Gangliosides of human, cat, and rabbit spinal cords and cord myelin

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Abstract Gangliosides were isolated from whole spinal cords and cord myelin of human, cat, and rabbit by a revised methodology. The method included the sequential application of DEAE–Sephadex column chromatography, base treatment, Sephadex G-50 column chromatography, and finally Iatrobeads column chromatography. The human whole spinal cord was found to contain about one-tenth of the ganglioside concentration as in cerebral gray matter and about one-third of that in cerebral white matter. Low levels of gangliosides were also found in cat and rabbit whole cords. Only *N*-acetyl neuraminic acid could be detected in the ganglioside fractions of all three species. The whole cords also possessed unique ganglioside patterns when compared with the patterns of cerebral tissues. The most prominent and consistent features were the reduced concentration of G_{D1a} and increased amounts of G_{M3} and G_{D3}. Human, but not cat and rabbit, spinal cord also contained G_{M4} as one of the major gangliosides.

Myelin prepared from the spinal cords of all three species also contained gangliosides. The amounts were only about half of those in the respective cerebral white matter myelin. The cord myelin ganglioside pattern was generally similar to the cerebral white matter myelin within the same species. G_{M1} was the most abundant ganglioside in the cord myelin. G_{M4} was found to be highly enriched only in myelin prepared from human sources.

Supplementary key words cerebral gray matter · cerebral white matter · cerebral myelin

The biochemical composition of the spinal cord (SC) has generally received less attention than other areas of the central nervous system (CNS) (1). This is perhaps due to the relative inaccessibility of this tissue and to its being considered a less important region of the CNS (1). Because the SC undergoes maturation at an earlier stage than other regions of the CNS (2–4), which include myelination of the axons, a number of investigators have sought to study the composition of the SC and cord myelin in comparison with cerebral tissues and myelin. Many studies have indeed shown that cord white matter and cord myelin possess compositional and metabolic characteristics that are different from their cerebral counterparts (1, 5–14). Very few studies, however, have dealt with the ganglioside composition of this tissue, despite the relatively high concentration of these lipids in other areas of the CNS. Schuwirth in 1943 (15) indicated that gangliosides were present in the human SC in rather low concentrations, but the ganglioside pattern was not investigated. More recently, Taranova, Katsnelson, and Belokhvastova (16) also investigated the quantitative and qualitative compositions of rabbit SC gangliosides.

We are particularly interested in the question of whether gangliosides are present in cord myelin. Gangliosides are known to be present in myelin prepared from cerebral tissues (17–24) and in myelin prepared from peripheral nerves (PNS) (25). In general, cerebral myelin prepared from higher vertebrates contains G_{M1} as the predominate ganglioside species.[2] However, primate and avian cerebral myelin contain high concentrations of an additional ganglioside, sialosylgalactosyl ceramide (G_{M4}, or G_7) (22, 24, 26). Peripheral nerve myelin, on the other hand, is characterized by having a more complex ganglioside pattern and the absence of CNS specific ganglioside G_{M4} (25). SC myelin gangliosides, to our knowledge, have not been studied. It is, therefore, of special interest to investigate the ganglioside compositions of mammalian cord and cord myelin. An improved method for the isolation and purification of gangliosides from small samples is also described. A preliminary account of portions of this work has recently been reported (27).

Abbreviations: TLC, thin-layer chromatography; SC, spinal cord; CNS, central nervous system; PNS, peripheral nervous system.

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[2] The nomenclature system of Svennerholm (63) is used. Other gangliosides that have not been officially designated by Svennerholm include the following: G_{M4}, I^3NeuAc-Gal Cer; G_{D1b}-Fuc, IV^2Fuc, $II^3(NeuAc)_2$ $GgOse_4Cer$; G_{T1a}, $IV^3(NeuAc)_2$, II^3NeuAc-$GgOse_4$ Cer; and G_{Q1b}, $IV^3(NeuAc)_2$, $II^3(NeuAc)_2GgOse_4$ Cer. (64).

MATERIALS AND METHODS

Materials

Human brains and SC were obtained at autopsy from patients who died without neurological diseases. Fresh specimens of cat and rabbit were obtained from normal adult animals. Frozen animal tissues were purchased from Pel-Freez Biologicals, Inc. (Rogers, AR). For myelin isolation, only fresh tissues were used. Precoated HPTLC and silica gel 60 (F-254) thin-layer plates, Iatrobeads (6RS-8060), and Sephadex resins were bought from Merck AG (Darmstadt, Germany), Iatron Lab., Inc. (Tokyo, Japan), and Pharmacia Fine Chemicals (Piscataway, NJ), respectively. All chemicals were of the reagent grade and solvents were redistilled before use.

