Brain ceramide hexosides in Tay-Sachs disease and generalized gangliosidosis (G_{M1}-gangliosidosis)

KUNIHIKO SUZUKI and GLORIA C. CHEN

The Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, New York 10461

ABSTRACT The carbohydrate composition was determined for ceramide hexosides isolated from brains of patients with Tay-Sachs disease and generalized gangliosidosis (hereby named G_{M1}-gangliosidosis).

Gray matter of patients with each disease showed a characteristic abnormal ceramide hexoside pattern. In Tay-Sachs gray matter, ceramide trihexoside is the major component, whereas ceramide tetrahexoside is barely detectable. In G_{M1}-gangliosidosis, ceramide tetrahexoside is the major ceramide hexoside, while ceramide trihexoside is present only in small amount. These two major components have been characterized as the asialo derivatives of, respectively, the "Tay-Sachs ganglioside" (G_{M2}-ganglioside) and the normal major monosialoganglioside (G_{M1}-ganglioside).

In both diseases, more than half the ceramide monohexoside of gray matter was glucocerebroside. Gray matter ceramide dihexoside, present in both diseases at higher than normal levels, was mostly ceramide lactoside, with possibly a small amount of ceramide digalactoside. Sulfatide contained only galactose.

The abnormal ceramide hexoside pattern is limited to gray matter: white matter showed normal ceramide hexosides, i.e. a preponderance of monohexosides and sulfatide, with no detectable glucocerebroside.

KEY WORDS Tay-Sachs disease (G_{M2}-gangliosidosis) · generalized gangliosidosis (G_{M1}-gangliosidosis) · systemic late infantile lipidosis · ceramide hexosides · glucocerebroside · ganglioside · gray matter · white matter · man

TWO CHEMICALLY SPECIFIC inborn errors of metabolism that result in an abnormal accumulation of gangliosides are now known. The classical infantile amaurotic idiocy (Tay-Sachs disease) has long been known clinicopathologically, and it was in a brain of a patient with this disease that Klenk first discovered gangliosides (1). In this disorder the total level of brain ganglioside is greatly elevated, and one species of monosialoganglioside accumulates. This Tay-Sachs ganglioside (G_{M2}) has been shown to be identical with the normal major monosialoganglioside except that it lacks the terminal galactose (2, 3). In 1963, Jatzkewitz and Sandhoff reported a "biochemically special form of infantile amaurotic idiocy" where, instead of the Tay-Sachs ganglioside, the normal major monosialoganglioside (G_{M1}) is stored (4). Because their specimen had been preserved in formalin for a prolonged period (26 yr), their finding was only suggestive (long exposure of brain tissue to formalin partially destroys gangliosides and produces a ganglioside pattern in which the normal major monosialoganglioside predominates) (5). However, recently the disorder of two patients was simultaneously and independently identified (6, 7) as a gangliosidosis characterized by storage of the normal major monosialoganglioside G_{M1}. In both cases unfixed, frozen brains were analyzed; in one of them the accumulated ganglioside was identified, beyond any reasonable doubt, as G_{M1} ganglioside (8). Since then we have examined four additional fresh-frozen specimens in which the accumulations of G_{M1}-ganglioside could be established. Thus, the existence of this newly recognized entity has been well established.

Gatt and Berman (9) isolated and characterized two glycolipids from two brains of patients with Tay-Sachs disease. These were ceramide trihexoside, which has the same carbohydrate composition of the asialo derivative of Tay-Sachs ganglioside, and a ceramide dihexoside, which they characterized as ceramide digalactoside. Makita and Yamakawa (10) also isolated the ceramide

Abbreviation: TLC, thin-layer chromatography.

Nomenclature of gangliosides is that of Svennerholm (21).

trihexoside from a brain of a patient with Tay-Sachs disease and characterized it as *N*-acetylgalactosaminyl (1 → 4) galactosyl (1 → 4) glucosylceramide.

On the other hand, Jatzkewitz, Pilz, and Sandhoff (11) reported an abnormally high level of ceramide tetrahexoside in their "biochemically special form of infantile amaurotic idiocy." No carbohydrate composition was given, and the reservation with regard to prolonged formalin preservation of their material mentioned above applies here too.

The ceramide hexosides from a brain with G_{M1}-gangliosidosis were therefore isolated and characterized as the preliminary step of our detailed lipid analysis of this disease. For reference and comparison, the brain of a patient with Tay-Sachs disease was similarly investigated.

MATERIAL

The pathological brains used for this study were obtained post mortem and kept frozen at −60°C until analysis. A normal 3 yr old control brain was similarly obtained and analyzed simultaneously with the pathological specimens. The diagnoses of Tay-Sachs disease (G_{M2}-gangliosidosis) and generalized gangliosidosis (G_{M1}-gangliosidosis) were established unequivocally by the pathognomonic abnormal ganglioside patterns of the respective diseases.

