Fatty acid composition of human brain sphingomyelins: normal variation with age and changes during myelin disorders

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SUMMARY Sphingomyelins have been isolated in almost quantitative yield from normal and pathological human nervous tissues, and their fatty acid compositions determined by gas–liquid chromatography. In normal frontal lobe the proportion of stearic acid (18:0) decreases with increasing age from about 80% in the newborn to about 40% in the adult, whereas the C_{22}–C_{26} acids increase from about 10 to about 50%. In dysmyelinating diseases or malformations of the nervous system the content of C_{22}–C_{26} acids was much smaller than in normal brains of the same age. In normal cortex 18:0 constitutes at least two-thirds of the sphingomyelin fatty acids at all ages. In normal white matter from adults C_{22}–C_{26} acids represent two-thirds of the acids present. We conclude that sphingomyelin of cytoplasm and that from myelin sheath show striking differences in the chain-lengths of their fatty acids. In patients who had died from dysmyelinating and demyelinating diseases the deviation from the normal pattern was much more pronounced in cerebral white matter than in total brain.

Sphingomyelins of spinal medulla have a fatty acid pattern similar to that of adult brain but contain relatively higher amounts of 18:0 and 24:1. Sphingomyelins of peripheral nerve have a distinctly different fatty acid pattern, with much less 18:0 than in cerebral white matter.

KEY WORDS sphingomyelins · fatty acid composition · man · brain · cerebral cortex · white matter · age variation · dysmyelinating · demyelinating · disease · spinal medulla · peripheral nerve · encephalomyelitis · leukoencephalitis · multiple sclerosis

EARLY STUDIES of the fatty acids of sphingomyelins from nervous tissue by Merz (2), Rennkamp (3), and Thannhauser and Boncoddo (4) gave important information but before the advent of gas–liquid chromatography (GLC) the limitations of the analytical techniques did not allow a detailed quantitative analysis.

Using GLC a much more complete analysis of the fatty acid pattern can be obtained, but no systematic investigation of the fatty acids of sphingomyelins in man has so far been performed. Bernhard and Lesch (5) have reported the fatty acid composition of brain sphingomyelins from four regions of a brain of a 69 yr old man. In a preliminary communication, O'Brien (30) gives data for the frontal lobe of a 9 yr old normal child and of two children of similar age with metachromatic leukodystrophy. For comparison their values for gray and white matter have been included in our Tables 5 and 6.

MATERIALS AND ANALYTICAL METHODS

Normal Nervous Tissues

The normal human brains were obtained from the Institute of Pathology, Sahlgren's Hospital, Gothenburg. It was planned to use only brains from persons killed in accidents but after a year it was evident that enough material could not be obtained. For this reason nervous tissues from persons who had died suddenly after surgical operations or from vascular diseases have also been used. All the material was subjected to pathological-anatomical examinations and quantitative analysis of the main lipids. Sphingomyelins were isolated only from material that showed no pathological changes. The brains of the three premature and the three full term infants were macroscopically normal, but no microscopical examination was performed. The samples were taken from the frontal lobe or, in a few cases, the temporal lobe (Table 1).

A preliminary report of the results obtained was given at VII International Congress of Neurology in Rome (1961) (1).

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TABLE 1 Source of Normal Tissue Used for the Isolation of Sphingomyelins