Preparation of myelin

The procedure of Norton and Poduslo (28) was used for myelin preparation. Fresh cerebral white matter and whole SC (cervical and thoracic) were employed. The latter was carefully freed of meningeal layers, spinal roots, and blood vessels before use. The human cerebral white matter and the whole SC preparations had characteristics of highly purified myelin structures and were morphologically indistinguishable from each other under electron microscopic examination.

Ganglioside isolation and purification

The basic procedure was that of Ledeen, Yu, and Eng (22) with modifications for small-sized samples. Mixed segments of SC taken from cervical and thoracic regions (ca. 1 g wet wt) were used. The tissue was weighed in a tared 30-ml Euclid centrifuge tube and lyophilized to dryness in order to obtain the dry weight. The dried tissue was softened by the addition of a volume of water equivalent to that removed by lyophilization. Chloroform–methanol 1:1, (v/v, 20 ml per g wet tissue) was then added. Homogenization was carried out by a magnetic stirring bar at room temperature overnight. The homogenate was centrifuged at low speed and the supernatant was pipetted out. The sediment was washed twice with 10 ml of chloroform–methanol 1:1. The supernatant and washings were combined and the solvent was adjusted to chloroform–methanol–water 30:60:8 (Solvent A) by the addition of appropriate volumes of methanol and water. The lipid solution was applied to a DEAE–Sephadex (A-25, acetate form, 0.5 g) column (bed volume 2 ml) prepared as described previously (22). After the sample solution was passed through the column slowly (flow rate about 1 ml/min), the column was further eluted with 10 bed volumes (20 ml) of Solvent A followed by 3 bed volumes (6 ml) of methanol. Acidic lipids were then eluted from the column with 5 bed volumes (10 ml) of methanol containing 0.2 M sodium acetate and the eluant was collected in a 50-ml culture tube. Two ml of 1 N NaOH in methanol was added to the acidic lipid fraction and the solution was incubated at 37°C for 1 hr to destroy alkali-labile phospholipids. The solution was then neutralized by 2 ml of 1 N acetic acid in methanol. The resulting fatty acid methyl esters were removed by partitioning three times with 15 ml of *n*-hexane. The methanolic phase, which contained mild alkali-stable lipids, was evaporated to dryness with a nitrogen evaporator.

The dried sample was desalted on a Sephadex G-50 (medium) column which had a bed dimension of 2.0 × 21 cm and a bed volume of 80 ml. The residue was applied to the column in 4 ml of water. After the sample solution was absorbed into the top layer of the resin bed, the column was then eluted with distilled water. The first 21 ml of eluant was discarded and the next 32 ml, which contained gangliosides and sulfatides, was collected. This fraction was evaporated with a rotary evaporator or lyophilized to dryness. The dried residue was redissolved in 2 ml of chloroform–methanol 85:15 with the aid of a bath-type sonicator. The sample solution was then applied to an Iatrobead column (bed volume, 2 ml, 1.0 × 2.5 cm) (29) prepared in the same solvent. Prior to preparing the column, the Iatrobeads were first washed with chloroform–methanol–2.5 N ammonium hydroxide 30:60:10 and then with chloroform–methanol–water 30:60:10 in order to remove contaminants. After the application of the sample solution, the column was eluted with another 18 ml of chloroform–methanol 85:15 to remove sulfatides. Pure gangliosides were eluted from the column with 20 ml of chloroform–methanol 1:2.

For isolation of myelin gangliosides, the myelin sample (50–200 mg) was dissolved in 40 ml of Solvent A with the aid of sonication. The solubilized sample solution was applied to the DEAE–Sephadex column as described above.

