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# Isolation of Gangliosides

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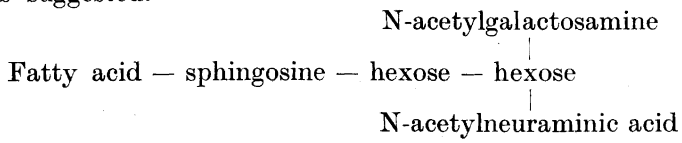
A method for the extraction, purification and separation of gangliosides on silicic acid in mono- and disialogangliosides is described in detail. Other procedures used for the extraction and purification of gangliosides are compared with the present method. Analysis of the gangliosides from human and calf brains shows a marked similarity in respect to composition.

In 1942 Klenk [1] isolated from beef brain a new type of glycolipid with a relatively large carbohydrate moiety which he named ganglioside. Among the products of hydrolysis Klenk identified fatty acids, sphingosine, hexoses, consisting mainly of galactose, and methoxy neuraminic acid. Lipids of the same type were already encountered in 1925 in horse kidney by Levene and Landsteiner [2], and independently, by Walz [3] in spleen and brain. Before Klenk had isolated and elucidated the composition of gangliosides from normal brain tissue he had demonstrated increased amounts of unidentified glycolipids in brains of patients with Tay-Sachs and Niemann-Pick disease [4]. In 1938, Blix [5] found 9 % of hexosamine in a lipid preparation similar to that investigated by Klenk, but not until ten years later was the hexosamine isolated and identified as D-galactosamine [6].

Brain gangliosides were generally considered to have a uniform carbohydrate structure [7,8] but Svennerholm [9] was able to separate human brain gangliosides into two fractions with different carbohydrate composition by chromatography on cellulose. The fast moving gangliosides were further purified and a homogeneous major fraction was obtained in crystalline form. From the analytical data the following structure of this ganglioside was suggested:




The occurrence of different gangliosides have since been confirmed by Kuhn and associates [10], Klenk and Gielen [11] and several other workers. Kuhn

*et al.* obtained a fast moving ganglioside from crude beef brain gangliosides which was thought to have the same composition as that isolated by Svennerholm [9] from the human brain, and three additional slow moving gangliosides.

The slow moving gangliosides of human brain can be converted to fast moving gangliosides [12,13] by enzymic hydrolysis with neuraminidase. Because the molar ratio of sialic acid to sphingosine was 1 in the fast moving gangliosides and nearly 2 in the slow moving ones, they were provisionally termed mono- and disialogangliosides, respectively [13]. Kuhn, Wiegandt and Egge [14] have also reported on a ganglioside from beef and human brain with three moles of sialic acid. In the same report they also concluded that the gangliosides contain three nitrogen-free sugars instead of two which had been assumed earlier.

When it appeared likely that the gangliosides comprised a family of glycolipids with different carbohydrate moities, we began in 1955 to develop methods for the quantititive isolation of the individual gangliosides. Since there is at present considerable interest in the biochemistry of gangliosides, we feel that it is desirable to present at this time a detailed description of the procedure developed *in this laboratory* [15] for the isolation and characterization of gangliosides.

## EXPERIMENTAL

*Analytical methods.* Hexose was determined by orcinol [16] or anthrone [17] methods after hydrolysis according to Radin *et al.*[18] Glucose was assayed in the following manner. The glycolipids were hydrolysed with chloroform-ethanol-conc. hydrochloric acid [18], the organic solvents were removed and the fatty acids and sphingosine were extracted into light petroleum. The aqueous phase was hydrolysed with 1 N HCl for one additional hour. After neutralization, glucose was determined with glucose oxidase [19]. Galactose did not react with this preparation.Hexosamine and sialic acids were determined by methods described elsewhere [20,21]. Total nitrogen was determined by a micro-Kjeldahl method. When there was a scarcity of material, the ammonia formed after digestion was analysed with Nessler's reagent [22]. Sphingosine was assayed in the organic solvent phase after acid hydrolysis according to Robins *et al.*[23].