The patient with Tay-Sachs disease died at the age of 30 months after exhibiting a typical clinical picture of Tay-Sachs disease. Post-mortem examination revealed no histological involvement of liver, spleen, or other visceral organs. The patient with generalized gangliosidosis was first admitted to the hospital at the age of 14 months for evaluation of mental and developmental retardation, difficult breathing, and the inability to eat solid food without regurgitation. The child appeared to have developed reasonably well up to 7 months, but then growth was arrested and the patient could never sit up. The liver was palpable 1 cm below the right costal margin, but the spleen was not palpable. Head support and the control of pharyngeal mucus were poor. Myoclonic jerks were present and deep tendon reflexes were hyperactive throughout. There was heavy pigmentation around the optic discs at the age of 16 months, but no cherry-red spots. Bone survey by X-ray revealed a 7 month bone age at the chronological age of 14 months, but none of the deformities that O'Brien, Stern, Landing, O'Brien, and Donnel (7) considered characteristic of this disease. Electroencephalograms taken at 14 and 16 months were interpreted as normal for the age. Rectal biopsy and brain biopsy from the right temporal lobe 18 months before death revealed lipid accumulation in the ganglion cells. In addition, foamy macrophages containing cytoplasmic periodic acid–Schiff (PAS)-positive material were present in the rectal mucosa. The patient gradually became blind, and showed fasciculations of tongue beginning at the age of 17 months. The neurological condition worsened to the decerebrate state. The patient developed pneumonia repeatedly and died of pneumonia and cachexia at the age of 37 months.

Examined at autopsy, all organs were markedly atrophic. There were no definite skeletal abnormalities, although bony growth was retarded. Hematoxylin–eosin-stained sections taken from liver, spleen, lymph nodes, bone marrow, lungs, and colon revealed swollen foamy cells that contained faintly eosinophilic, granular material, which was strongly PAS-positive. Nearly all of the neurons of the cerebral cortex, basal ganglia, cerebellum, thalamus, brain stem, and spinal cord were abnormally swollen. The PAS-positivity of the cytoplasm was variable; most neurons were negative or only faintly positive, while a few were strongly positive. Many swollen glial cells in the cerebral cortex and cerebellar granular layer were strongly PAS-positive and stained faintly with Sudan, while similar swollen cells in the white matter were strongly sudanophilic and only slightly PAS-positive. Severe demyelination and gliosis were present in the white matter.

Further details of the clinical, histochemical and electron microscopic observations will be reported elsewhere (K. Suzuki, K. Suzuki, and G. C. Chen, manuscript submitted for publication).

METHODS

Extraction and Isolation of Ceramide Hexosides

Gray and white matter were carefully separated, and approximately 5 g wet weight of tissue was extracted with 19 volumes of chloroform–methanol 2:1 at room temperature for 5 min in a Lourdes homogenizer (Lourdes Instrument Corp., Brooklyn, N.Y.) (12). The extract was filtered through a sintered glass funnel and separated into two phases after the addition of 0.2 volume of water. The upper phase was removed, and the lower phase was washed four times with solvent equivalent to the upper phase in composition but containing no inorganic salt (Folch's "pure solvent upper phase") (12). This extensive washing of the lower phase was needed to ensure complete extraction of the large amounts of relatively nonpolar gangliosides into the upper phase (5). The lower phase was evaporated to dryness and the residue was dissolved in chloroform–methanol 2:1 (saturated with water). Proteolipid protein was denatured and rendered insoluble by repeated drying of the sample in the above solvent system.

After proteolipid protein had been removed by centrifugation, the lower phase lipids were subjected to the $HgCl_2$-saponification procedure essentially as described

by Abramson, Norton, and Katzman (13). This procedure eliminated all glycerophosphatides and left a mixture of fatty aldehydes, fatty acids, cholesterol, sphingomyelin, ceramide hexosides, and sulfatide.

The above mixture of lipids was subjected to TLC on 0.25 mm layers of Silica Gel G, in chloroform–methanol–water 70:30:4. Fatty aldehydes, fatty acids, and cholesterol all ran close to the solvent front. Ceramide mono-, di-, and tri-, and tetrahexosides, as well as sulfatide, were cleanly separated from each other. Sphingomyelin and ceramide tetrahexoside ran close together, although the separation was clean. Since the carbohydrate chains of ceramide hexosides were the main concern of this phase of the investigation, possible slight contamination of ceramide tetrahexoside by sphingomyelin was judged unobjectionable. As the chromatographic standard, a mixture of ceramide hexosides was used; it was prepared from the total ganglioside of a normal human brain by partial acid hydrolysis as described by Ledeen, Salsman, Gonatas, and Taghavy (8). 20 plates were used for the preparative TLC for each sample. Ceramide hexosides were located by brief exposure of the plates to iodine vapor. Again, iodine was judged to be unobjectionable for the study of carbohydrate moieties of ceramide hexosides. Zones of silica gel scraped off the plates were extracted with chloroform–methanol–water 10:20:3 with agitation at 37°C, centrifuged, and extracted twice more in the same manner. The combined extracts were evaporated to dryness under nitrogen. To the samples, dissolved in chloroform–methanol 2:1 and filtered through sintered glass, 0.2 volume of water was added. The upper phase was discarded, and the lower phase washed twice with the "pure solvent upper phase" without salt. The final lower phase was dried under nitrogen.