Sephadex column chromatography

The efficiency of the Sephadex column in removing water-soluble nonlipid contaminants in the ganglioside fraction was tested by applying synthetic mixtures of water-soluble materials, which are frequently encountered in the ganglioside isolation, to a Sephadex column. The column had bed dimensions and bed volume as described above. The following test

samples, each dissolved in 2 ml of water, were used: *a*) 1 mg of blue dextran, 184 μg of free sialic acid, 20 mg of NaCl, 5 mg of sucrose, and 2 mg of L-tryptophan; *b*) ganglioside mixture of the normal human white matter (containing 310 μg of sialic acid) and 180 μg of free sialic acid; and *c*) 1 mg of blue dextran, 0.2 mg of NaCl, and 1.2 mg of UDP-galactose. After the applied sample had been absorbed into the gel bed, the column was eluted with distilled water. Another test sample was a ganglioside mixture of normal human white matter (containing 100 μg of sialic acid) and 0.12 mg of UDP-galactose, made up to 2 ml with 0.02 M NaCl. The column was eluted with 0.02 M NaCl. The eluant was collected as 3.1-ml fractions at a flow rate of 1.3 ml per min. The chromatographic separations were monitored by methods described below. The ganglioside recovery from the Sephadex column was estimated by applying known amounts (2–506 μg as sialic acid) of mixed gangliosides of normal human white matter in a similar manner. Elution was carried out with distilled water. The first 21 ml of eluant was discarded and the next 32 ml was collected. This fraction, which contained the gangliosides, was measured for its ganglioside sialic acid content.

Silica gel thin-layer chromatography

Thin-layer chromatography (TLC) was performed on precoated silica gel TLC plates. The plates were activated at 100°C for 30 min before use. The plate was developed with chloroform–methanol–water 55:45:10 (containing 0.02% $CaCl_2 \cdot 2H_2O$) or chloroform–methanol–2.5 N ammonium hydroxide 60:40:9 in an ascending manner. Gangliosides were visualized by spraying the plate with the resorcinol–HCl reagent followed by heating the covered plate at 95°C for 30 min.

Analytical methods

Ganglioside sialic acid was determined by a gas–liquid chromatographic method (30) or by the resorcinol method (31, 32). The latter method was also used for the estimation of free sialic acid. The method of Jourdian, Dean, and Roseman (33) was used to distinguish bound sialic acid from total sialic acid. Sucrose was measured by the anthrone method (34). Sodium chloride was estimated by turbidity test as follows. Aliquots (20-μl) were removed and each was diluted with 1 ml of water. To the diluted solution, 1 drop of 5% silver nitrate was added and the turbidity was measured spectrophotometrically at 703 nm. Blue dextran, L-tryptophan, and UDP-galactose were measured spectrophotometrically at 625 nm, 280 nm, and 260 nm, respectively.

TABLE 1. Ganglioside sialic acid contents of CNS tissues and myelin

		Human	Cat	Rabbit
		μg lipid-bound sialic acid/g (wet wt)[a]		
Tissue	Cerebral gray matter	875	1302	916
	Cerebral white matter	275	535	436
	Spinal cord	87	165	195
		μg lipid-bound sialic acid/g (dry wt)[a]		
Myelin	Cerebral	632	793	969
	Spinal cord	322	442	467

[a] Values are averages of at least two determinations.

Densitometric scanning of ganglioside pattern

The percent distribution of ganglioside sialic acid in a given sample was determined by direct densitometric scanning of the resorcinol positive bands on TLC plates. The instrument used was a Transidyne RFT Scanning Densitometer (Ann Arbor, MI). Peak areas were measured with a Hewlett-Packard 3380A electronic integrator and were calibrated with a mixture of pure gangliosides.[3]

RESULTS

Ganglioside contents of CNS tissues and myelin

Table 1 shows the ganglioside concentrations of whole SC and cord myelin of human, cat, and rabbit. The ganglioside contents of cerebral gray matter, white matter, and cerebral white matter myelin from the three mammalian species are also presented. Among the tissues examined, the whole SC contained the lowest concentrations of gangliosides. In human samples, the cord ganglioside concentration was only one-tenth of the ganglioside concentration in the cerebral gray matter and only one-third of the concentration in the cerebral white matter. Species variations were also evident: human SC contained considerably lower amounts of gangliosides than the SC of cat and rabbit. *N*-Acetylneuraminic acid was the only type of sialic acid detected in the ganglioside fractions of the three species.

The cord myelin of each species contained only about half the amount of gangliosides as their cerebral white matter myelin counterparts. Quantitative differences in SC myelin gangliosides among the three mammalian species were not as prominent as the differences in the whole SC gangliosides.

[3] Ando, S., N.-C. Chang, and R. K. Yu. *Anal. Biochem.* In press.

