Gangliosides in subacute sclerosing leukoencephalitis: isolation and fatty acid composition of nine fractions

ROBERT LEDEEN, KENNETH SALSMAN, and MARIA CABRERA

The Saul R. Korey Department of Neurology and the Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York 10461

ABSTRACT Gangliosides from brain of an 8 yr old boy with subacute sclerosing leukoencephalitis have been studied in terms of pattern and structure. Thin-layer chromatography showed that both gray and white matter have a highly abnormal pattern, with elevation of the relative proportion of four gangliosides corresponding to minor species in normal brain. The total level of lipid-bound sialic acid, however, was not increased, which indicated a compensating loss of other gangliosides. Two of the proliferating species were monosialogangliosides (G_5 and G_6) (Korey nomenclature), and two were disialo types (G_{2A} and G_{3A}). Studies of their carbohydrate structures are described.

Nine ganglioside fractions were isolated by preparative TLC in combination with column chromatography, and the fatty acid compositions were determined. Seven contained stearate as the major component, while two (G_{3A} and G_6) had relatively large proportions of oleate and palmitate. Five of the fractions contained two fatty acids of long chain-length and unknown structure.

KEY WORDS gangliosides · abnormal pattern · subacute sclerosing leukoencephalitis · ganglioside fractions · separation · fatty acid composition · carbohydrate structure · brain · disease · glycolipids · demyelination

THE DISEASE first described by Van Bogaert (1) and later named by him (2) "subacute sclerosing leukoencephalitis" (SSLE) is characterized by gliosis, inflammation, and demyelination. Many areas of the brain may be affected but the major lesions are usually in white matter. The etiology is not yet clear but some evidence points to a viral origin (3,4).

Recently the brain lipids of a case of SSLE were analyzed in some detail (5), and among the abnormalities noted was an unusual ganglioside pattern in the white matter. Gray matter, on the other hand, was reported to have a normal pattern. In the present study of an SSLE brain we found similar ganglioside distortion, but in this case both white and gray matter were similarly affected. The abnormality is manifested chiefly in proliferation of four gangliosides which correspond to minor components of normal brain.

For purposes of structure comparison we have isolated nine ganglioside fractions from this brain and determined the fatty acid composition of each. To further characterize the "abnormal" or proliferating species we have also studied some aspects of their carbohydrate structures. A preliminary description of some portions of this work has been presented (6).

METHODS

The patient was an 8 yr old boy who died after 9 months of illness. Following autopsy half of the brain was frozen and kept in a sealed plastic bag at −50°C for a few months before analysis. The normal brain for control was that of an 8 yr old girl whose death did not involve pathology of the nervous system; it was preserved in similar fashion.

Ganglioside Extraction

Portions of cerebral tissue were carefully dissected into gray and white matter and the lipids were extracted

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; SSLE, subacute sclerosing leukoencephalitis; NANA, *N*-acetyl neuraminic acid; NGNA, *N*-glycolyl neuraminic acid.

with 15 volumes of chloroform–methanol 1:1. The mixture was filtered through a fritted glass funnel of medium porosity, and sufficient chloroform was then added to the filtrate to bring the composition to 2:1. Partitioning was carried out according to Folch, Lees, and Sloane Stanley (7) with 0.2 volume of 0.88% aqueous potassium chloride, and then with "pure solvents upper phase" containing no salt. Each upper phase was evaporated to near dryness, taken up in water, and dialyzed for 2 days in the cold against distilled water. The bag contents were lyophilized to dryness which left the crude ganglioside mixture in the form of a fluffy white solid. The upper phases that lacked salt had considerable lipid contamination, and these were not used for preparative isolation of individual gangliosides; the contaminants did not interfere, however, with ganglioside estimation by the resorcinol method (8).

Thin-Layer Chromatography

Plates were coated with a 250 μ layer of Silica Gel G (Merck) which had been previously extracted with chloroform–methanol 2:1 and acetone to remove organic impurities. Before use, the plates were activated at 110°C for 40 min. For pattern determination and preparative separation two different systems were used:

System A. 20 × 20 cm plates were first developed (ascending) in chloroform–methanol–2.5 N aqueous ammonia 60:40:9. After drying for 1 hr the plate was inverted and run in descending fashion with *n*-propanol–water 7:3, according to the method of Korey and Gonatas (9). This procedure proved particularly suitable for separating the eight major gangliosides from the SSLE brain, and was therefore used for overall pattern depiction. The use of two systems in this manner was first described by Suzuki and Chen (10).