Subject	Sex	Age and Cause of Death	Tissue Used
FB 101	F	Premature, 29 wk. Died 2 days after delivery	Whole brain
FB 102 } Twins	M	Premature, 33 wk. Died 1 day after delivery	Whole brain
FB 103 } Twins	M	Premature, 33 wk. Died 1 day after delivery	Whole brain
FB 104–106	M, F	Newborn, fullterm. Lived for 1–3 days (Combined)	Three cerebral hemispheres
CB 101	F	14 months. Esophageal atresia	Right cerebral hemisphere
CB 102	F	17 months. Congenital heart disease	Frontal lobe, total Temporal lobe, cortex and white matter
CB 103	M	4 yr. Road accident	Frontal lobe, total
CB 104	M	4 yr. Congenital heart disease	Frontal lobe, total
CB 106	M	8 yr. Road accident	Frontal lobe, total
CB 107	F	15 yr. Congenital heart disease	Central lobe, total Frontal + temporal lobe, cortex and white matter
CB 108	F	8 months. Accident	Frontal lobe, cortex and white matter
AB 102	M	16 yr. Road accident	Frontal lobe, total Temporal lobe, cortex " " , white matter
AB 103	M	22 yr. Accident	Frontal lobe, total
AB 104	F	32 yr. Cancer ovarii. No involvement of CNS	Frontal lobe, cortex " " , white matter
AB 105	F	33 yr. Acute nephritis. No pathological changes of nervous system	Frontal lobe, total Central lobe, cortex " " , white matter
AB 106	F	39 yr. Cancer ventriculi. No involvement of CNS	Frontal lobe, total
AB 107	M	64 yr. Cancer. No involvement of CNS	Total brain
AB 108	F	77 yr. Bronchopneumonia	Frontal lobe, cortex " " , white matter
AB 109	F	77 yr. Emphysema + Bronchopneumonia	Spinal medulla + spinal nerve roots
AB 110	M	70 yr. Acute myocardial infarction	Femoral nerves
AB 111	M	72 yr. Cerebral hemorrhage	Femoral nerves

FB, fetal brain. CB, child's brain. AB, adult brain.

Pathological Nervous Tissue

The clinical diagnosis was verified in all but one case by pathological-anatomical examination carried out by Dr. P. Sourander of the Department of Neuropathology at this University. One case (CB 221) was examined by Dr. L. Zettergren of the Institute of Pathology, Sahlgren's Hospital. Literature references to published case reports are given in Tables 6 and 7.

Preparation of Specimen for Isolation of Lipids

Within 48 hr of death the desired tissues were isolated and stored at $-20°$ if they were not to be handled immediately. In general the frontal lobe of the right cerebral hemisphere was used for the isolation of the sphingolipids. From the same region samples were taken for quantitative lipid analyses. The analytical procedure and methods used have been described elsewhere (6). In the more recent part of the work the brain tissue was separated into cerebral cortex and cerebral white matter. If the frontal lobe had already been used for lipid isolation the temporal lobe was used instead (noted in Table 1 for normal material; Case CB 213 in the pathological material). In the normal material it was rather difficult to achieve a complete separation of cortex and medulla. In one case (CB 107) only the outer 1–2 mm of the outer cerebral cortex was taken for analysis. In order to obtain enough material in this case it was necessary also to use material from the temporal lobe. In the pathological material the separation of cerebral cortex and white matter was much easier to perform because of the severe gliosis.

Chemicals

Reagent grade methanol was stored over NaOH pellets and distilled before use. It contained 0.3–0.5% of water. Chloroform was dried over calcium chloride and distilled. After distillation the chloroform was not stored for more than a week. Tetrahydrofuran was redistilled and hydroquinone (1 g/liter) was added. These solvents were stored in brown bottles and a stream of nitrogen was passed through the solvents for 15 min when a new bottle was used. All other solvents were redistilled and stored without additives.

Silicic acid (Baker, A. R., Lot No. 4680) was treated in the manner described recently (13). In the alumina (standardized according to Brockmann) (Merck) the finest particles were removed by suspending the material in chloroform–methanol 1:1 and decanting the solvent

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TABLE 2 MELTING POINTS OF REFERENCE COMPOUNDS

	Acid		Methyl Ester	
	Present Work	Literature	Present Work	Literature*
		degrees C		
16:0†			30.0	30.55 (γ)
18:0‡			38.9	39.1 (γ)
20:0‡			46.4–46.6	46.6 (γ)
21:0‡			47.2	47.2 (β)
22:0‡			52.6–52.9	52.7 (β)
22:1‡	33.3–33.6	33.15§	—‖	
23:0‡			54.0	54.0 (β)
23:1¶	43.8–44.2		—‖	
24:0‖			57.6–57.8	57.8 (β)
24:1¶	43.4–43.6	**	—‖	
25:0‡			59.3–59.5	59.5 (β)
25:1¶	52.7–52.9		28.3–28.5	
26:0‡	87.6–87.9	87.7*	62.7–62.9	62.9 (β)
26:1¶	51.5–51.7		19.7–20.1	

* F. Francis, and S. H. Piper *J. Am. Chem. Soc.* **61:** 577, 1939.
† Mann Research Lab.
‡ Prepared in our laboratory.
§ Melting point of sample of erucic acid obtained from Prof. F. Francis in 1938.
‖ Liquid at room temperature.
¶ Compound prepared by lengthening the chain of oleic or erucic acid. The synthesis will be described in a separate communication.
** Melting points found in the literature range from 39 to 45°. Cf. Bounds, D. G., R. P. Linstead, and B. C. L. Weedon, *J. Chem. Soc.* **no vol:** 448, 1954.

after 5 min. This was performed twice, shortly before use.