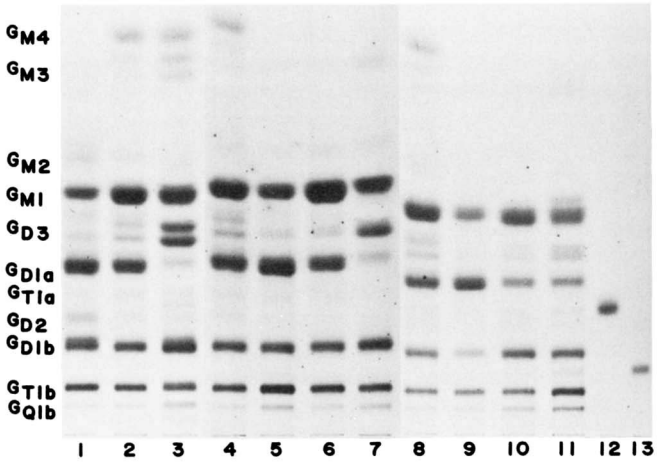

Fig. 1. TLC of the total gangliosides from cerebral gray matter, white matter, and whole spinal cord. Each lane contained 7 μg of lipid-bound sialic acid except for lanes 12 and 13 which contained 1 μg. The plate was developed in chloroform–methanol–water 55:45:10 (containing 0.02% $CaCl_2 \cdot 2H_2O$) for 3 hr in an ascending manner. Spots were visualized with resorcinol–HCl reagent. Lane *1*, normal human cerebral gray matter; lanes *2*, *4*, and *8*, normal human cerebral white matter; *3*, normal human SC; *5*, cat cerebral gray matter; *6*, cat cerebral white matter; *7*, cat spinal cord; *9*, rabbit cerebral gray matter; *10*, rabbit cerebral white matter; *11*, rabbit SC; *12*, human brain G_{T1a}; and *13*, human brain G_{D1b}-Fuc.

Ganglioside composition

The thin-layer chromatogram of cerebral gray matter, cerebral white matter, and whole SC gangliosides is shown in **Fig. 1.** The percentage distributions of lipid-bound sialic acid in these samples, as determined by densitometry, are shown in **Table 2.** The SC ganglioside patterns of all three species were different from the cerebral gray and white matter patterns. The most conspicuous and consistent features of the cord patterns were the reduced concentration of G_{D1a} which is one of the major gangliosides in cerebral tissues, and the increased concentrations of the usually minor species, G_{M3} and G_{D3}. These features were particularly striking in human and cat SC and somewhat less so in rabbit SC. The human SC also contained a high level of G_{M4}. This ganglioside, which is one of the major gangliosides in primate and avian cerebral white matter (22, 24, 26, 35), was only present in minute quantities (about 1%) in cat cerebral white matter and whole cord. It was present in rabbit tissues in even lower concentrations (<0.1%), which could not be measured accurately by the densitometric scanning. The concentration of G_{M4} was exceedingly low in the cerebral gray matter of all three species.

Other major gangliosides in the whole SC as well as in the cerebral tissues were G_{M1}, G_{D1b}, and G_{T1b}. G_{T1a}, whose structure was recently characterized by Ando and Yu (36) was also present in small concentrations in the CNS tissues of all three species. Another minor ganglioside, G_{D1b}-fucose (37), was present in measurable amounts in cat and rabbit cerebral white matter and whole SC.

The myelin ganglioside pattern of the SC was generally similar to that of the cerebral white matter myelin in the same species (**Fig. 2** and **Table 3**). In all three species, G_{M1} was the most abundant ganglioside. This feature appears to be characteristic of CNS type myelin. G_{M4} was found to be highly enriched in human SC as well as cerebral myelin. Slight enrichment of this ganglioside could also be seen in cat but not rabbit CNS myelin. Decreased amounts of G_{D1a} in SC myelin as compared with cere-