System B. Double-length plates (20 × 40 cm) were employed with chloroform–methanol–2.5 N aqueous ammonia 60:40:9. The latter was allowed to ascend 4/5 of the length during a period of 4–6 hrs., and the plate was then dried for 1 hr in the atmosphere. To improve resolution the procedure was repeated for a second and frequently a third run.

Ganglioside Separation

TLC system B was employed in the first step for preparative isolation of the nine ganglioside fractions. Approximately 2 mg of ganglioside mixture could be run on each plate without danger of overloading. After two or three successive ascending runs the plates were sprayed with Rhodamine 6G (0.001% aqueous) and viewed under UV light for band detection. The zones were scraped from the plates with a razor and eluted with methanol–chloroform–water pyridine 56:40:12:2. All TLC fractions except G_5 and G_6 were then subjected to Folch partitioning in which a drop of ammonia but no salt was present in the upper phase. Most of the rhodamine and other impurities remained in the lower phase. G_5 and G_6 were not so treated because of the small proportions of these two which partition into the upper phase; they were purified directly by chromatography on a small column of activated silicic acid (Unisil, Clarkson Chemical Company, Inc., Williamsport, Pa.). First rhodamine was eluted from the column with chloroform–methanol 9:1 and then ganglioside with the same solvents in the ratio 3:2. Most of the other fractions were similarly chromatographed after Folch partitioning, except for G_0 and G_{2A} which showed a tendency to undergo decomposition on Unisil. By discarding the first portion of the 3:2 eluate before ganglioside appeared we removed any small remainder of nonganglioside lipid contaminant.

A difficulty was encountered in the separation of G_3 and G_{3A}, which formed overlapping bands with TLC system B. They could, however, be resolved with the Unisil column by eluting first G_{3A} with chloroform–methanol 3:1, and subsequently G_3 with a higher proportion of methanol. An alternative procedure was to use TLC system A, or descending TLC alone in which G_{3A} migrates clearly ahead of G_3.

The gangliosides employed for fatty acid analysis were derived from gray matter, while for other purposes the source was mixed white and gray.

Fatty Acid Analysis

Approximately 0.2 μmole of each ganglioside was treated with 2 ml of boron trifluoride–methanol (14% by weight, Applied Science Laboratories Inc., State College, Pa.) in a small tube with a Teflon-lined screw cap. Air was displaced by nitrogen and the mixture heated for 3 hr at 100°C. This procedure was previously shown to be suitable for cleaving the amide bond of gangliosides, in addition to other sphingolipids (11). The cooled mixture was treated with 2 ml of water and extracted four times with 2-ml portions of hexane. The combined hexane phases, after washing with water, were evaporated to a small volume and applied as a 1 inch streak to a thin-layer plate coated with Silica Gel HR (no binder). One ascending run was carried out with heptane–ethyl ether 2:1; the plate was then dried and sprayed with water. Standards of methyl stearate and methyl 2-hydroxystearate were used as markers, and the corresponding zones were scraped off the plate and eluted with methylene chloride. After filtration and evaporation the residue was transferred in hexane to 5 ml tubes and evaporated with nitrogen to a small volume for GLC. The sample was kept cool throughout to avoid loss of shorter-chain fatty acids.

Each mixture was chromatographed on polar and nonpolar columns using a hydrogen flame detector. The stationary phases were 15% EGSS-X (an ethylene glycol succinate polyester combined with a silicone) on Gas-Chrom P and 6% JXR on Gas-Chrom Q (Applied Science Laboratories, Inc.). Two temperatures were used, one for analyzing C_{14}–C_{20} and the other for longer chain-lengths. Column peaks were identified by comparison with standards and by plots of logarithm retention time versus carbon number. Quantification was obtained by multiplication of peak height by width at half-height. Each value is an average of at least two GLC determinations. Data are reported as weight percentages (no correction for differences in detector response). Equivalent chain lengths of the unknown fatty acids *X* and *Y* were determined by the method of Miwa, Mikolajczak, Earle, and Wolff (12).