Analytical Methods

Phosphorus was determined by a modification of the method described by Lowry et al. (14, 15) and nitrogen with a ninhydrin procedure (16). In the initial stage of this work (1960–61) analytical partition chromatography was run on Schleicher & Schüll paper 2045b with tetrahydrofuran-diisobutylketone–water 45:5:6 (v/v/v) and diisobutylketone–acetic acid–water 40:25:5 as solvents (17). Later (from 1962 onward) only thin-layer plates of Silica Gel G (Merck) have been used with chloroform–methanol–water 65:25:4 (18). In a second series of analyses the water of the solvent mixtures was replaced with 4 N ammonia.

Isolation of the Sphingomyelins

Chromatography of total lipid extract on silicic acid. A crude sphingolipid extract was made by mild alkaline hydrolysis and solvent partition (13). The silicic acid columns were loaded with 10–50 mg of sphingolipids per g of silicic acid, which corresponded to 2–10 mg of sphingomyelin per g of silicic acid. The glycosphingolipids (cerebrosides and sulfatides) were eluted with chloroform–methanol (C–M) 4:1, 1-alkyl 3-glycerophosphoryl ethanolamine and monosialogangliosides with C–M 1:1, and sphingomyelins, di- and trisialogangliosides, and some 1-alkyl 3-glycerophosphoryl choline, with C–M 1:4. The effluent was collected in tubes on a fraction collector. When the amount of sphingomyelin was large, some sphingomyelin appeared already with C–M 1:1, but this was detected by paper or thin-layer chromatography. The contents of all tubes containing sphingomyelins were combined and evaporated to dryness. The crude sphingomyelins (50–500 mg) were dissolved in 100 ml of C–M 2:1, and 20 ml of water was added. The mixture was shaken and left standing for 24 hr before separating the lower phase. The upper phase was extracted twice with 10 ml of chloroform, each time allowing separation to take place for 1 hr. The lower phases were all combined. The upper phase contained most of the gangliosides and low molecular weight compounds.

Chromatography on alumina. After evaporation of the solvent the lower phase was dissolved in 25 ml of C–M 1:1 and applied to a column of 10 g of alumina. The sphingomyelins were eluted with 200 ml of the same solvent and the remaining lipids (gangliosides) with chloroform–methanol–water 2:5:2 (19). The purity of the sphingomyelin fraction was checked by TLC. Usually the fraction was contaminated with material that moved with the solvent front and often also with traces of material having the same R_F value as 1-alkyl 3-glycerophosphoryl choline. Final purification was carried out on silicic acid. Silicic acid (10 g) was slurried in C–M 9:1 and the sphingomyelins were applied to the column in the same solvent. The impurities were eluted with 100 ml of C–M 9:1, and the sphingomyelins with 200 ml of C–M 1:2 followed by 100 ml of methanol. If the extract contained more than 2% of nonsphingomyelin phospholipids, which occurred when the sphingomyelins were prepared from infant brains, the eluate was collected as 10 ml samples on a fraction collector and the samples were tested by chromatography. Otherwise only three fractions were taken.

The isolated sphingomyelins were assayed for phosphorus and nitrogen. Analytical chromatography was performed on all samples with two different solvents and with three different amounts applied to the chromatograms. The yields of sphingomyelins were calculated by determining the total phosphorus in the lipid extract before chromatography and in the separated fractions of 1-alkyl 3-glycerophosphoryl ethanolamine, 1-alkyl 3-glycerophosphoryl choline, and sphingomyelins. The recovery of lipid bound P was 95–101% except for some of the preparations from infant brains. Such preparations were discarded and new ones made. After

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addition of sphingomyelins to the lipid extract the recovery of added sphingomyelins was 99–102%.