TABLE 2. Percent distribution of ganglioside sialic acid in CNS tissues

	Human			Cat			Rabbit		
	Cerebral GM[a]	Cerebral WM[b]	SC	Cerebral GM	Cerebral WM	SC	Cerebral GM	Cerebral WM	SC
	% of total ganglioside sialic acid[c]								
G_{M4}	1.5	8.6	12.8		0.8	1.1			
G_{M3}	2.7	4.8	14.0	1.3	0.8	3.5	1.1		2.9
G_{M2}	4.1	2.5	3.7	1.3	3.0	3.2	1.5	1.6	1.3
G_{M1}	14.9	21.6	16.8	20.9	39.5	27.8	26.5	34.6	29.0
G_{D3}	5.5	8.8	16.4	4.8	5.0	19.5	3.9	5.4	10.5
G_{D1a}	22.1	17.8	4.2	26.5	18.4	6.1	35.6	16.4	14.7
G_{T1a}	1.8	2.2	0.5	2.7	1.6	0.3	2.1	1.8	2.0
G_{D2}	8.0	3.1	0.9	1.8	0.6		1.3	1.1	1.2
G_{D1b}	18.2	16.9	18.7	18.5	16.8	21.1	9.8	18.6	14.3
G_{D1b}-Fuc								1.0	2.3
G_{T1b}	16.3	11.1	9.1	18.0	11.6	13.6	14.0	13.3	15.6
G_{Q1b}	5.1	2.8	2.9	4.2	1.9	3.8	2.9	5.5	6.1
Others							1.5	1.3	

[a] GM, gray matter.
[b] WM, white matter.
[c] Each value represents average of at least two determinations.

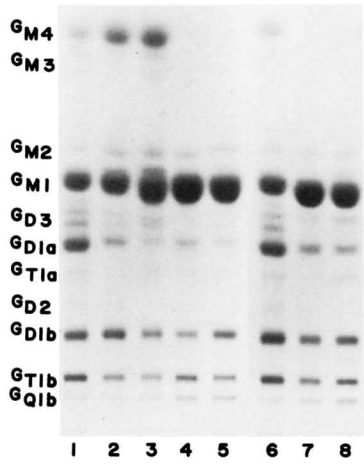

Fig. 2. TLC of the total gangliosides from cerebral and SC myelin. The conditions were the same as in Fig. 1. Lanes *1* and *6*, normal human cerebral white matter ganglioside mixture; *2*, human cerebral myelin; *3*, human SC myelin; *4*, cat cerebral myelin; *5*, cat SC myelin; *7*, rabbit cerebral myelin; and *8*, rabbit SC myelin.

bral white matter myelin were evident in human and cat, but less so in rabbit. Human SC myelin also contained higher levels of G_{M3} and G_{D3} than cerebral white matter myelin, but such a trend was not prominent in cat and rabbit. G_{T1a} again appeared in all the myelin samples examined, but G_{D1b}-fucose was found in measurable amounts only in cat and rabbit myelin.

Sephadex column chromatography

Fig. 3 shows the elution profile for the chromatographic separation of blue dextran, free sialic acid, sucrose, sodium chloride, and L-tryptophan by a Sephadex G-50 column with water as the eluting solvent. Blue dextran was eluted at the void volume of 37.2 ml (fraction no. 12). Sodium chloride had a

TABLE 3. Percent distribution of ganglioside sialic acid in CNS myelin

	Human		Cat		Rabbit	
	Cerebrum	Spinal Cord	Cerebrum	Spinal Cord	Cerebrum	Spinal Cord
	% of total ganglioside sialic acid[a]					
G_{M4}	26.6	25.3	1.0	1.7		
G_{M3}	1.2	3.5				
G_{M2}	3.7	6.6	4.7	4.3	3.2	3.5
G_{M1}	34.7	38.2	51.1	52.1	55.6	44.6
G_{D3}	2.7	5.0			2.7	5.3
G_{D1a}	6.9	0.7	4.3	2.1	8.1	7.8
G_{T1a}	0.5	0.1	2.0	1.1	0.5	2.4
G_{D2}	0.3	0.4	1.9	2.7	0.2	1.0
G_{D1b}	16.1	11.1	13.4	18.2	14.9	15.3
G_{D1b}-Fuc			1.8	2.4	0.9	2.4
G_{T1b}	6.3	7.1	13.7	11.0	10.8	12.5
G_{Q1b}	1.0	2.2	6.3	4.4	3.2	5.3

[a] Each value represents average of at least two determinations.