*Partition chromatography.* Paper chromatography was run with the following solvents (all proportions by volume): Di-isobutylketone-acetic acid-water 40:30:7 [24], tetrahydrofuran-di-isobutylketone-water 45:5:6 [25] or 50:5:9, 1-butanol-pyridine-water 3:1:1 or 6:5:4 [26]. Thin layer chromatograms on silica gel plates were run with the following solvents: chloroform-methanol-water 60:35:8 [27], 1-propanol-water 7:3 [14] and 1-propanol-ammonia-water 7:2:1 [28]. Gangliosides and other lipids were located with the following reagents: ninhydrin, dipikrylamin, Rhodamin B [25] and cresyl violet. With the latter reagent, air-dried papers were immersed in 0.02 % w/v cresyl violet in 1 % v/v acetic acid and heated at 60° for 10 min and then washed in 1 % acetic acid. Monosialogangliosides appear as mauve spots while those of the disialogangliosides are redder. The ammonium molybdate-perchloric acid spray [27] was used as a general test for all lipids on thin layer chromatograms. Gangliosides were identified by spraying the plates with the resorcinol reagent [21] and then the plates were heated at 120° for 30 min horizontally in a covered glass jar. Gangliosides give red-violet and neutral aminoglycolipids brown-yellow spots.

### Materials

*Chromatographic materials.* Silicic acid (Baker AR, lot No. 4680) was milled in a ball mill and screened. Particles passing a 120 DIN sieve (German standard) were used. The smallest particles were removed by suspending the silicic acid in methanol. The methanol was decanted after 20 min. Then the procedure was repeated once. The remaining silicic acid was dried on a Büchner funnel, and a 2 cm thick layer on an enamel plate was activated at 120° for 16 h.

Alumina (standardized according to Brockmann) was manufactured by E. Merck AG., Darmstadt. The finest particles were removed by suspending the alumina twice in methanol and decanting the supernatant after 5 min.

Thin layer chromatography was performed with the equipment manufactured by C. Desaga GmbH, Heidelberg, Germany. Paper partition chromatograms were run on Schleicher & Schüll 2045b papers.

*Solvents.* Acetone, chloroform and methanol used for the extraction of lipids from tissues were of reagent grade quality and they were not redistilled. All solvents used for chromatography were redistilled. Chloroform was dried over anhydrous calcium chloride before distillation. After the distillation it was stored in brown flasks and used within a week. Methanol, reagent grade, was dried above anhydrous sodium sulfate and NaOH pellets before distillation. To tetrahydrofuran, 1 g hydroquinone/l solvent was added after distillation. Other solvents were stored without additives. All other chemicals were the best commercially available.

*Sources for gangliosides.* Human brains were obtained from the Institute of Pathology, Sahlgren's Hospital, Göteborg. The extraction of the materials was initiated within 48 h after death. Material was taken only from patients without signs or symptoms of mental, neurological or known metabolic diseases. Brains from calf, sheep and pig were removed at the slaughter-house within 1 h after sacrifice.

## Procedure

*Extraction of gangliosides.* The brains were freed from blood and meninges under running tap water. For the preparation of gangliosides from the adult brains grossly grey matter was obtained by cutting a 3–4 mm thick outer layer of the cerebral cortex. The dissection was rather gross and about 500 g of tissue was obtained from an adult human brain. White matter from the centrum semiovale which was carefully dissected free from all visible grey matter was used in small scale preparations.

All materials were homogenized in a Turmix blendor and poured into four volumes of cold acetone (+ 4°) or lyophilized directly. The acetone-treated material was stored at + 4° until it was used. At that time the acetone was filtered off and 4 volumes of fresh acetone were added. After two to three days' storage in the fresh acetone, the mixture was filtered and the residual material was dried in air. No gangliosides were lost by treating the tissue with acetone. 100 g acetone-dried or lyophilised material was extracted in a Soxhlet apparatus (Quickfit & Quartz Ltd.) with 500 ml chloroform: methanol 2:1, v/v (C–M) for 8 h and for 16 h with 300 ml C–M 1:2. The latter extract was evaporated to half its original volume and dialysed for at least three days against running water. Then the solvent was removed in a rotating evaporator. The residue was extracted several times with boiling C–M 2:1, and then the solvent was evaporated. This residue contained mainly slow moving gangliosides and nonlipid material.