Preparation of Methyl Esters of Fatty Acids

Sphingomyelin, 50 mg, was methanolyzed with 5 ml of HCl–methanol (5%) in a sealed tube for 4 hr in a boiling water bath. The tube was cooled to room temperature and opened, and the methyl esters were extracted in the tube with 3 × 10 ml of light petroleum (bp 40–60°). The combined extracts were rinsed with water followed by 5% sodium bicarbonate and water, and then dried over anhydrous Na_2SO_4. The methyl esters from sphingomyelins of cerebral gray and white matter were tested for purity by thin-layer chromatography (TLC) with light petroleum–ethyl ether–acetic acid 90:10:1 as solvent. If the sample contained 3-*O*-methyl sphingosine (which occurred in about 50% of the preparations), it was chromatographed on silicic acid (5 g). The methyl esters were eluted with 50 ml of chloroform. The weight of methyl esters was 37–49% of the weight of the sphingomyelins (theoretical amount for C_{18}-sphingomyelin = 39% and for C_{24}-sphingomyelin = 46%). The higher figures were found for white matter, and the lowest figures for the fetal and premature material. The recovery of free or methylated fatty acids added in the methanolysis step was 99–103%.

Gas–Liquid Chromatography

Perkin-Elmer gas chromatographs model 154 B (modified) equipped with flame ionization detectors were used. Most of the analyses have been performed with Reoplex 400 (5%) as stationary phase (20). A large number of other phases have been tried (see, for example, references 21, 22) but we found no reason to abandon Reoplex 400. The same batch of Reoplex 400 polyester has been used throughout the study. Bleeding was considerably reduced if the chloroform solution of the polyester had been carefully washed with water, and dried with sodium sulphate. The dried solution was then directly used for coating the support. Chromosorb W 80–100 mesh or Gas Chrom A 80–100 mesh was used as support. They were treated with dimethyldichlorosilane before use (23). The columns were made from aluminum tubes 2 m × 4 mm i.d. They had an efficiency of 2500 to 3000 theoretical plates (for methyl *n*-tricosanoate). The analyses were carried out at a temperature of 210° with helium as carrier gas. The retention time for methyl *n*-hexacosanoate was 30–45 min. Each column used was tested with an artificial mixture having a composition closely similar to the mixtures analyzed (cf. reference 24). The source and mp of acids and/or methyl esters used are listed in Table 2 and the result of a typical test run is shown in Table 3.

TABLE 3 GLC Analysis of Mixture of Known Composition

Fatty Acid Methyl Ester	Weight Per Cent of Component	Found	Difference
16:0	2.0	2.2	+0.2
18:0	28.9	29.6	+0.7
20:0	1.9	1.8	−0.1
21:0	0.2	0.2	±0
22:0	1.7	1.8	+0.1
22:1	0.8	1.1	+0.3
23:0	2.4	2.6	+0.3
23:1	2.1	1.7	−0.4
24:0	5.8	5.6	−0.2
24:1	38.1	37.3	−0.8
25:0	2.5	2.6	+0.1
25:1	5.7	5.7	±0
26:0	0.9	1.5	+0.6
26:1	7.0	6.3	−0.7

All samples of natural esters were examined both before and after hydrogenation over Adam's catalyst. It was found that for each chain length the quantitative figure obtained after hydrogenation agreed with the combined figures for the unsaturated and the saturated esters of the same chain length. Peak areas were determined by weighing. In order to reduce cutting out errors the paper speed of the recorder was altered during the run to give peaks of suitable width for the measurements. Before the systematic quantitative analyses were started, great care was taken to identify the components responsible for the peaks observed in the gas chromatograms. The retention times were compared with those of synthetic compounds using both silicone and polyesters as stationary phase. In several cases the components were collected and subjected to mass spectrometric analysis.

RESULTS

The fatty acid composition of the sphingomyelins is given in Tables 4–7. In addition to the acids listed in the tables there are also present small amounts of mainly C_{24}- and C_{26}-dienoic acids but these, together with other acids present, constitute less than 2% of the total fatty acids and have not been analyzed. The routine analysis of the methyl esters by thin-layer chromatography gave no evidence for the occurrence of hydroxy fatty acids in sphingomyelins. As a further check sphingomyelins were isolated from total brain of CB 103 and AB 101 with great care to obtain also the most slowly eluted sphingomyelins, which might contain the hydroxy fatty acids. After methanolysis thin-layer plates were run with up to 200 μg of fatty acid methyl esters. There was no sign of any hydroxy esters although as little as 1 μg of a mixture of hydroxy esters was clearly visible under the conditions used.