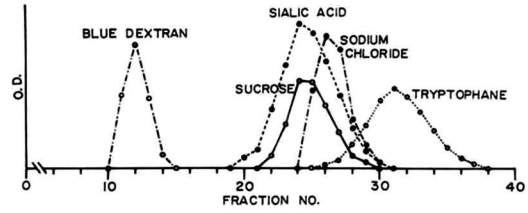

Fig. 3. Sephadex G-50 column chromatography of blue dextran, sialic acid, sucrose, tryptophan, and sodium chloride. A mixture of 1 mg of blue dextran, 184 μg of sialic acid, 20 mg of NaCl, 5 mg of sucrose, and 2 mg of L-tryptophan were applied to the column (bed vol. 80 ml, bed dimension 2 × 21 cm) in 2 ml of water and eluted with distilled water. Fractions of 3.1 ml were collected and analyzed for each component as described in the text.

peak at fraction no. 26 (80.6 ml). Sialic acid and sucrose peaked at fraction no. 24 (74.4 ml). L-Tryptophan lagged behind the sodium chloride peak due to the well-known aromatic interaction between tryptophan and the resin.

The elution profile for the separation of gangliosides and free sialic acid is shown in **Fig. 4.** The first peak, eluted at the void volume, contained only ganglioside-bound sialic acid as assayed by the method of Jourdian et al. (33). Free sialic acid was eluted in the second fraction which did not contain any lipid-bound sialic acid.

Chromatography of the blue dextran, NaCl, and UDP-galactose mixture (not shown) yielded two peaks when monitored at 260 nm. The first peak coincided with the blue dextran peak and was due to the adsorption of blue dextran. UDP-galactose gave a rather broadened peak which came off slightly ahead of the NaCl peak. Improved separation of gangliosides and UDP-galactose could be achieved by using 0.2 M NaCl solution as the eluant (Fig. 4). The ganglioside, monitored by resorcinol assay, peaked at the void volume. Two sharp peaks were obtained when the eluant was monitored spectrophotometrically at 260 nm.

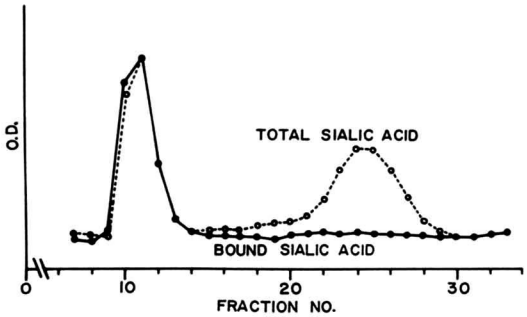

Fig. 4. Sephadex G-50 column chromatography of ganglioside and sialic acid. Ganglioside mixture of the normal human white matter (310 μg as sialic acid) and 180 μg of sialic acid was chromatographed in the same manner as described in Fig. 3. Total and bound sialic acids were assayed by the method of Jourdian et al. (33).

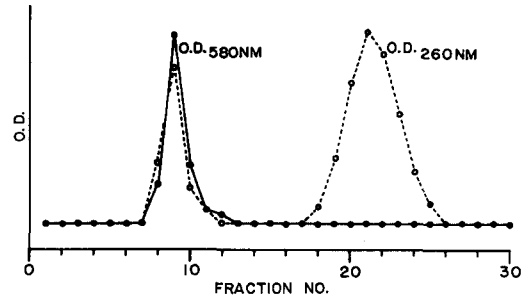

Fig. 5. Sephadex G-50 column chromatography of ganglioside and UDP-galactose. A ganglioside mixture of normal human white matter (100 μg as sialic acid) and 120 μg of UDP-galactose in 2 ml of 0.02 M NaCl was chromatographed as described in Fig. 3 except for eluting solvent. In this case, the column was eluted with 0.02 M NaCl. Fractions were analyzed by the resorcinol method (O.D. 580 ● —— ●) and absorption at 260 nm (O - - - O).

The first peak was due to the absorption by gangliosides. The second peak was due solely to UDP-galactose. This was shown in a separate experiment in which the same amount of UDP-galactose was chromatographed alone; it gave a peak at the identical position with the same size as shown in **Fig. 5.**

The ganglioside recovery rate is shown in **Fig. 6.** Recoveries of mixed gangliosides were 80–100% (2–506 μg lipid-bound sialic acid). In the higher concentrations tested (above 65 μg), the recovery was essentially quantitative. In the lower concentration range, recovery decreased to 80% or better, according to the amount of sample applied.