Carbohydrate Analysis and Structure

Hexoses and hexosamine were identified by paper chromatography of acid hydrolysates, prepared by heating a small amount of the ganglioside in 1 N hydrochloric acid at 100°C for 14 hr. *n*-Butanol–pyridine–water 6:4:3 was used as descending solvent in a 24 hr run, and spots were detected with silver nitrate–sodium hydroxide. Hexose, glucose, and hexosamine were determined according to previously described methods (13). Galactose was calculated as the difference between total hexose and glucose (from glucose oxidase), because of the observation that these were the only neutral sugars detected by paper chromatography. Sialic acid was determined by the resorcinol method of Svennerholm as later modified by Miettinen and Takki-Luukkainin (8). TLC with *n*-propanol–aqueous ammonia (1 N)–water 6:2:1 (14) was used to differentiate NANA from NGNA; prior to TLC, sialic acid was split off by mild acid hydrolysis with 0.03 N HCl at 85°C for 2 hr.

The oligosaccharide chain of G_{3A} was degraded by periodate–borohydride treatment according to methods previously outlined (13). The water-soluble products were identified by GLC of the acetate derivatives. The fate of NANA during the periodate treatment was determined by subjecting the product to mild acid hydrolysis, followed by TLC with the system described above.

RESULTS

Ganglioside Pattern and Level

The TLC pattern of gangliosides from the SSLE brain is compared with that of normal brain in Fig. 1. The diseased tissue shows a striking abnormality in the elevation of four species which migrate parallel to four of the minor gangliosides of normal brain; the latter were

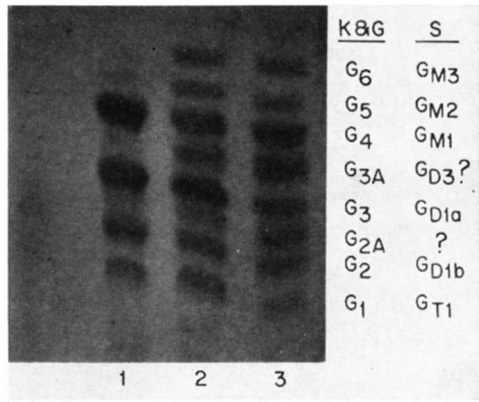

FIG. 1. TLC of SSLE gangliosides. *1*, normal human brain; *2*, SSLE gray matter; *3*, SSLE white matter. Ganglioside symbols under K & G, Korey and Gonatas (9); under S, Svennerholm (15). Conditions: TLC system A (see *Methods*); resorcinol–HCl spray.

faintly visible on the chromatogram. We have designated these as G_{2A}, G_{3A}, G_5, and G_6 according to the Korey system of nomenclature (9); the corresponding symbols of Svennerholm (15) are also shown. In this case both white and gray matter exhibited the same abnormality. Despite the marked increase of four species, total ganglioside level (as measured by resorcinol assay of lipid-bound sialic acid) was not elevated in either white or gray matter (Table 1); indeed, gray matter showed a slight decrease while white matter was close to normal.

Ganglioside Separation

TLC system B with double-length plates was used for preparative isolation. Fig. 2 shows a typical chromatogram with this system for gangliosides from SSLE and normal brain. The fractions were usually well separated, with the exception of G_3 and G_{3A}; these two were scraped and eluted together and subsequently separated by the two procedures described. Following elution from the silica gel, all fractions were subjected to one or more additional purification steps (see Methods). The nine fractions thus isolated are shown in Fig. 3. All bands, including those only faintly visible, were resorcinol-positive (purple) and hence ganglioside. Be-

TABLE 1 GANGLIOSIDE CONTENT OF BRAIN TISSUE

	Wet Weight		Dry Weight		H_2O Content (%)	
Brain	Gray	White	Gray	White	Gray	White
SSLE	0.672	0.226	3.93	0.88	82.9	74.4
Normal	0.785	0.232	4.43	0.89	82.3	73.3

Values are expressed as mg of lipid-bound sialic acid per g of tissue. Sialic acid was determined by resorcinol method (8). Both "normal" and diseased tissue were from brains of 8-yr old children.