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TABLE 4 FATTY ACID COMPOSITION OF SPHINGOMYELINS FROM NORMAL HUMAN NERVOUS TISSUE
Cerebral cortex and white matter not separated
Weight percentages

	Brain															Spinal Medulla	Peripheral Nerves	
	FB 101 29 Fetal Wks	FB 102 33 Fetal Wks	FB 103 33 Fetal Wks	FB 104-6 Full-Term Combined	CB 101 14 Months	CB 102 17 Months	CB 103 4 Yr	CB 104 4 Yr	CB 106 8 Yr	CB 107 15 Yr	AB 102 16 Yr	AB 103 22 Yr	AB 105 33 Yr	AB 106 39 Yr	AB 107 64 Yr	AB 109 77 Yr	AB 109 77 Yr	AB 110 70 Yr, AB 111 72 Yr Combined
14, 15	0.4	0.6	0.2	0.2	0.1	0.2	0.3	0.1	0.1	0.4	0.1	0.4	0.1	0.3	0.1	0.2	0.2	0.2
16:0	11.9	8.3	9.0	7.3	3.0	3.2	2.3	2.5	2.2	3.0	2.3	2.7	2.3	3.6	2.4	2.9	4.9	6.9
17:0	0.1	—	—	0.1	0.1	—	0.1	0.2	0.1	0.3	0.1	0.2	0.2	0.4	0.1	0.1	0.1	0.2
18:0	70.5	78.2	75.7	84.8	65.7	63.2	49.0	48.3	41.1	49.3	39.9	48.8	38.8	40.0	34.6	41.1	18.9	13.8
19:0	Tr.	0.1	0.1	0.1	—	—	0.1	0.2	0.2	0.4	0.2	0.3	0.3	0.4	0.3	0.4	0.5	0.4
20:0	2.0	2.2	2.2	1.6	2.1	1.7	1.7	1.8	1.8	2.1	1.4	1.8	1.5	1.6	1.5	1.5	6.1	5.1
20:1	0.3	—	—	—	—	0.1	—	—	—	—	—	0.1	0.1	0.5	—	0.1	—	—
21:0	0.1	0.1	0.1	Tr.	0.1	0.2	0.1	0.2	0.3	0.6	0.1	0.2	0.3	0.1	0.2	0.1	0.5	0.5
22:0	2.7	1.9	2.3	1.8	2.1	1.9	1.9	1.8	1.9	2.0	1.6	2.0	1.6	2.0	1.8	1.7	11.1	10.7
22:1	0.6	0.3	0.3	0.1	0.9	0.7	0.6	0.8	1.0	0.6	0.8	0.6	0.6	0.7	0.6	0.6	0.8	0.7
23:0	0.4	0.4	0.4	0.2	1.0	1.0	1.6	1.6	2.0	2.1	1.8	2.4	2.1	2.2	2.1	1.1	5.8	6.9
23:1	0.2	0.1	0.2	Tr.	0.5	0.5	0.8	1.0	1.1	0.9	1.1	0.7	1.0	1.0	1.2	0.9	0.5	0.4
24:0	2.5	1.6	2.3	1.2	3.9	4.1	5.5	4.8	4.7	4.8	4.9	5.4	4.3	8.1	5.6	3.3	15.6	17.9
24:1	6.9	5.7	6.5	2.2	15.9	15.5	26.4	25.2	29.0	22.0	33.1	22.9	31.7	25.1	37.2	40.6	31.4	32.6
25:0	0.3	0.2	0.3	—	0.5	1.4	1.8	2.9	2.8	2.1	2.6	3.0	3.7	3.0	1.4	0.5	1.6	1.9
25:1	0.4	0.1	0.1	0.1	1.0	2.3	3.4	3.7	5.7	4.6	5.3	4.1	6.4	4.8	6.4	2.6	0.5	0.6
26:0	—	0.2	0.1	—	0.4	0.9	0.3	0.4	0.2	0.5	0.4	0.1	0.2	0.9	0.6	0.3	0.3	0.2
26:1	0.6	0.1	0.3	0.2	2.5	3.1	4.2	4.5	5.8	4.3	4.3	4.4	4.9	4.4	4.1	1.9	1.1	1.0
22–26	14.6	10.6	12.8	5.8	28.7	31.4	46.5	46.7	54.2	43.9	55.9	45.6	56.5	52.2	61.0	53.5	68.8	72.9