DISCUSSION

Spinal cord gangliosides

Schuwirth (15) first reported the presence of small amounts of gangliosides in human whole SC. He reported a value of 0.03 g of ganglioside per 100 g wet tissue based on Bial's reaction of rather crude preparations, which corresponds to about 100 μg lipid-bound sialic acid per g. This value is somewhat higher than our value for human whole SC. The only other report on SC gangliosides was provided by Taranova et al. (16) on rabbit SC. They found 0.515 mg ganglioside per g fresh tissue, corresponding to about 170 μg lipid-bound sialic acid per g.

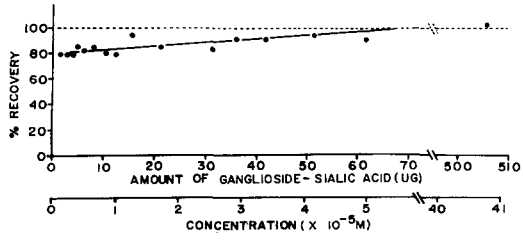

Fig. 6. Percent recovery of ganglioside from a Sephadex G-50 column as a function of applied amount or concentration. Known amounts of ganglioside mixture of normal human white matter (2–506 μg as sialic acid) were applied to the column (see Fig. 3) in 2 ml of water and eluted with distilled water. The first 21 ml was discarded and the next 32 ml was collected as the ganglioside fraction. This fraction was measured for sialic acid by the resorcinol method.

In the present study, whole SC of three mammalian species were found to contain considerably lower amounts of gangliosides than the corresponding cerebral gray matter. Since the SC gray matter contains primarily motoneurons, which are characterized by their relatively large size and modest dendritic arborizations, the contribution from cord gray matter would be expected to be smaller than the contribution of cerebral gray matter to the cerebrum. The SC myelin had a lower ganglioside content than cerebral white matter myelin, suggesting a lower SC white matter ganglioside concentration (see below). These factors could therefore contribute to the low ganglioside levels in the whole SC. It is interesting to note that the ganglioside concentrations in the whole SC are in the range of mammalian peripheral nerves (25, 38–41).

The strikingly different ganglioside pattern obtained from human whole SC when compared with cerebral tissues was unexpected. The unusual features were the reduced concentration of G_{D1a} and the elevated levels of G_{D3} and G_{M3}. Such dissimilarities were also noted for cat and to a lesser extent, for rabbit whole SC gangliosides. These differences are probably characteristic of SC. All CNS tissues examined contained small amounts of G_{T1a}, which had recently been isolated and characterized as a minor ganglioside in human brain (36). The cat and rabbit cords and cerebral white matter also contained an additional band that comigrated on TLC with a novel fucose containing G_{D1b} in two different solvent systems. This is a minor ganglioside in the human brain and its structure was recently elucidated as a G_{D1b} derivative containing an additional fucose moiety attached to the terminal galactose through an (α 1–2) linkage (37). G_{M4} ganglioside, which is abundant in human white matter, was also one of the major gangliosides in human SC. It was present in cat and rabbit cerebral white matter and cord only in very low concentrations. This is in line with our earlier finding that its presence in CNS tissue was species dependent (24, 35). G_{M4} ganglioside has recently been shown to be absent in PNS tissues and myelin (25, 42).

We have demonstrated the presence of gangliosides in SC myelin. The ganglioside contents in SC myelin were only about one-half of that in cerebral white

matter myelin in all three species, despite the generally higher lipid content in the cord myelin than in cerebral white matter myelin (43). The low ganglioside content is therefore parallel to the lower protein content in cord myelin (7, 10, 12, 14, 44–46). This correlation may be functionally significant in view of the recent calculation that an equimolar relationship exists between myelin basic protein and ganglioside sialic acid (25). Such regional differences in the heterogeneity of myelin compositions could be related, as suggested by Norton (43), to the phylogenetic age difference between the cord and cerebral tissues. The SC generally myelinates early, and is phylogenetically older than the cerebral white matter. The SC may then have a myelin with a different and more "primitive" composition.

Although the SC and cerebral white matter myelin gangliosides were quantitatively different, the ganglioside patterns were quite similar. This similarity in ganglioside patterns might reflect their common oligodendroglial origin. This was particularly evident in human CNS myelin in which G_{M4} was highly enriched. G_{M4} was present in rather low concentrations in cat and rabbit myelin. In all species studied, however, G_{M1} was the most abundant ganglioside in the SC as well as in cerebral white matter myelin.