Identification and Determination of Carbohydrates

Appropriate amounts of the isolated ceramide hexoside fractions were hydrolyzed in sealed ampules with 1 N HCl at 100°C for 16 hr. After hydrolysis, the samples were extracted with the addition of chloroform. The chloroform phase was discarded, and this washing procedure was repeated three more times.

For the qualitative identification of carbohydrates, portions of the washed hydrolysate were applied to Whatman 3MM paper for descending paper chromatography for 20–24 hr in *n*-butanol–pyridine–water 6:4:3. A mixture of standard monosaccharides was chromatographed on the same sheet, and the spots were located by the silver nitrate–sodium hydroxide method. The above solvent separated mannose, glucose, galactose, glucosamine, and galactosamine satisfactorily for qualitative purposes.

Once the carbohydrate compositions were known qualitatively, the remainder of the hydrolysate was used for the quantitative determination of each monosaccharide. Determination methods used were: glucose oxidase reagent (Glucostat, Worthington Biochemical Corporation, Freehold, N.J.) for glucose; orcinol method (14) for neutral hexoses; and a modified method using the Elson-Morgan reaction (15) for hexosamine. With the authentic standard monosaccharides either alone or as mixtures of various concentrations, it was found that glucose and galactosamine could be determined directly on any mixture of these three sugars without interference, and that galactosamine interfered in the orcinol procedure to a negligible extent unless it was present in great excess over the neutral hexoses. For the Glucostat reagent, it was necessary to include standard glucose treated in exactly the same way as the samples from the acid hydrolysis step, because we found that the standard glucose thus treated consistently gave only 71% of the optical density of untreated glucose. This finding is in agreement with that reported by Suomi and Agranoff (16). The relative molar extinction for galactose and glucose was 1:0.77 in the orcinol procedure. Therefore, when the amount of glucose was known from the Glucostat determination, the amount of galactose could be calculated. Thus, reasonably reliable sugar ratios could be determined directly on the total mixture of the three sugars without further separation.

Study of Partial Hydrolysis Products

The products of partial hydrolysis were investigated, whenever a sufficient quantity of ceramide hexoside was available, in order to determine the sequence of monosaccharides. The samples were suspended in 0.1 ml of methanol in tubes with tight-fitting screw caps with Teflon lining, and 2.0 ml of 0.1 N HCl was added. The tubes were heated at 100°C for 15 min. Samples were partitioned by addition of 10 ml of chloroform–methanol 2:1. The upper phase was discarded, and the lower phase was washed twice with the "pure solvent upper phase" without salt, and dried at 37°C under nitrogen. The products of partial hydrolysis (ceramide hexosides with shorter carbohydrate chains) and the remaining unhydrolyzed original material were then separated and purified as described for the preparation of ceramide hexosides. The partial hydrolysis procedure was repeated if necessary until a sufficient amount for carbohydrate identification had been obtained. The carbohydrate compositions of the partial hydrolysis products were qualitatively determined by acid hydrolysis followed by paper chromatography as described above.

Quantitative Analysis of Ceramide Hexosides

The procedures used for extraction and purification of individual ceramide hexosides were not quantitative.

Significant amounts of ceramide tetrahexoside, and, to a lesser extent, ceramide trihexoside and sulfatide, were lost, mostly during the partition steps that occur in the procedure (the extraction, the $HgCl_2$-saponification, and the elution of ceramide hexosides from TLC). In order to obtain satisfactorily quantitative data, we took several 1 g tissue samples and prepared the lower phase lipids from them in the same way as described above, except that the lower phase was washed with "pure solvent upper phase" only twice. The lower phase lipids were chromatographed without $HgCl_2$-saponification, and the TLC zones were scraped and extracted as described, but without the partition procedure. Each fraction was analyzed for hexose by the orcinol method (14). The only ceramide hexosides present in detectable amounts in the upper phase (ganglioside) fraction were ceramide trihexoside in Tay-Sachs gray matter and ceramide tetrahexoside in gray matter of G_{M1}-gangliosidosis. Therefore, the upper phase fractions of these samples were similarly analyzed.

As the carbohydrate composition of each ceramide hexoside had been determined before this analytic step, the actual amount of the individual ceramide hexosides could be calculated from the simple orcinol values on the basis of the carbohydrate composition and the relative molar extinctions of glucose and galactose. The over-all recovery of these glycolipids, when eluted from the TLC plates as described above, was about 90%. The following molecular weights were used as approximations: galactocerebroside, 823; glucocerebroside, 727; ceramide dihexoside, 889; ceramide trihexoside, 1092; ceramide tetrahexodide, 1254; and sulfatide, 933. These molecular weights were based on the following assumptions: (*a*) except for galactocerebroside and sulfatide, the only fatty acid present is stearic acid; (*b*) the long-chain base is sphingosine; (*c*) the aminosugar is *N*-acetylated. The molecular weights given for galactocerebroside and sulfatide are our empirical values obtained for normal brains: two-thirds of galactocerebroside is assumed to contain hydroxy fatty acid, and sulfatide is presumed to be an equimolar mixture of sodium and potassium salts. Because of all these assumptions, the analytical values given for each compound could have an error of ±10%.