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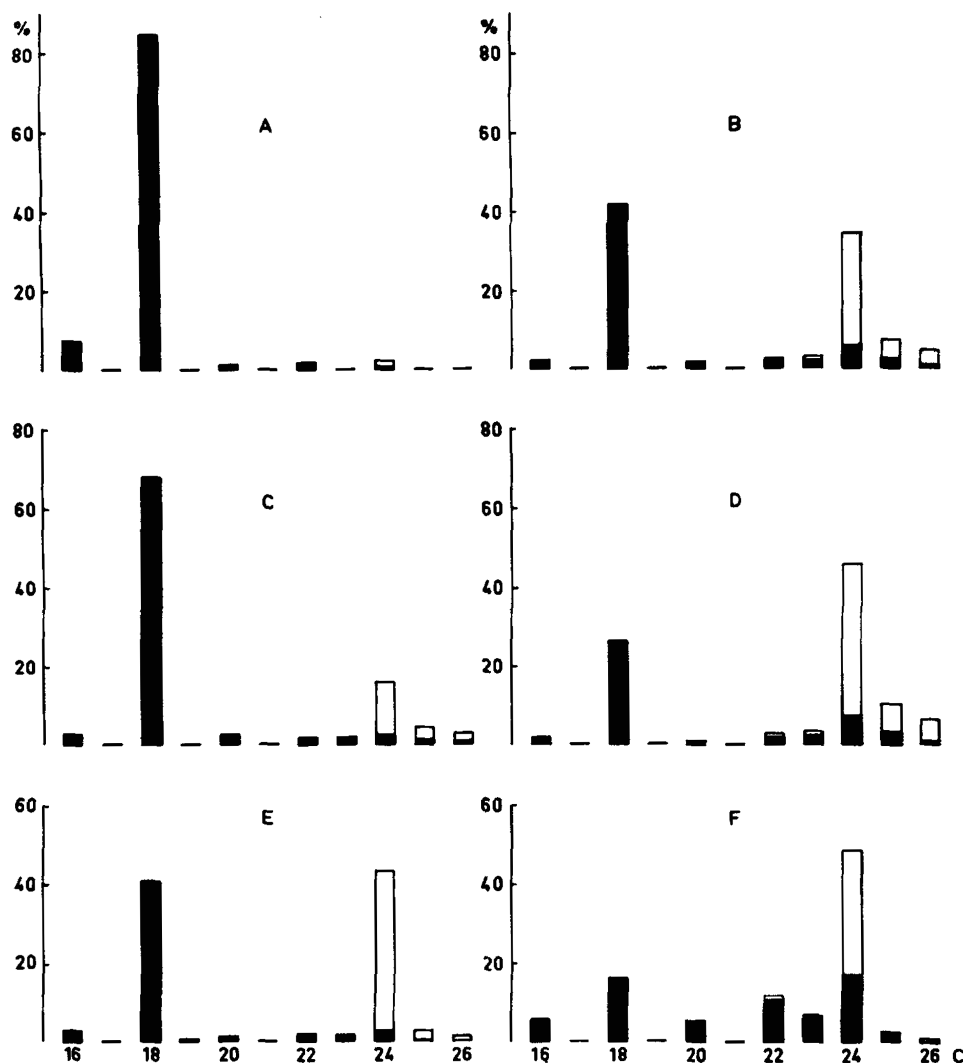

FIG. 1. Fatty acid composition of sphingomyelins from normal human nervous tissue. Solid bars, saturated acids; open bars, monounsaturated acids. *A*, full term infants FB 104–6; whole brain. *B*, adult brains AB 102–103, 105–106; cerebral cortex and white matter not separated. *C*, adult brain AB 102, 104, 105, 108; cerebral cortex. *D*, adult brain AB 102, 104, 105, 108; white matter. *E*, spinal medulla AB 109. *F*, peripheral nerves AB 109–111.