DEAE–Sephadex and Iatrobead column chromatography

We have modified the original method of Ledeen, Yu, and Eng (22) for the isolation and purification of gangliosides from tissue lipid extracts. This modification was necessitated by the fact that only a small amount of SC and SC myelin could be obtained. Following an initial elution from the DEAE–Sephadex column of all the neutral (nonionic and zwitterionic) lipids with Solvent A, the acidic lipids were eluted with 0.2 M sodium acetate in methanol. Quantitative recovery was achieved with only five bed volumes of this salt solution. The use of methanol also facilitated the removal of fatty acid methyl esters formed by alkaline methanolysis of the acidic phospholipids. In addition, the methanolic solution is easier to evaporate than the chloroform–methanol–0.8 M sodium acetate 30:60:8 (Solvent B) used in the original method. We have also introduced the recently developed porous silica gel spheres, Iatrobeads, in place of Unisil for the final separation of sulfatides and gangliosides. Sulfatides could be quantitatively eluted from the column with 10 bed volumes of chloroform–methanol 85:15. An additional 10 bed volumes of the same solvent did not elute any more sulfatides, nor gangliosides, including the least polar G_{M4}. The gangliosides could then be quantitatively eluted with 10 bed volumes of chloroform–methanol 1:2. Further elution with the same solvent or more polar solvent such as chloroform–methanol 1:3 did not reveal any gangliosides.

Sephadex column chromatography

We have studied the application of Sephadex column chromatography for removing sodium acetate and other water-soluble contaminants from the acidic lipid fraction eluted from the DEAE–Sephadex column. We have also developed this technique as a general procedure for ganglioside purification.

Dialysis in a Visking tube is the most popular method for removing inorganic salts as well as water-soluble small molecules. However, when the ganglioside concentration is below 150 μg sialic acid per ml, considerable loss of gangliosides can occur (47). In addition, sugar nucleotides, which are frequently encountered in ganglioside biosynthesis experiments, are not readily dialyzable (48). The sugar nucleotides can be destroyed by treatment with snake venom phosphodiesterase (48). Alternate procedures such as electrophoresis (49) trichloroacetic acid–phosphotungstic acid precipitation (50) are either very tedious or may cause artifacts and abnormal TLC patterns (51–53). Other methods used for the removal of contaminants include solvent partitioning (54) and gel permeation chromatography. In our hands, Sephadex column chromatography in organic solvents (55–58) has proven to be difficult to apply because the flow rate is extremely slow and alterations of the gel swelling properties always occur. deRaveglia and Ghittoni (59) reported the purification of gangliosides on a Sephadex G-100 column that employed water as the eluting solvent, and McCluer, Coram, and Lee (60) reported a similar method employing Sephadex G-50. These investigators indicated that gangliosides were eluted as micelles at the void volume. We have reinvestigated this technique using various types of Sephadex G resins (G-25, G-50, G-100, G-200). Sephadex G-50 (medium) was finally chosen because of the excellent separation and flow rate. In general, chromatography takes only 1 hr for one complete cycle and the column is ready to be reused. Complete separation of gangliosides and other small molecules such as inorganic salts, sucrose, amino acids, and free sialic acid could be achieved. UDP-galactose was eluted as a broadened peak between blue dextran and NaCl when the column was eluted with distilled water. This was probably because the Sephadex contained small amounts of carboxyl groups which prevented the highly negatively charged UDP-galactose from entering the gel matrix. This problem was overcome by using 0.02 M NaCl as the eluting solvent

(to increase the ionic strength).[4] This resulted in baseline separation between the gangliosides and the UDP-galactose without cross contaminations. Excess sodium chloride present in the ganglioside fraction can then be removed by rechromatography using water for elution. This method for removing sugar nucleotides seems to be simpler and more quantitative than the enzyme method or paper electrophoresis as mentioned earlier.

The ganglioside recovery from the Sephadex column is quantitative above the concentration of 65 μg lipid-bound sialic acid per 2 ml (volume dissolved). This corresponds to a concentration of about 5×10^{-5} M or in the order of the critical micelle concentration (61, 62). At even lower concentrations, the recovery of gangliosides at the void volume is still better than 80%. This observation favors the concept that gangliosides below the critical micelle concentration could exist as submicellar complexes (61) rather than monomers (62). The Sephadex column method, therefore, appears to be excellent for the separation of gangliosides from other water-soluble nonlipid contaminants when dealing with minute amounts of the material.

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