cells (Table 2). For each sample examined, SM, PI, and PS accounted for 5–9% each of total PL.

4. Nonsialylated Sphingolipids

These lipids were the most difficult group to separate and identify. Many NSL migrated as several bands, particularly the cerebrosides (CMH, CDH, and CTH), GLOB, and SPH, due to the presence of hydroxylated and nonhydroxylated species (e.g., Fig. 5a). Furthermore, the use of a single solvent mixture led to overlapping migration positions for certain NSL. These difficulties necessitated the use of several different solvent combinations to unambiguously discriminate between the eight different molecular species of retinal and brain NSL (Figs. 5–7). The relative distributions of each NSL are depicted in Fig. 8, with the values for CMH, GLOB, CTH, and SM being taken from the densitometric analysis of Figs. 5a and 5b; those for CDH, SULF, and SPH from analysis of Fig. 6; and that of CER from Fig. 7. Retinal tissues and glial cells showed the presence of CER, CMH, CDH, SULF, CTH, SPH, SM, and GLOB, this latter NSL being present only at trace levels in rat brain. Retinas and RMG showed roughly similar profiles for the relative abundance of NSL, although on a unit protein basis RMG contained onefold higher SM levels (Table 2). When expressed in terms of relative abundance, whole retinas showed a predominance of SM (31–35%), while RMG were enriched in GLOB (15%) (Fig. 8). NSL of rat brain were mostly CMH (42%) and SULF (26%).
of GM3 and moderate levels of GD1a and GD2, with a total absence of tri- and tetrasialo species.

The quantitative data concerning retinal and brain GG samples are illustrated in Figs. 10 and 11. The relative distributions show clearly the contrasting profiles between the samples: in pig retinas, GD3 represented about 30% and was the major species, rat brain contained abundant GD1a (30%), and RMG contained mostly GM3 (70%). RMG was the only sample expressing detectable levels of GM2 and GD2, whereas GD1b, GT1b, and GQ1 were below detection limits (these three GG accounted for more than 40% total GG in retina and brain) (Fig. 10). When these results were expressed as nmol GG/mg protein, the highest amounts of GM1, GD1a, GD1b, GT1b, and GQ1 were found for brain, whereas RMG showed the highest levels of GM3 and pig retina the largest amounts of GD3 (Fig. 11).

DISCUSSION

We present here a modified, rapid, and reliable technique for lipid analysis which permits efficient extraction (90–97%) of lipid components including GG, without preferential loss of any one group, and which is applicable to biological samples of limited availability (e.g., small tissues and cultured cells). The protocol is easy to perform, does not require any special apparatus, and enables characterization of each lipid fraction.
FIG. 8. Histogram showing relative percentages of NSL in different retinal and brain fractions. Data (means ± SD) are from four to seven separate experiments, each performed in duplicate as indicated in Table 2. Each NSL percentage was determined as described in Material and Methods, analysis of NSL. Abbreviations as in text.

FIG. 9. HPTLC plate of GG isolated from RMG (lane 2), pig retina (lane 3), and rat brain (lane 4). About 3–5 μg GG-NeuAc was spotted by lane. Standard GG from bovine brain supplemented with pure GM2, GM3, and GQ1b (Matreya, USA) were cochromatographed on lanes 1 (run in parallel with RMG) and 5 (run in parallel with pig retina and rat brain). The different GG were separated using three successive runs in (1) C:M (2:1, v/v); (2) C:M:H₂O (65:25:4, v/v/v); and (3) C:M:0.25% CaCl₂ in H₂O (50:37:10, v/v/v) as solvent systems. The differences between RMG (high levels of GM3 and GD1a), pig retina (substantial amounts of GD3), and rat brain (appreciable amounts of GM1, GD1a, and GT1b) are easily visible. The arrowhead between lanes 1 and 2 indicates the presence of GD2 in RMG. Abbreviations as in text.