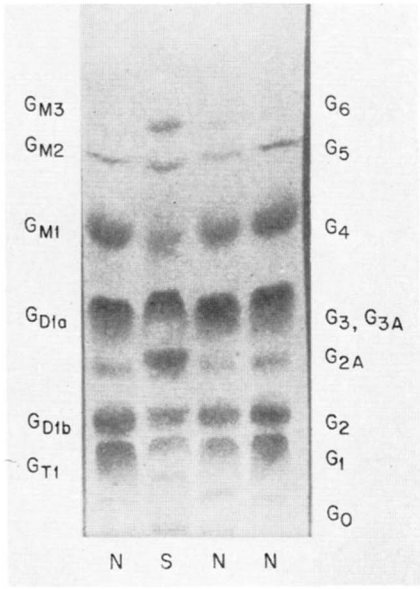

FIG. 2. Separation of gangliosides with TLC system B (see *Methods*) and resorcinol–HCl spray: N, normal brain; S, SSLE. Symbols: as in Fig. 1.

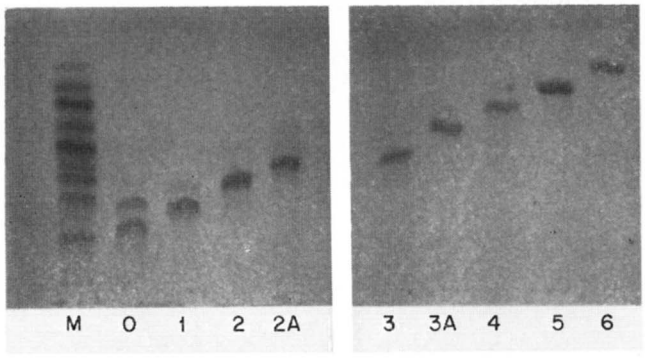

FIG. 3. TLC of separated ganglioside fractions. M, SSLE mixture. Conditions: TLC system A (see *Methods*); each fraction about 8 μg of sialic acid (20–40 μg of ganglioside). Ganglioside symbols are those of Korey and Gonatas (9). Spray: resorcinol–HCl.

fore being sprayed with this reagent the plate had been sprayed with aqueous rhodamine and viewed under UV light, but no bands other than those which subsequently appeared with resorcinol were visible. This TLC system was different from that used originally for separation, and the ganglioside fractions appeared to be free of lipid contamination.

The G_0 fraction consisted of two major gangliosides and one minor slow-moving species; whether this was due to decomposition or failure initially to separate G_0 from G_1 is not known. The other fractions showed mainly one species with minor amounts of other gangliosides in some cases. G_{2A} was particularly prone to suffer breakdown during purification, sometimes generating four or five new gangliosides during chromatography on a Unisil column. G_3, G_4, G_5, and G_6 appeared as homogeneous fractions.

Carbohydrate Structures

Analyses obtained for G_{2A} and G_{3A} from the SSLE brain are given in Table 2, along with a G_{3A} sample from normal brain for comparison. The G_{3A} samples were similar, both lacking hexosamine and containing glucose, galactose, and NANA in molar ratios approximating 1:1:2, respectively. Neuraminidase removed both sialic acids from G_{3A} and yielded an asialo product which ran parallel on TLC to ceramide lactoside. This product appeared in most instances as two closely-running spots on TLC.

Mild acid hydrolysis of G_{3A} produced the ceramide monohexoside (among other products), which was isolated by TLC. Vigorous acid hydrolysis of this product liberated glucose as the sole carbohydrate, thereby establishing the sequence ceramide–glucose–galactose. Periodate–borohydride treatment of the ganglioside gave rise to water-soluble products which were identified by paper chromatography and GLC of the acetates. These proved to be galactose, erythritol, and a small amount of unchanged glucose (sialic acid was destroyed by the vigorous acid hydrolysis). In another run periodate treatment was followed by mild acid hydrolysis, and the products in this case were found to include unchanged NANA as well as a periodate-cleaved derivative of the latter which migrated ahead of NANA on TLC and stained light brown with resorcinol. A similar light brown spot was obtained by parallel treatment of Tay-Sachs ganglioside which contains a single, terminal NANA (13). These results were entirely consistent with a NANA(2 → 8)NANA linkage, already reported to be present in a number of gangliosides (16,17). The total structure of G_{3A} is therefore:

$$\begin{matrix} \text{NANA} \\ \begin{pmatrix} 2 \\ \downarrow \\ 8 \end{pmatrix} \\ \text{NANA}(2 \rightarrow 3)\text{GAL}(1 \rightarrow 4)\text{GLU}(1 \rightarrow 1)\text{ceramide.} \end{matrix}$$

Still undetermined are the configurations of the glycosidic and ketosidic bonds, and the long-chain base structure (fatty acid composition is discussed below). One or both of these aspects might explain the formation of two asialo products with neuraminidase, an unsolved matter referred to previously.