Other Analytical Procedures

For the separation of gluco- and galactocerebrosides, borate-impregnated Silica Gel G plates (17, 18) were developed with chloroform–methanol–2.5 N ammonia 70:30:3. In general, spots were located by means of a 50% sulfuric acid spray followed by heating. For selective detection of glycolipids, either the diphenylamine method of Jatzkewitz and Mehl (19) or an α-naphthol spray (20) was employed.

RESULTS

Ceramide Hexoside Patterns

The over-all glycolipid patterns of gray and white matter of normal, Tay-Sachs, and G_{M1}-gangliosidosis are shown semiquantitatively in Fig. 1. Approximately the same amounts of total lipid were applied for each of the gray matter samples and for each of the white matter samples; spots are comparable within gray matter or white matter but not between gray and white matter. From this figure, it is clear that the abnormal patterns of ceramide hexosides are limited chiefly to the gray matter of these pathological brains; white matter lipids exhibit essentially normal glycolipid patterns. In the gray and white matter of normal brain, the major glycolipids are cerebrosides (ceramide monohexosides) and sulfatides. In gray matter of Tay-Sachs brain, the major glycolipid is ceramide trihexoside; a significant amount of ceramide dihexoside is present. On the other hand, only one band of ceramide monohexoside is visible and its amount appears to be lower than normal. In Tay-Sachs gray matter, ceramide tetrahexoside is barely visible. Apart from a faint spot showing the presence of ceramide trihexoside, the glycolipids of Tay-Sachs white matter appear to be normal. In G_{M1}-gangliosidosis, the major ceramide hexoside in gray matter is ceramide tetrahexoside, and ceramide trihexoside is hardly detectable. Interestingly, ceramide dihexoside is present in similar relative amounts in G_{M1}-gangliosidosis gray matter and in Tay-Sachs gray matter. The glycolipids in white matter of G_{M1}-gangliosidosis are, as in Tay-Sachs disease, essentially normal except for a faint ceramide tetrahexoside spot. The pattern of Tay-Sachs gray matter is what was expected from the data reported by Gatt and Berman (9), and that of G_{M1}-gangliosidosis is consistent with the high ceramide tetrahexoside data reported by Jatzkewitz et al. (11) on a formalin-preserved specimen. These investigators, however, did not point out the clear-cut confinement of these abnormal patterns to gray matter. We have since confirmed these abnormal glycolipid patterns in four additional cases of G_{M1}-gangliosidosis. A detailed analysis of the lipids found in these fives cases will be reported separately (K. Suzuki and G.C. Chen, data in preparation).

Carbohydrate Analysis

The qualitative paper chromatogram for ceramide hexosides in gray matter of G_{M1}-gangliosidosis is shown in Fig. 2 (carbohydrates from gray matter ceramide hexosides of Tay-Sachs disease gave an identical paper chromatogram). A few aspects are noteworthy. More than half the cerebroside in gray matter in both diseases appears to be glucocerebroside, in contrast to normal brain cerebroside, which is all galactocerebroside. In