The 2:1 C–M extract was transferred to a separatory funnel and 1/6 volume of 0.1 % NaCl solution was added. The funnel was shaken vigorously for one minute. After standing for 12 h, the lower phase was transferred to a new separatory funnel containing 1/3 volume (calculated from the original volume of the C–M 2–1 extract) of fresh upper phase (chloroform-methanol-water 1:10:10, by volume). The lower phase was extracted in all 5 to 7 times with this fresh upper phase. The combined upper phases were evaporated *in vacuo* until the foaming became disturbing. Then the residual extract was dialysed and treated as the 1:2 C–M extract. The two extracts were combined and termed crude gangliosides. They were assayed for hexose, hexosamine, N-acetylneuraminic acid and examined by paper and thin layer chromatography.

## Chromatography on silicic acid

*Isolation of total gangliosides.* 60 g silicic acid was slurried in C–M 4:1 v/v and poured into a column 30 mm in diameter equipped with a Teflon stopcock at the bottom and a standard taper female ground glass joint at the top for the solvent reservoir. 2.0–3.0 g crude gangliosides were dissolved in 50 ml C–M 4:1 with gentle warming and 10 g silicic acid were added. When the solution had cooled to room temperature, it was poured on

the silicic acid column. After several rinsings with small volumes of the same solvent, the following elution scheme was used:

| | | |
|---|---|---|
| C—M | 4:1 | 600 ml |
| C—M | 2:1 | 300 ml |
| C—M | 1:2 | 600 ml |
| Methanol | | 300 ml |

Fractions of 25 ml were collected on a fraction collector. An aliquot from every third tube was analysed chromatographically on paper or silica gel plates. The tubes containing the major portion of gangliosides but no sphingomyelins were pooled. The tubes containing sphingomyelins and the tailing gangliosides were combined from several runs, evaporated to dryness and dissolved in C—M 2:1. 1/6 volume of water was added and after shaking was allowed to stand for 16 h. The lower phase was taken to dryness, the residue dissolved in 25 ml C—M 1:1 and poured on to a column containing 10 g alumina. The sphingomyelins were eluted with 150 ml of the same solvent. Gangliosides were eluted with 150 ml chloroform-ethanol-water 2:5:2 (by volume). This latter eluate was combined with the upper phase from the preceding partition procedure, and dialysed against distilled water. The solvents were removed with a rotating evaporator and the residue extracted with C—M 2:1.

*Separation of the gangliosides.* A column containing 60 g silicic acid was prepared in C—M 4:1. About 600 mg gangliosides were dissolved in 5 ml C—M 4:1 and transferred to the column with a pipet. After rinsing, the column was developed with the following solvents:

| | | |
|---|---|---|
| C—M | 4:1 | 600 ml |
| C—M | 3:2 | 600 ml |
| C—M | 1:2 | 600 ml |

Every second tube was tested by chromatography on paper or silica gel plates. The monosialogangliosides were eluted with C—M 3:2 and the disialogangliosides with C—M 1:2. There was considerable overlapping. Tubes with chromatographically uniform fractions were combined and evaporated *in vacuo* to a small volume. During the evaporation, the gangliosides crystallized out on the walls of the flask. The gangliosides were dissolved with warming by the addition of chloroform and the solution was filtered through a sintered glass filter. The filtrate was concentrated to a small volume and ethanol was added gradually without allowing any precipitation to occur. When the smell of chloroform was negligible, the flask with the gangliosides was allowed to stand at room temperature. Crystallization soon occurred and it was continued at + 4°. The crystallized material was collected on a glass filter and the mother liquor was concentrated to half its original volume and kept 48 h longer at + 4°.

At least three silicic acid chromatographic separations have been made of the total ganglioside fraction from every source available. From human brains about 20 separations have been run. The reproducibility of the chromatographic separation on silicic acid has been good.

## RESULTS AND DISCUSSION

*Extraction of gangliosides from biological materials.* In the original methods for the preparation of gangliosides from brain, the starting material contained in the protagon fraction (mainly the sphingolipid fraction according to present nomenclature) was obtained by fractionate solvent extraction. It was demonstrated in 1957 [29] that large losses of gangliosides could occur before obtaining the protagon fraction. The subsequent purification of gangliosides from this fraction was tedious and the yield of gangliosides was low.