G_{2A} contained hexosamine and appeared to be a disialo species with possibly one unit each of glucose and galactose. However, the analytical results departed significantly from these simple integral ratios and an unambiguous formulation is therefore not yet possible.

This species was notably unstable during isolation, a factor which may have contributed to the analytical difficulties. Neuraminidase treatment gave rise to two major products, both of which still contained some sialic acid; they migrated closely on TLC and were somewhat similar to Tay-Sachs ganglioside (G_5). The data are thus suggestive of a disialo analogue of the latter, though it is also possible that more than one ganglioside was present in this fraction.

The carbohydrates of G_5 and G_6 were studied qualitatively by paper chromatography of their acid hydrolysates. G_5 had glucose, galactose, and galactosamine while G_6 had only the first two. G_5 was resistant to neuraminidase whereas the NANA of G_6 was readily removed to yield a product that migrated on TLC at the same rate as ceramide lactoside. Both gangliosides ran together with their normal brain counterparts in TLC systems A and B, and since all other properties were in agreement the structures were concluded to be the same.

Fatty Acids

The compositions obtained for the nine fractions are shown in Table 3. Stearate predominated by a wide margin in most of the gangliosides, but two notable exceptions were G_{3A} and G_6, which had considerably more 16:0 and 18:1 than the others. A portion of G_6 was treated with neuraminidase to form ceramide lactoside, the latter being purified by TLC and analyzed by GLC. The fatty acid composition, shown in the last column of Table 3, is in good agreement with that of the original G_6 ganglioside, and we therefore conclude that the unusual pattern is genuine and not due to lipid contaminants.

Two new fatty acids of unknown structure, *X* and *Y*, were found to be present in five of the fractions. They emerged from the chromatogram after 24:0 and 24:1 but their retention times did not fall on semilog plots for either normal saturated or monosaturated acids. Components *X* and *Y* had equivalent chain lengths of 25.8 and 27.3 on the polar column and 24.9 and 24.6 on the nonpolar column, respectively. Together they comprised approximately 5% of total fatty acid.

Attempts were made to isolate hydroxy fatty acids from the total ganglioside mixture and the individual fractions. The thin-layer plates used to purify the fatty acid methyl esters contained standards of methyl 2-hydroxystearate, and although the samples showed no visible bands in that region the zones were eluted and analyzed with the flame detector at high sensitivity. No hydroxy fatty acids were found.

TABLE 2 CARBOHYDRATE COMPOSITION OF GANGLIOSIDES

Ganglioside	Glucose	Galactose	NANA	Hexosamine
G_{2A} SSLE	1.0	0.96	1.66	0.84
G_{3A} SSLE	1.0	1.33	2.01	0.09
G_{3A} NHB*	1.0	1.22	1.97	0.13

Expressed as molar ratios, relative to glucose as 1.
* Normal human brain.

DISCUSSION

The ganglioside pattern change we have observed in both gray and white matter is quite similar to that re-