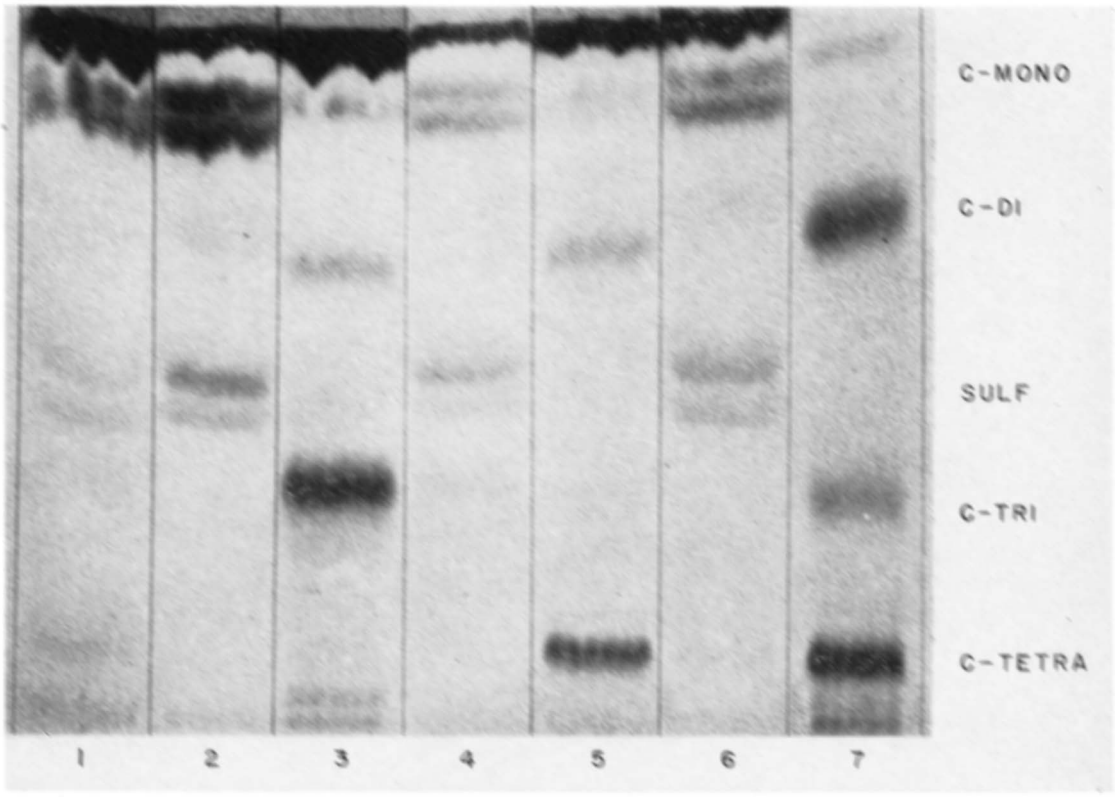


FIG. 1. Thin-layer chromatogram of brain ceramide hexosides of gray and white matter of normal subject, Tay-Sachs patient, and G_{M1}-gangliosidosis patient. Solvent, chloroform–methanol–water 70:30:4; α-naphthol spray. *1*, normal gray matter; *2*, normal white matter; *3*, Tay-Sachs gray matter; *4*, Tay-Sachs white matter; *5*, G_{M1}-gangliosidosis gray matter; *6*, G_{M1}-gangliosidosis white matter; *7*, mixture of ceramide hexosides prepared by partial acid hydrolysis from gangliosides. C-mono, ceramide monohexoside; C-di, ceramide dihexoside; Sulf, sulfatides; C-tri, ceramide trihexoside; C-tetra, ceramide tetrahexoside.

spite of such a large amount of glucocerebroside, the sulfatide contains only galactose. Ceramide dihexoside contains both glucose and galactose. Ceramide trihexoside and ceramide tetrahexoside contain, in addition to glucose and galactose, galactosamine. The galactose spot in ceramide tetrahexoside appears to be darker than the other two, which suggests that this molecule might contain two galactose moieties.

Glucocerebroside is present only in gray matter. Fig. 3 shows a paper chromatogram of the sugars of ceramide monohexoside from gray and white matter of Tay-Sachs disease and G_{M1}-gangliosidosis. Although in both diseases glucose is predominant in ceramide monohexoside of gray matter, only galactose was detected from cerebroside of white matter. This finding is also corroborated by the borate-impregnated silica gel TLC, which showed large amounts of glucocerebroside in gray matter of both diseases but not in white matter of either disease.

That these carbohydrate patterns were not the result of artifacts due to breakdown of higher ceramide hexosides or of gangliosides during the extraction and isolation procedure was ascertained in the following manner. (*a*) When isolated gangliosides, isotopically labeled in vivo by injection of either D-glucose-U-^{14}C or D-glucosamine-1-^{14}C, were added to brain tissue being extracted with chloroform–methanol 2:1, all the radioactivity was recovered in the dialyzed upper phase and none in the lower phase (K. Suzuki, unpublished data). (*b*) Portions of isolated ceramide di-, tri-, and tetrahexosides were once more carried through the $HgCl_2$-saponification procedure in the manner described. No breakdown of any ceramide hexosides to those of shorter carbohydrate chains was observed by TLC. (*c*) The same carbohydrate patterns were obtained when ceramide hexosides were isolated from total lipids before the $HgCl_2$-saponification treatment. (*d*) The presence of glucose in the cerebroside fraction from gray matter was also demonstrated by means of the Glucostat reagent when cerebroside was prepared from TLC of total lipids without the $HgCl_2$-saponification procedure.

Table 1 summarizes the quantitative data on the carbohydrate compositions of these ceramide hexosides from gray matter. The amounts of ceramide tetrahexoside of Tay-Sachs disease and the ceramide trihexoside of G_{M1}-gangliosidosis were small, and the error in the analytical results for these compounds would be larger than in others. Although by no means conclusive,

the data are consistent with the ideas (*a*) that corresponding ceramide hexosides in these two diseases are the same compound, and (*b*) that they have carbohydrate compositions which result from sequential removal of monosaccharide units from the asialo derivative of the normal major monosialoganglioside (G_{M1}). Ceramide dihexoside contains both glucose and galactose; ceramide trihexoside contains glucose, galactose, and galactosamine in equimolar amounts, and ceramide tetrahexoside has another galactose moiety added. The meaning of the molar ratio of glucose and galactose in ceramide monohexoside is, of course, entirely different, because this fraction is a mixture of two different compounds, gluco- and galactocerebrosides. The sugar ratio of this fraction indicates the relative amounts of these cerebrosides present. Thus, the previous impression that more glucocerebroside than galactocerebroside is present in gray matter of these disorders was quantitatively confirmed.