A significant advance was made when Folch and associates [30] developed a partition technique for the isolation of gangliosides (strandin) from tissue lipid extracts. In their original procedure, the lipid extract was prepared by


*Fig. 1.* Soxhlet extraction of brain tissue. Lipid extracts after *a*) 2 h, *b*) 2—5 h, and *c*) 5—12 h, extraction with chloroform-methanol 2:1 v/v, and *d*) after 4 h, and *e*) 4—8 h extraction with chloroform-methanol 1:2 v/v. Paper partition chromatogram. Solvent: tetrahydrofuran-diisobutylketone-water 45:5:6 v/v.

mixing one volume of tissue with 20 volumes of chloroform-methanol 2:1 v/v, at + 4°. Then the gangliosides were separated by partition dialysis. There are two main disadvantages with this procedure, *i.e.*, the use of large volumes of solvents and the difficulties in removing nonlipid contaminants from the gangliosides.

In order to avoid these disadvantages we [9] prepared a total lipid extract from lyophilized or acetone dried tissue by Soxhlet extraction. As shown in Fig. 1, the gangliosides were quantitatively extracted from the tissue only by prolonged extraction with chloroform-methanol, 1:2, v/v. For the quantitave estimation of gangliosides, only the extraction with chloroform-methanol 1:2 was used [29]. With large scale preparations, it was found convenient to extract most of the other lipids and the easily soluble gangliosides separately with chloroform-methanol 2:1 v/v, and extract the remaining gangliosides separately with methanol-chloroform 1:2 v/v.

Folch, Meath and Bogoch [31] later developed a simpler extraction procedure which is similar in principle to the original method of Klenk [1]. Brain tissue is extracted with hot methanol, the filtrate stored in the cold and the precipitate which is formed is used as starting material for the isolation of gangliosides by the partition technique. The same method has been used by Bogoch [8], Folch and Lees [32] and Thannhauser and associates [33], but no figures have been given for the loss of gangliosides at the precipitation. In our experience with this method there was a rather large loss of gangliosides, especially of the monosialogangliosides.

*Preparation of a crude ganglioside extract.* Several procedures have been applied for the primary purification of gangliosides. The partition dialysis method introduced by Folch and associates [30] did not work satisfactorily when applied to concentrated lipid solutions. Chromatography on cellulose columns [9] gave a high recovery and considerable purification but the capacity of the column was low. The capacities of anion exchange resins [34] were much larger

but other acid lipids were also retained on the columns. During elution of the gangliosides, a resinous material was released from the column which was very difficult to remove from the gangliosides.

The best recovery of gangliosides, as evaluated from the determination of hexosamine and sialic acid, was obtained by a modification of Folch's partition method. Folch and associates [31,35] have published a procedure similar to that used by us, the main difference being that we increased the number of extractions of the chloroform phase with fresh upper phase (up to seven times).

The final lower phase contained a residual 5—10 % of the original hexosamines and 2—5 % of the total sialic acid. The recovery of gangliosides in the upper phase depended on the concentration of lipids, the ratio of gangliosides to total lipids, and the types of the lipids and gangliosides. In one case of Tay-Sachs disease the recovery of gangliosides in the upper phases was not more than 80 %. Low recoveries of gangliosides were also obtained in lipid extracts of liver and spleen.

From each species 2 kg portions of brain tissue were worked up. The yield of crude gangliosides was about 10 g. The concentration of N-acetylneuraminic acid was 9—12 mg/100 mg and of hexosamine 3—4 mg/100 mg. Besides gangliosides, the crude material contained kephalins, sulfatides, nonlipid material, and, in lesser amounts, cerebrosides, lecithins and sphingomyelins. The nonlipid material constituted more than 10 %. Part of it could be removed from the gangliosides by chromatography on cellulose [36]. After elution of the gangliosides, free amino acids, small peptides and sialic acid-containing glycopeptides were isolated. The difficulty in removing low molecular weight organic material from ganglioside solutions by dialysis is probably due to their participation in the well-known micelle formation of gangliosides in aqueous solutions.

*Chromatography on silicic acid.* The purpose of the chromatography on silicic acid was to remove other lipids from the gangliosides and to separate the ganglioside fraction into three components: Neutral aminoglycolipids, monosialogangliosides and disialogangliosides (eventually higher).

The best separation and the highest capacity of silicic acid columns were achieved by using small particle sized silicic acid. Other workers have added filter aid as Hyflo Supercel or Celite to this type of silicic acid to avoid a very slow flow of solvents at chromatography. Then the chromatographic separation of gangliosides was unsatisfactory. By sedimentation of the screened silicic acid in large volumes of methanol the fine particles were removed, the solvent flow of the silicic acid columns was adequate and only small amounts of silicic acid were released into the eluate.