TABLE 3 FATTY ACID COMPOSITIONS OF INDIVIDUAL GANGLIOSIDES

Fatty acid	Gangliosides									
	G_0	G_1	G_2	G_{2A}	G_3	G_{3A}	G_4	G_5	G_6	G_6*
	wt % of fatty acids									
14:0	5.9	2.3	2.3	3.6	2.1	8.2	1.0	2.2	5.5	5.9
14:1		3.5		tr.	1.9		1.8			
15:0						2.3		0.5	1.3	2.5
16:0	3.7	2.2	2.1	2.8	7.1	27.8	2.6	9.5	24.5	26.9
16:1					1.0	7.7		2.0	3.0	7.0
18:0	64.3	68.0	77.6	75.2	68.0	24.7	80.9	70.4	39.1	35.7
18:1	1.0	1.3	3.5	tr.	5.1	20.2	0.5	2.6	14.1	10.9
19:1						2.7		1.3	1.8	1.5
20:0	6.3	8.3	8.8	7.2	5.9	2.0	6.7	3.9	3.0	2.7
22:0	2.3	2.2	1.6	1.3	0.4	1.2	1.1	0.7	2.8	2.0
22:1			0.2					0.4		
23:0	3.5	3.2	1.0	1.9	1.2	0.8	2.5	1.2	2.2	1.9
23:1				0.9						
24:0	1.4	1.3	0.8	0.7	1.0	1.2	1.1	0.7	1.5	1.6
24:1	4.7	2.4	2.1	2.6	2.1	1.1	1.9	1.0	1.4	1.5
X	2.4	1.5		1.3	1.8			1.0		
Y	4.4	3.9		2.9	3.1			2.6		

Each value is the mean of two determinations. tr., trace. Fatty acids designated by chain length: no. of double bonds.
* Purified via cytoside.

cently found in the white matter only of another SSLE brain (5). The latter was reported to show a two-fold elevation of total ganglioside in white matter based on dry weight, whereas the present study gave normal levels for white matter and slightly subnormal for gray. This indicates that pattern change is probably more characteristic of the disease than is total ganglioside level. Whether or not this altered pattern is a pathognomonic feature of SSLE is not known since these appear to be the only two cases whose gangliosides were studied from this point of view.

Wender (18) has described two studies of SSLE which showed an elevation of lipid hexosamine in cerebral white matter. Cumings (19) reported increased ganglioside of cerebral cortex in one out of three cases, and increased total hexosamine in all three. This was amplified in a later report (20) which specified that the increased hexosamine was due to residual and not lipid or water-soluble hexosamine. The nature of the residual substance was not specified.

The change of ganglioside pattern in this disease may be contrasted with those of genetically induced gangliosidoses, such as Tay-Sachs disease (13,21) and late infantile systemic lipidosis (22, 23) [i.e., "G_{M1}-gangliosidosis" (24); "generalized gangliosidosis" (25)]. These two diseases involve proliferation of a single and specific (though different) ganglioside, and the total level in brain is usually increased several-fold. Despite the presence of four elevated gangliosides, SSLE cannot be classified with these as a true lipidosis; the changes appear to be a secondary consequence of degeneration and inflammation in contrast to "primary" accumulation through enzymic deficiency.

Our structural studies were designed in part to determine whether the "abnormal" or proliferating gangliosides correspond to species normally present in brain, or whether they represent new types which may have derived from an exogenous source (e.g. virus, lymphoplasmocytic infiltrates, etc.). Three were clearly shown to be in the former category while the fourth (G_{2A}) appears also to have its normal brain counterpart in terms of TLC pattern, although its structure is still uncertain. In normal brain the species parallel to these four are all minor components and together constitute approximately 10% of total ganglioside.

G_5 and G_6 are monosialo species whose carbohydrate compositions agree with those previously reported (15,17). G_5 contains hexosamine (*N*-acetylgalactosamine) and has properties which parallel the major ganglioside of Tay-Sachs brain (13) as well as the minor G_5 fraction of normal brain (26). G_6 lacks hexosamine and is similar to the ganglioside of equine erythrocytes, termed "hematoside" by Yamakawa and Suzuki (27). G_{3A} also lacks hexosamine and was analyzed as the disialo analogue of G_6. More detailed study of its structure lead us to conclude that it is identical to the "G'_{Lact}" of Kuhn and Wiegandt (17), obtained from normal brain. We have also isolated the same fraction from normal brain and found it has comparable properties to the SSLE sample. It is interesting to note that a ganglioside bearing much resemblance to this species was found in large amounts in brain of a case of congenital amaurotic idiocy (28). However, its structure was not fully determined, and the claim that brief reaction with neuraminidase produced two monosialo products indicates that it may differ from G_{3A}.