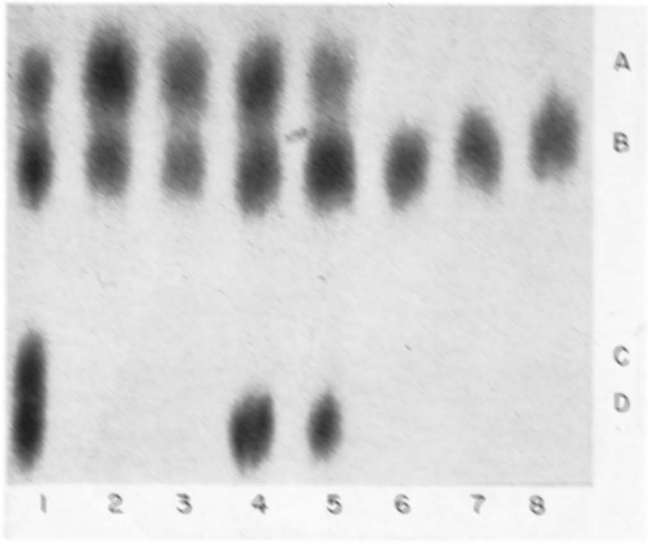


FIG. 2. Paper chromatogram showing the carbohydrate compositions of ceramide hexosides from gray matter of G_{M1}-gangliosidosis. Solvent, *n*-butanol–pyridine–water 6:4:3, descending. *1*, a mixture of standard monosaccharides; *2*, ceramide monohexoside; *3*, ceramide dihexoside; *4*, ceramide trihexoside; *5*, ceramide tetrahexoside; *6*, sulfatide; *7*, ceramide monohexoside from normal brain; *8*, sulfatide from normal brain. A, glucose, B, galactose; C, glucosamine; D, galactosamine.

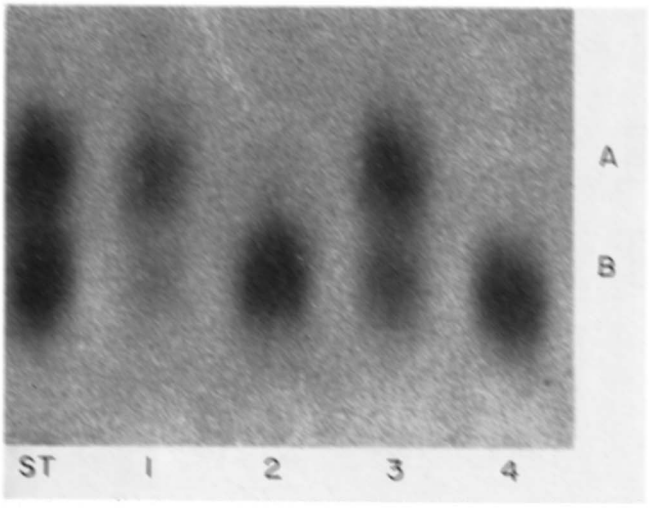


FIG. 3. Paper chromatogram of the carbohydrate compositions of ceramide monohexosides. Solvent, *n*-butanol–pyridine–water 6:4:3, descending. *ST*, standard glucose and galactose; *1*, Tay-Sachs gray matter; *2*, Tay-Sachs white matter; *3*, G_{M1}-gangliosidosis gray matter; *4*, G_{M1}-gangliosidosis white matter. A, glucose; B, galactose.

Partial Hydrolysis Study

Since the yields of the partial hydrolysis products were poor, a relatively large quantity was required for the study. Because of this limitation, the study of partial hydrolysis products could not be carried out on ceramide tetrahexoside of Tay-Sachs disease or ceramide trihexoside of G_{M1}-gangliosidosis. The ceramide tetrahexoside of G_{M1}-gangliosidosis gave rise to a ceramide trihexoside containing glucose, galactose, and galactosamine, a ceramide dihexoside containing glucose and galactose, and a ceramide monohexoside containing only glucose. The ceramide trihexoside of Tay-Sachs disease produced a ceramide dihexoside containing glucose and galactose, and a ceramide monohexoside containing glucose only. Ceramide dihexoside from both brains was split to a ceramide monohexoside that was mostly glucocerebroside. However, on the paper chromatogram, faint spots corresponding to galactose were visible; this suggests that the ceramide dihexoside fraction may contain, as a minor component, a ceramide digalactoside that was originally reported in Tay-Sachs brain by Gatt and Berman (9). Therefore, most of ceramide dihexoside in the two brains studied appears to be ceramide lactoside rather than ceramide digalactoside.

Relative Amounts of Ceramide Hexosides in Brains

The relative amounts of these ceramide hexosides are compared with the control values from a 3 yr old normal brain (Table 2). The values given for the ceramide trihexoside of Tay-Sachs gray matter and for the ceramide tetrahexoside of G_{M1}-gangliosidosis gray matter are the sums of those found in the upper and lower phases. Approximately 5% of ceramide trihexoside and 30% of ceramide tetrahexoside were found in the upper phase. Although these ceramide hexosides were expected to be present in similar quantities in the upper phases of white matter samples or of normal gray matter, the actual amounts were too small for quantitative determination. In none of the samples was ceramide mono- or dihexoside, or sulfatide present in the upper phase in sufficient amounts for quantitative determination.