Several different solvent mixtures were tried such as with tetrahydrofuran, di-isobutylketone and pyridine-butanol, which have been successfully applied for the separation of gangliosides by paper partition chromatography, but they did not give any appreciable separation. Propanol-ammonia-water used by the Thannhauser group [28] gave several fractions but they were mixtures of the types mentioned above. Besides, it was very difficult to remove all the silicic acid eluted in the fractions.

From our experience the best results were obtained with chloroform-methanol mixtures. Since the gangliosides vary in their composition and crude

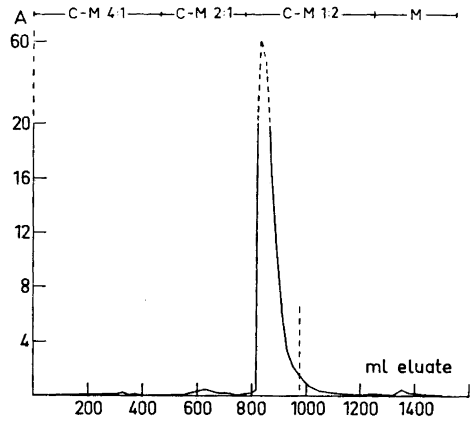



*Fig. 2.* Elution of total gangliosides from a silicic acid column. Ordinate: Concentration of N-acetylneuraminic acid determined by the resorcinol method [21] expressed in absorbancy/ml eluate.

ganglioside extracts contain varying concentrations of other lipids it is not possible to devise a solvent system which is optimal in all instances. The solvent mixtures were selected according to the composition of the total ganglioside extract estimated by paper or thin layer chromatography: If large amounts of cerebrosides and sulfatides were present, the columns were eluted with larger volume of C—M 4:1. When the crude ganglioside extract was contaminated with large amounts of sphingomyelins, the major portion of the gangliosides was eluted with 15—20 column volumes of C—M 3:2. Extracts with large amounts of fast moving gangliosides, such as found in Tay-Sachs disease, were eluted with increased quantities of chloroform-rich solvents.

The chromatography on silicic acid was attended by considerable tailing. We tried to eliminate this disadvantage by the addition of water to the silicic acid and to the solvent mixtures. In general there was no decrease of the tailing, but autohydrolysis of the gangliosides occurred and the eluate fractions were heavily contaminated with silicic acid.

By charging the column with less than 10 mg crude gangliosides per g silicic acid, a separation was achieved but some of the disialogangliosides were contaminated with sphingomyelins and nonlipid material which made it necessary to purify the latter fraction further. Therefore, we have preferred to make a preliminary separation of the gangliosides from other lipids by loading the columns with 25—50 mg crude gangliosides per g silicic acid and elute the gangliosides in a main fraction. About 80 (— 90) % of the gangliosides are recovered in this peak. The rest of the gangliosides tailed together with sphingomyelins and nonlipid material, Fig. 2. The tubes containing the tailing gangliosides from several runnings were combined and evaporated to dryness and dissolved in chloroform-methanol 2:1 v/v. By the addition of one volume water to six volumes of extract, a two phase system appeared. The lower phase contained the sphingomyelins and part of the gangliosides, while most of the gangliosides and nonlipid material went to the upper phase. If there were any sphingomyelins in the upper phase they could be removed by the addition of a small portion of chloroform to the upper phase and new

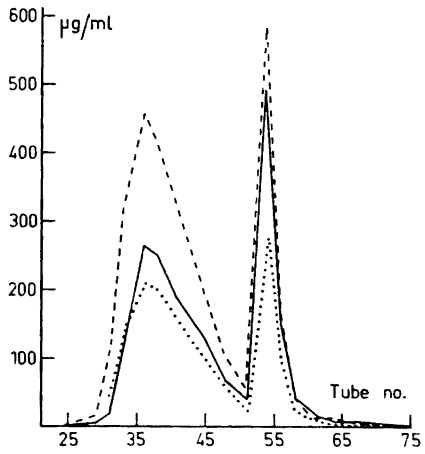



*Fig. 3.* Separation of gangliosides on silicic acid in two main fractions. ————N-acetylneuraminic acid, - - - - - - - hexose and ..... hexosamine. The scale for hexosamine is doubled.

partition. The gangliosides in the lower phase could then be separated from the sphingomyelins by chromatography on alumina. The nonlipid material was removed from the gangliosides by dialysis but still more was freed from the gangliosides by the extraction of the dialysed and lyophilised ganglioside fraction.