Fraction G_{2A} presented a complex picture structurally. It appears to be a disialo species containing hexosamine, but the analytical data departed significantly from integral ratios. However, they were suggestive of a disialo analogue of Tay-Sachs' ganglioside (G_5), similar to the minor component "G'_{GNTrII}" isolated and partially characterized by Kuhn and Wiegandt (17). This was supported to some extent by our observation that neuraminidase gave rise to products that migrated close to G_5, though it is not clear why two such products were formed. Instability was a notable feature of this fraction which, during purification, would frequently break down into several components. These observations are difficult to reconcile with conventional ganglioside structure and suggest the possibility that G_{2A} may be a larger complex of different gangliosides held together by weak bonds; however, other possibilities have not been ruled out.

The very unusual pattern of this brain presented an opportunity for comparing certain aspects of the structures of gangliosides which would include minor fractions difficult to isolate in quantity from normal brain. Comparison of lipophilic units was particularly desirable since most studies to date have dealt with ganglioside mixtures, and little is known of possible differences among individual species. Of the nine fractions isolated here seven were found to contain stearate as the predominating fatty acid, consistent with the findings for total brain ganglioside (for review see ref. 29). However, G_{3A} and G_6 had compositions notably different from the rest, though similar to each other; the relative increases in 16:0 and 18:1 (and to a lesser extent 14:0 and 16:1) at the expense of 18:0 result in compositions that are most unusual among the brain gangliosides. It is interesting to note that these two species are also distinctive in being the only ones (of the nine isolated here) which lack hexosamine and which yield ceramide lactoside with neuraminidase.

This study also revealed the presence of two fatty acids of long chain-length (*X* and *Y*) not previously reported in gangliosides. Their structures are not yet determined but they are thought to contain branched chains.

They were found in only five of the fractions, comprising approximately 4–6% of total fatty acid in each case. Where present they occurred together and in approximately the same ratio.

We do not know to what extent these results may reflect the influence of disease, since fatty acids of certain brain lipids are known to suffer changes in some neurological disorders. However, according to preliminary work we have carried out with bovine brain gangliosides it appears that the fatty acid compositions observed here are inherent properties of the individual ganglioside structures and apply as well to the normal state.

Concerning the origin of the four elevated gangliosides, it is useful to correlate the chemical findings with histological studies of this disease (30). Widespread gliosis has been shown to accompany severe neuronal degeneration, and it is conceivable that proliferation of one or more glial elements is the morphological correlate of the increase in gangliosides G_{2A}, G_{3A}, G_5, and G_b. There is evidence that most of the brain gangliosides in the normal state reside in neurons (31), but it is thought unlikely that these four elevated species in SSLE are part of the greatly depleted neuron population. It also seems improbable that they accumulate through breakdown of other species; this applies particularly to G_{3A} and G_6, which have their own characteristic fatty acid patterns. The presence of gangliosides in glia has received strong support from a recent study of several types of glial tumors (32), many of which were found to contain abnormal patterns of ganglioside.

The fact that total ganglioside in this case was not elevated, despite a sharp increase in four species, requires a compensatory decrease elsewhere. This evidently applies to G_1, G_2, G_3, and G_4, which have suffered both relative and absolute depletion as a result of the disease. They are the major species in normal brain, and their decline here associated with neuron destruction suggests their morphological identity as the neuron-specific gangliosides.

Finally, it should be noted that ganglioside changes of a somewhat similar nature have been observed in Creutzfeldt-Jakob disease (10), and metachromatic leukodystrophy (33), although the magnitude of the effect in these cases was much lower than observed here. In Creutzfeldt-Jakob disease the increases of G_{2A}, G_{3A}, G_5, and G_6 were actually relative (in comparison to the major gangliosides, which decreased), but not absolute in terms of tissue content; in metachromatic leukodystrophy the elevation, though slight, appeared to be absolute. It is possible that the SSLE pattern is an extreme manifestation of a generalized phenomenon which characterizes in various degrees a number of degenerative brain disorders, but the number of diseases studied from this standpoint is not yet sufficient to establish such a conclusion. It is also not known whether these ganglioside changes are merely incidental to brain damage or whether they constitute an integral part of the brain's defense mechanism.

This investigation was supported by NIH grants NB-04834, NB-01006, and NB-03356.

Manuscript received 19 June 1967; accepted 18 September 1967.