TABLE 1 CARBOHYDRATE COMPOSITION OF ISOLATED CERAMIDE HEXOSIDES

Ceramide Hexosides	Molar Ratio		
	Glucose	Galactose	Galactosamine
Tay-Sachs disease			
Monohexoside	1.00	0.69	—
Dihexoside	1.00	0.94	—
Trihexoside	1.00	0.96	1.12
Tetrahexoside	1.00	1.79	1.11
G_{M1}-gangliosidosis			
Monohexoside	1.00	0.87	—
Dihexoside	1.00	1.06	—
Trihexoside	1.00	1.17	1.15
Tetrahexoside	1:00	2.02	1.01

TABLE 2 GLYCOLIPIDS IN TAY-SACHS DISEASE, G_{M1}-GANGLIOSIDOSIS, AND A NORMAL CONTROL BRAIN (3 YR OLD)

Ceramide Hexosides	Gray Matter			White Matter		
	Normal	Tay-Sachs Disease	G_{M1}-Gangliosidosis	Normal	Tay-Sachs Disease	G_{M1}-Gangliosidosis
Ceramide monohexoside						
Glucoside	0	0.53	0.74	0	0	0
Galactoside	1.55	0.37	0.64	23.3	7.42	7.10
Ceramide dihexoside	0.23	0.98	1.17	tr.	0.83	0.16
Ceramide trihexoside	0.32	2.86	0.43	0	0.91	0.59
Ceramide tetrahexoside	0.09	0.41	4.10	0	0.75	0.40
Sulfatide	0.45	0.36	1.08	4.0	3.77	2.26

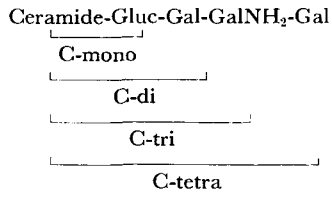
Expressed as weight per cent of total lipids based on the approximate molecular weights of individual ceramide hexosides, as given under Methods (*Quantitative Analysis of Ceramide Hexosides*).

The glycolipid patterns of white matter in these diseases are similar, and both can be considered close to normal in that they show no glucocerebroside and very low proportions of ceramide di-, tri-, and tetrahexosides. Actual amounts of cerebroside and sulfatide are lower than the control, which probably reflects the considerable degree of demyelination observed histologically in both cases. In gray matter of Tay-Sachs disease, ceramide trihexoside constitutes over half the total glycolipid in the lower phase, whereas in G_{M1}-gangliosidosis gray matter, half of the total glycolipid is ceramide tetrahexoside. Significant amounts of ceramide hexosides of various carbohydrate chain lengths are present in the control 3 yr old gray matter.

DISCUSSION

The disease characterized by excessive storage of the normal major monosialoganglioside (G_{M1}) has been variously termed generalized gangliosidosis (7), systemic late infantile lipidosis (6), and biochemically special form of infantile amaurotic idiocy (4). The first two names were based on the fact that not only the nervous system but other visceral organs such as liver or spleen are also involved in this disorder. None of these terminologies, however, indicates the specific chemical abnormality found in this disease, and all of them may become the source of confusion if other types of gangliosidoses are discovered in the future. We propose, therefore, a systematic and unambiguous nomenclature for chemically delineated gangliosidoses. They are to be called gangliosidosis prefixed by the specific ganglioside involved in each disorder. Since there is at present no standard nomenclature for gangliosides, our proposed system for gangliosidoses is inevitably tentative, and we have adopted Svennerholm's nomenclature (21) [for various nomenclatures for gangliosides, see Ledeen (22)]. According to our system, Tay-Sachs disease should be called G_{M2}-gangliosidosis, and generalized gangliosidosis, G_{M1}-gangliosidosis. As the term Tay-Sachs disease is well established and gives little danger of confusion at this time, we use this term here. Our nomenclature can be subclassified with suitable adjectives, if subclassification becomes necessary in the future.

Among the glycolipids found in these diseases, galactocerebroside, sulfatide, and the possible small amount of ceramide digalactoside belong to a group chemically unrelated to other ceramide hexosides or major gangliosides of the brain because they have galactose next to ceramide. The rest of the ceramide hexosides appear to be chemically related, and in regard to the carbohydrate compositions and sequence, they fit into the following scheme.



The ceramide tetrahexoside in brain of G_{M1}-gangliosidosis is not the type found in erythrocyte stroma (23) and named globoside by Yamakawa, Yokoyama, and Handa. Globoside has the same carbohydrate composition as the ceramide tetrahexoside discussed, but the galactosamine moiety is terminal. The ceramide tetrahexoside in human kidney (Cytolipin K) (24) is probably identical with globoside and also possesses the terminal *N*-acetylgalactosamine. It must be pointed out, however, that, although likely, the above conclusion regarding the chemical relationship among ceramide hexosides is still tentative, because the limited amount of each ceramide hexoside available did not permit the study of carbohydrate linkages, sphingosine content, or fatty acid composition.