The yield of purified brain gangliosides, calculated from the concentration of N-acetylneuraminic acid and hexosamine, was 85—90 %. (The remaining 10—15 % were mainly lost in the testing of the eluates.)

When the crude gangliosides showed neutral reaction in water solutions, no measurable hydrolysis of the gangliosides occurred during the chromatography on silicic acid with the procedure described. But if the extract was acid, sialic acid was split off, the amount depending on pH, temperature and velocity of elution. The largest liberation of sialic acid occurred from the slowest moving gangliosides.

The final separation of the gangliosides was achieved by charging the silicic acid column with not more than 10 mg gangliosides/g silicic acid. As is evident from Figs. 3 and 4, there still was an incomplete separation between the neutral aminoglycolipids and the fastest moving monosialogangliosides. Then without sharp boundary the major portion of the monosialogangliosides appeared. After this peak a mixture of mono- and disialogangliosides appeared, and then came the second main peak containing mainly disialogangliosides. There was some separation of the three different slow-moving gangliosides, but in general, we have not separated them but combined them. The tubes containing the two main peaks of gangliosides were each evaporated, and the gangliosides were crystallized from methanol-ethanol. The yield of mono- and disialo-gangliosides from human and calf brain gangliosides was 25—30 % and 30—35 %, respectively.

The mother liquors from crystallization of the monosialogangliosides were pooled together with all forefractions, and after evaporation, exhaustive dialysis

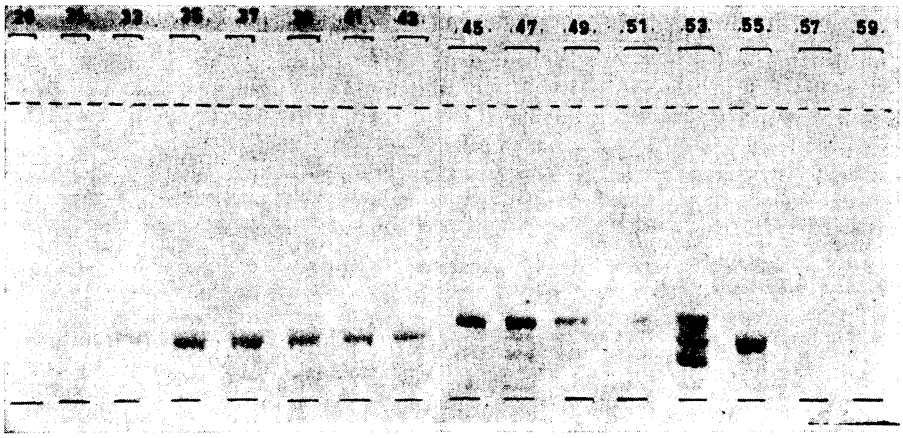



*Fig. 4.* Thin layer chromatogram of eluates from the silicic acid column shown in Fig. 3. Solvent: propanol-water 3:1 v/v. Spray: resorcinol reagent.

against distilled water and lyophilisation, a rechromatography on silicic acid was performed. At this time some tubes with sialic acid-free aminoglycolipid (neutral) was obtained. In the subsequent tubes there was a mixture of neutral aminoglycolipids and a fast running monosialoganglioside which was later shown to have the same $R_F$-value as the predominating ganglioside of a Tay-Sachs brain *. Sometimes this ganglioside was obtained in a rather pure state between the neutral aminoglycolipids and the main type of monosialogangliosides.

In the same manner, the mother liquors from the crystallization of the disialogangliosides and the tubes containing mixtures of mono- and disialogangliosides were recromatographed and further amounts of mono- and disialogangliosides were collected.

From 1 g of unseparated gangliosides a total of 300—350 mg of monosialogangliosides and 400—450 mg of disialogangliosides were obtained in this manner. The yield of neutral aminoglycolipids was not more than 1—2 % of total gangliosides. However, from small scale preparations it is evident that their concentration in brain tissue is higher, perhaps up to 5 %. It is probable that a loss of them occurred at the partition of the lipids during the preparation of the crude ganglioside extract. The greater loss of hexosamine than of sialic acid at this stage of the preparation is in consistence with this assumption. A hexosamine-containing, sialic acid-free glycolipid has been observed by Weiss [37] during the separation of brain glycolipids on silicic acid columns and it is probably the same type of compound.