References

1. Van Bogaert, L., and J. D. Busscher. 1939. *Rev. Neurol.* **71:** 679.
2. Van Bogaert, L. 1945. *J. Neurol. Neurosurg. Psychiat.* **8:** 101.
3. Gonatas, N., and G. M. Shy. 1965. *Nature.* **208:** 1338.
4. Tellez-Nagel, I., and D. H. Harter. 1966. *Science.* **154:** 899.
5. Norton, W. T., S. E. Poduslo, and K. Suzuki. 1966. *J. Neuropathol. Exptl. Neurol.* **25:** 582.
6. Ledeen, R., K. Salsman, and M. Cabrera. 1966. *Federation Proc.* **25:** 601.
7. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. *J. Biol. Chem.* **226:** 497.
8. Miettinen, T., and I. T. Takki-Luukkainen. 1959. *Acta Chem. Scand.* **13:** 856.
9. Korey, S. R., and J. Gonatas. 1963. *Life Sci.* **5:** 296.
10. Suzuki, K., and G. Chen. 1966. *J. Neuropathol. Exptl. Neurol.* **25:** 396.
11. Rosenberg, A., and N. Stern. 1966. *J. Lipid Res.* **7:** 122.
12. Miwa, T. K., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff. 1960. *Anal. Chem.* **32:** 1739.
13. Ledeen, R., and K. Salsman. 1965. *Biochemistry.* **4:** 2225.
14. Granzer, E. 1962. *Z. Physiol. Chem.* **328:** 277.
15. Svennerholm, L. 1963. *J. Neurochem.* **10:** 613.
16. Kuhn, R., and H. Wiegandt. 1963. *Z. Naturforsch.* **18b:** 541.
17. Kuhn, R., and H. Wiegandt. 1964. *Z. Naturforsch.* **19b:** 256.
18. Wender, M. 1961. *Psychiat. Neurol.* **141:** 381.
19. Cumings, J. N. 1961. *In* Encephalitides. L. Van Bogaert, J. Radermecker, J. Hozay, and A. Lowenthal, editors. Elsevier Publishing Co., Amsterdam. 638–647.
20. Cumings, J. N. 1963. *In* Mechanisms of Demyelination. A. S. Rose and C. M. Pearson, editors. McGraw-Hill, Inc., New York. 58–71.
21. Svennerholm, L. 1962. *Biochem. Biophys. Res. Commun.* **9:** 436.
22. Ledeen, R., K. Salsman, J. Gonatas, and A. Taghavy. 1965. *J. Neuropathol. Exptl. Neurol.* **24:** 341.
23. Gonatas, N. K., and J. Gonatas. 1965. *J. Neuropathol. Exptl. Neurol.* **24:** 318.
24. Suzuki, K., and G. C. Chen. 1967. *J. Lipid Res.* **8:** 105.
25. O'Brien, J. S., M. B. Stern, B. H. Landing, J. K. O'Brien, and G. Donnell. 1965. *Am. J. Diseases Children.* **109:** 338.
26. Ledeen, R., K. Salsman, and M. Cabrera. 1967. *In* Inborn Disorders of Sphingolipid Metabolism. S. M. Aronson and B. W. Volk, editors. Pergamon Press Inc., Oxford and New York. 231–240.
27. Yamakawa, T., and S. Suzuki. 1951. *J. Biochem.* (*Tokyo*). **38:** 199.
28. Hagberg, B., G. Hultquist, R. Ohman, and L. Svennerholm. 1965. *Acta Paediat. Scand.* **54:** 116.
29. Ledeen, R. 1966. *J. Am. Oil Chemists' Soc.* **43:** 57.

30. Tellez-Nagel, I., and D. H. Harter. 1966. *J. Neuropathol. Exptl. Neurol.* **25:** 560.
31. Lowden, J. A., and L. S. Wolfe. 1964. *Can. J. Biochem.* **42:** 1587.
32. Seifert, H. 1966. Unterschungen uber die Gangliosidzusammensetzung bei verschiedenen Hirntumoren. Ph. D. Thesis. Max-Planck-Institute für Hirnsforschung, Abteiling für Tumorforschung und experimentelle Pathologie, Köln.
33. Suzuki, K. 1967. *In* Inborn Errors of Sphingolipid Metabolism. S. M. Aronson and B. W. Volk, editors. Pergamon Press Inc., Oxford and New York. 215–230.