These ceramide hexosides are present only in trace amounts in gray matter of adult brains. In a 2.5 month old normal brain, we found the proportions of ceramide hexosides to be higher than those in the 3 yr old control brain. Svennerholm (25) reported even higher levels of various ceramide hexosides in normal fetal, premature, and full-term human infant brains. His analytical data were on whole brains and, therefore, not directly comparable to our data. On TLC, Svennerholm showed two bands corresponding to ceramide dihexoside, but ceramide dihexoside in our pathological brains exhibited only one band (Fig. 1). Svennerholm (25) also reported a small but definite amount of glucocerebroside as a normal constituent in human brains until birth. No glucocerebroside was found in our 3 yr old control brain. However, Schwarz, Dreisbach, Barrionuevo, Kleschick, and Kostyk (26) reported glucocerebroside in human brains in old age. Nishimura, Ueta, and Yamakawa (27) recently found a small but metabolically active glucocerebroside fraction in rat and guinea pig brains. Svennerholm found only galactose in the sulfatide fraction from the human fetal brains in which a significant quantity of glucocerebroside was present. Our finding on the sulfatide in these pathological brains is the same as in his finding on fetal brains.

The presence of a large amount of glucocerebroside in Tay-Sachs disease brain has not been well documented. Makita and Yamakawa (10) found only galactose after hydrolysis of cerebroside from a brain with Tay-Sachs disease. Samuels, Korey, Gonatas, Terry, and Weiss (28) found only glucocerebroside in the isolated Tay-Sachs membranous cytoplasmic bodies. In the gray matter of the Tay-Sachs brain we examined, more glucocerebroside than galactocerebroside was present. This finding is unequivocal, since artifactual breakdown of higher glycolipids are ruled out, and the sugar chromatogram, borate-impregnated TLC, and glucose oxidase reagent all indicated the same conclusion.

Our findings on ceramide trihexoside in Tay-Sachs brain are in agreement with those of Gatt and Berman (9). However, our findings on ceramide dihexoside differ somewhat. Gatt and Berman reported a very small amount of ceramide digalactoside, about one-tenth of ceramide trihexoside, but no ceramide lactoside. In gray matter of Tay-Sachs brain, we found ceramide dihexoside to be mostly ceramide lactoside. The study of partial hydrolysis products suggested the presence of ceramide digalactoside as a very minor component. The relative amount of total ceramide dihexoside was also different: approximately one-third of ceramide trihexoside. We ruled out the possibility of inadvertent breakdown of higher ceramide hexosides or gangliosides during the preparative and analytical procedures, as described in the Results section. Gatt and Berman fractionated glycolipids by column chromatography. Although the relative quantitative data were not given, the hexose peaks for various glycolipids suggest that, in their specimen, cerebroside constituted almost the same amount as ceramide trihexoside; the ceramide digalactoside peak was very small. In the brain we analyzed, cerebroside and ceramide dihexoside were present in almost equal amounts, both about one-third of ceramide trihexoside. The most likely explanation is the fact that they analyzed the whole brain, including white matter, which is rich in cerebroside. Another possible explanation is, however, the degree of severity of the disease process. Our case was considered to be in an advanced stage of the disease when the patient died. Accumulation of glucocerebroside and ceramide lactoside with concomitant decrease in galactocerebroside may occur as the disease progresses, thus obscuring the ceramide digalactoside that might be present in a constant amount throughout the disease process.

Our finding on the gray matter of G_{M1}-gangliosidosis is in agreement with that of Jatzkewitz et al. (11) in that ceramide tetrahexoside is the major glycolipid in the lower phase. The level of ceramide tetrahexoside in their specimen, however, was much higher than that found in our specimen. In view of the labile nature of gangliosides in formalin-fixed brains (5), ceramide tetrahexoside in their sample probably contained the artifactual breakdown product of gangliosides. In their study, the identification of each ceramide hexoside was solely by the mobility on TLC, and no analytical data were presented in regard to the sugar composition or sequence. We could characterize the ceramide tetrahexoside in our frozen brain of G_{M1}-gangliosidosis as identical in sugar composition and sequence with the asialo derivative of the accumulated ganglioside in this disorder. As in Tay-Sachs disease, ceramide dihexoside in gray matter of G_{M1}-gangliosidosis appears to be mostly ceramide lactoside, possibly with a small amount of ceramide digalactoside, and cerebroside in G_{M1}-gangliosidosis gray matter is predominantly glucocerebroside.

The relationship of these ceramide hexosides to the specific ganglioside abnormalities in these two disorders is a most intriguing question. Gangliosides are found predominantly in gray matter and localized mostly, if not entirely, in neurons (29). Such localization of gangliosides is also found in these lipidoses. The ceramide hexoside composition too is abnormal only in gray matter. This is consistent with the idea that these ceramide hexosides might be metabolically related to gangliosides. Except those glycolipids that have a galactose moiety next to ceramide, the series of the ceramide hexosides found in these diseases could derive, at least chemically, from ganglioside by sequential removal of *N*-acetyl neuraminic acid and then of monosaccharide units. The

fact that the major ceramide hexosides are probably the asialo derivatives of the accumulated gangliosides in each disease further suggests their metabolic relationship. The discussion of this aspect is inevitably limited at the present time, because the biosynthetic and degradative pathways of brain gangliosides have not been firmly established. The clear-cut delineation of both biosynthetic and degradative pathways of brain gangliosides is necessary before further speculation on the metabolic defects of gangliosidoses can be meaningful.

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