*Analytical chromatography of gangliosides.* During the first years of this work quantitative analysis of hexosamine, sialic acid and hexose, and paper

* Svennerholm, L. *Biochem. Biophys. Res. Comm.* **9** (1962) 436.

partition chromatography were used for checking the isolation steps. Of the solvents used for paper chromatography, tetrahydrofuran-di-isobutylketone-water 45:5:6 and di-isobutylketone-acetic acid-water 40:30:7 gave the best separation of neutral aminoglycolipids and monosialogangliosides. The disialogangliosides were better separated with tetrahydrofuran-di-isobutylketone-water 50:5:9 or with a pyridine-containing solvent [26].

In 1961 we changed from paper to thin layer chromatography which gives a more distinct separation and permits the detection of trace amounts of impurities. For the separation of neutral aminoglycolipids and the different monosialogangliosides, chloroform-methanol mixtures have been preferred, while the slow moving gangliosides have been best separated with propanol-water mixtures.

*The composition of gangliosides.* The fatty acid pattern of the gangliosides has been analysed with gas phase chromatography by Dr. S. Stenhagen and

*Table 1.* Carbohydrate composition of gangliosides.

| *Material* | Galactosamine % | Hexose [a] % | N-Acetylneuraminic acid % | Molar ratio (galactosamine: hexose:N-acetylneuraminic acid) |
|---|---|---|---|---|
| *Human brain:* | | | | |
| Total gangliosides | 7.9 | 25.7 | 24.0 | 1.0 : 3.2 : 1.8 |
| Monosialogangliosides | 11.9 | 35.5 | 20.6 | 1.0 : 3.0 : 1.0 |
| Disialogangliosides | 9.0 | 28.6 | 31.5 | 1.0 : 3.2 : 2.0 |
| Tay-Sachs gangliosides | 11.2 | 24.5 | 19.9 | 1.0 : 2.1 : 1.0 |
| *Calf brain* | | | | |
| Total gangliosides | 9.3 | 26.3 | 23.7 | 1.0 : 2.8 : 1.5 |
| » » [b] | 7.5 | 28.6 | 26.3 | 1.0 : 3.8 : 2.0 |
| Monosialogangliosides | 11.7 | 34.0 | 19.2 | 1.0 : 2.9 : 0.9 |
| Disialogangliosides | 9.6 | 28.6 | 32.0 | 1.0 : 3.0 : 1.9 |
| *Pig brain* | | | | |
| Total gangliosides | 9.6 | 33.8 | 22.4 | 1.0 : 3.5 : 1.4 |
| *Sheep brain* | | | | |
| Total gangliosides | 9.1 | 31.8 | 26.7 | 1.0 : 3.5 : 1.7 |

[a] As reference standard was used a mixture of two parts of galactose and one part of glucose.
[b] The crude ganglioside extract was isolated with the procedure developed by Rosenberg and Chargaff (*J. Biol. Chem.* **232** (1958) 1031).

will be reported on in a separate communication *. Stearic acid constituted from 80—90 % of the fatty acids.

The sphingosine has not yet been characterized. The quantitative analyses of sphingosine as long chain base nitrogen have not been reliable and recent experiments show the recovery to be only 80 %. With the procedure proposed by Long and Staples [39], the recovery was less, or about 70 %.

* Stenhagen, S., and Svennerholm, L. *To be published.*

The determinations of the carbohydrate components (Table 1) have given somewhat lower values than expected. In general it depends on the admixture of the compounds with column material. The admixture is rather low in the mono- and disialogangliosides but larger in the neutral aminoglycolipid preparations. The silicic acid content of the compounds also caused difficulties in the elemental analyses of carbon and hydrogen.

The available analytical data of the gangliosides show that the main type of monosialogangliosides are ceramide-pentasaccharides. The sequence of the neutral carbohydrates has been shown to be glucose—galactose—N-acetyl-galactosamine—galactose, by stepwise degradation of the gangliosides, isolation and analyses of the ceramide-saccharides. The disialogangliosides contain 2 moles of N-acetylneuraminic acid and can be converted by enzymic hydrolysis with neuraminidase to monosialogangliosides of the composition just mentioned. The experimental work on the elucidation of the structure of gangliosides will be described in a following communication.

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