We have used this technique to analyze all the lipids from retinal tissues and cells. We report in this study the lipid composition of whole pig retina and cultured RMG cells, which are the predominant glial cells type in retina. Except for NSL, the lipid composition of the brain is well documented (13, 14, 21, 27–29) and we have performed different analyses on rat brain for comparative purposes. Data for rat retinal lipids have been reported elsewhere and were very close to those found for pig retina in this study (30). Much data have been reported on PL and GG in retinas of different species (13, 15, 17, 27, 30–33). However, to the best of our knowledge only fragmentary data have been previously published on the various NL and NSL (27, 34–36). Within ocular tissues, glycosphingolipids of lens have been examined (37). This latter study described a different new technique, also dealing with the analysis of all separate lipid classes obtained from the same starting material. However, this study did not provide data on parallel analyses of cultured material.
**FIG. 10.** Histogram showing relative percentages of GG in different retinal and brain fractions, calculated from lipid-bound NeuAc amounts. Data (means ± SD) are from four to seven separate experiments, each performed in duplicate as indicated in Table 2. Each GG-NeuAc percentage was determined as described under Materials and Methods, analysis of GG. Abbreviations as in text.

**FIG. 11.** Histogram showing total GG contents (in nmol GG/mg protein) of different retinal and brain fractions. Data (means ± SD) are from four to seven separate experiments, each performed in duplicate as indicated in Table 2. Each GG was determined as described in the legends to Figs. 9 and 10. Abbreviations as in text.
Although the retina forms part of the CNS, brain contained higher levels of representative lipids such as CHOL, PL, SM, and GG. The PL profiles were similar, while among NL the main differences between the tissues concerned more abundant amounts of DG within the retina. Major differences were noted for the SL (NSL and GG). As expected, due to the presence of myelin and white matter, CER, CMH, and SULF were the characteristic NSL species of the brain, whereas the retina, which completely lacks myelinated glia, was characterized by SM and SPH. The main difference with respect to GG concerned GD3 (and also GM3) which are highly enriched in the retina, whereas they form only minor species in the brain. GD3 has been shown to be expressed by immature CNS cells, transformed tumorigenic lines, and reactive astrocytes (38-40), where it is speculated that its lipophilic structure would favor permeability to ions and metabolites. In a previous study (30), the possible role of GD3 in photoreceptor cells was also discussed. NL and PL distributions of cultured RMG resemble those of whole retina, whereas differences are noted for NSL and GG. RMG showed highest relative percentages of GLOB and GM3 and exhibited appreciable amounts of GD2, undetectable in whole retina. They resemble astrocytes in vitro in possessing a relatively simple GG profile dominated by GM3 and lacking the more highly sialylated species (GD1b, GT1b, and GQ1) (41). Under culture conditions glial cells show a relatively simple GG composition, whereas quantitative in vivo studies suggest a more complicated make-up closer to that reported for neurons (42). The absence of more highly branched GG in RMG probably reflects the absence of glycosyltransferase activities involved in their synthesis (41).

Due to the paucity of previously published data on classes of retinal lipids such as NSL, it was not possible to compare our data. Such comparisons were possible for GG as several studies on these lipids have been published by Daniotti and colleagues (17, 43-46). The variations in their reported GG-NeuAc levels [4.5-12 nmol/mg protein (17, 43)] make strict comparisons difficult, although our own measures of GG-NeuAc isolated using their technique resulted in 50% lower levels than those obtained by the protocol presented here. Relative levels of the labile GG GM3 also differed widely in the literature (17, 43), whereas we are confident that our recovery was almost total. GG profiles given in this study also show higher amounts of poly-sialoGG, probably due to the fact that the elution of GG from a silicic acid column by C:M (2:3, v/v) alone (17, 43-46) is insufficient to permit the complete recovery of poly-sialoGG such as GD1b, GT1b, and GQ1, which requires a mixture composed of the lowest possible concentration of M but containing H2O as found in C:M:H2O (48:35:10, v/v/v). The inclusion of small amounts of water in the elution buffer is sufficient to efficiently displace these GG species due to the increased polarity.

In conclusion, we report here a technique able to detect and quantify the major lipid classes in samples of biological tissues. This approach will permit subsequent analyses of the precise structures of the various molecules by more sophisticated techniques such as mass spectrometry or HPLC.

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