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Fatty Acids of Brain Sphingomyelins (Gray and White Matter Not Separated)

Brain sphingomyelins contain (Table 4 and Fig. 1) about 20 different fatty acids in measurable amounts, and 18:0 and 24:1 constitute together about 75% of the total acids at all ages. There is, however, a marked change in the fatty acid pattern with age. In fetuses and newborn infants the sum of C_{16} and C_{18} acids is larger than 80% and there are only small amounts of C_{24} acids. The concentration of stearic acid is about 10 times that of palmitic acid and this quotient is still higher in child and adult brain. In parenchymatous organs outside the nervous system the ratio C_{18} to C_{16} is reversed.[1] A tendency in this direction is the relatively greater concentration of 16:0 in peripheral nerve than in brain.

Before myelination begins the concentration of C_{24} acids is low but from this stage there is an increase with age up to a concentration of 28–43% in adults. In general 24:1 constitutes about 80% or more of the total C_{24} acids. The brain sphingomyelins contain con-

[1] S. Ställberg-Stenhagen and L. Svennerholm, unpublished results.

TABLE 5 Fatty Acid Composition of Sphingomyelins from Cortex and White Matter of Normal Human Brain
Weight percentages

	CB 108 8 Months		CB 102 17 Months		CB 107 15 Yr		AB 102 16 Yr		AB 104 32 Yr		AB 105 33 Yr		AB 108 77 Yr		69 Yr*		9 Yr†	
	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W	C	W
14, 15	0.2	—	0.4	0.2	0.1	0.1	0.1	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.7	0.6	1.4	7.8
16:0	5.6	2.9	7.1	3.1	2.2	1.2	2.3	2.1	2.4	2.1	2.7	2.1	3.1	2.1	4.4	4.0	2.4	6.7
16:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4	0.4	Tr.	0.5
17:0	0.1	0.1	—	—	0.2	Tr.	0.2	0.1	—	0.2	0.2	0.1	0.2	0.1	—	—	—	—
18:0	85.5	49.5	85.6	34.9	82.1	25.2	69.5	28.1	66.2	29.7	69.1	26.0	67.3	23.3	56.7	33.8	78.6	30.5
18:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.7	1.2	2.2	1.3
19:0	0.1	0.1	—	0.2	0.1	Tr.	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2	—	—	—	—
20:0	1.6	2.2	1.7	1.7	2.5	1.0	2.3	1.1	2.0	1.2	2.6	0.9	2.6	1.1	1.1	0.9	1.2	0.9
20:1	—	—	—	—	—	0.1	—	0.1	—	—	—	—	—	—	—	—	Tr.	Tr.
21:0	0.1	0.1	0.3	0.3	0.2	0.2	0.2	0.5	0.3	0.5	0.1	0.1	0.1	0.1	4.4	1.1		
22:0	1.0	2.7	1.0	2.7	0.8	2.0	1.1	1.8	1.2	1.8	1.6	1.8	1.4	2.0	0.7	1.9	0.5	1.4
22:1	0.3	2.2	0.2	1.3	0.2	0.7	0.3	0.9	0.3	0.7	0.3	0.6	0.3	0.9	0.5	1.3	Tr.	0.4
23:0	0.2	1.1	0.3	1.7	0.5	2.3	0.9	2.2	1.0	2.3	1.0	2.5	1.1	3.0	0.6	4.1	Tr.	1.6
23:1	0.1	0.9	0.2	0.9	0.2	1.2	0.5	1.3	0.5	1.0	0.3	1.2	0.4	1.5	0.3	1.6	Tr.	0.5
24:0	0.9	4.6	0.8	9.7	1.0	7.0	2.3	6.9	2.5	7.9	3.0	8.1	2.9	8.1	1.6	9.6	1.5	6.5
24:1	3.8	28.3	2.5	30.2	5.1	42.3	14.0	38.2	13.5	35.5	12.6	41.2	13.4	38.8	21.2	30.7	2.9	25.2
25:0	0.1	0.3	Tr.	2.7	1.3	3.3	1.2	3.5	1.8	2.9	1.1	2.6	1.0	3.3	0.5	1.2	Tr.	1.4
25:1	0.2	1.5	Tr.	3.4	2.0	7.4	2.7	7.1	4.1	6.1	2.8	6.8	3.2	7.8	4.1	4.2	0.8	3.2
26:0	0.1	0.2	—	1.5	0.8	—	0.2	0.4	0.7	1.8	0.3	0.7	0.2	0.7	—	—	Tr.	Tr.
26:1	0.2	3.4	Tr.	6.0	0.8	6.0	2.2	5.3	2.9	5.8	2.0	5.0	2.5	6.9	2.1	3.4	Tr.	2.3
22–26	6.9	45.2	6.8	60.1	12.7	72.2	25.4	67.6	28.5	65.8	25.0	70.5	26.4	73.0	36.1	58.0	7.2	52.4

C = Cerebral cortex, W = White matter.
* From Bernhard and Lesch (5).
† From O'Brien (30).

siderable amounts (10–15%) of odd-numbered straight chain fatty acids, mainly C_{25}. In general these acids are more saturated than the C_{24} and C_{26} acids. Radin and Akahori (25) obtained similar results in a study of the fatty acid composition of human cerebrosides.

Cerebral Cortex and White Matter

The fatty acid compositions of brain sphingomyelins are quite different in cerebral gray and white matter (Table 5 and Fig. 1). In gray tissue 18:0 predominates and constitutes about two-thirds of total fatty acids in adult human cerebral cortex, whereas 24:1 is less than 15%. The content of long-chain acids in gray matter is particularly low in case CB 107, where the sample was taken from the outermost part of the cortex. In cerebral white matter the ratio 18:0 to 24:1 is reversed: 24:1 constitutes about 40% and 18:0 25–30%.

Spinal Medulla and Peripheral Nerve

The spinal medulla was studied only in one case (Table 4 and Fig. 1). The acids 18:0 and 24:1 occur in about equal amounts and make up about 82% of the total fatty acids.

In peripheral nerve the pattern shows large deviations from that of brain. Apart from the difference in the ratio of 16:0 to 18:0 already mentioned there are relatively large proportions of all the even-numbered fatty acids, with C_{24} predominating. The concentration of C_{23} is higher than in brain, but percentages of C_{25} and C_{26} acids are lower. Further, the fatty acids of peripheral nerve are more saturated than those of brain.

Pathological Brain Material

Brain sphingomyelins prepared from patients who have suffered from inherited disorders of lipid metabolism and malformations of nervous system show in general an "immature" fatty acid pattern (Tables 6 and 7). This means that they contain smaller percentages of very long-chain fatty acids (C_{22}–C_{26}) and higher proportions of C_{18} acids than the brain sphingomyelins prepared from normal persons of the same age. An exception to this general rule is that the sphingomyelins from one patient who had suffered from juvenile amaurotic idiocy (CB 222, Table 7) had a normal fatty acid pattern, which tallies with other evidence that this form of amaurotic idiocy is not a primary lipidosis (26).

In two patients with disseminated demyelinating disease (disseminated encephalomyelitis, CB 252, and disseminated sclerosis, AB 251, Table 6) the brain sphingomyelins in regions without macroscopic signs of lesions had a normal fatty acid pattern for the age. It was not possible to obtain enough material from lesions for determination of sphingomyelin fatty acids.

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TABLE 6 Fatty Acid Composition of Sphingomyelins from Pathological Human Brain in Dysmyelinating and Demyelinating Diseases

Weight percentages

	Leukodystrophy Globoid Cell Type					Leukodystrophy: Metachromatic Type									Sub-acute Leuko-encephalitis	Dis-semi-nated Enceph-alomye-litis	Multiple Sclerosis	Leuko-dystrophy: M. T. Late Infantile	
						Late Infantile								Adult					
	CB 201 F Ref. 7, Case 4 15 Months	CB 202 F Ref. 7, Case 3 $2^2/_3$ Yr		CB 203 F Ref. 7, Case 5 $2^2/_3$ Yr		CB 211 M Case A. A. $2^1/_2$ Yr		CB 212 F Ref. 1, Case A. L. $3^3/_4$ Yr	CB 213 M Ref. 1, Case R. A. $4^1/_2$ Yr			CB 214 F Case I. M. $7^1/_2$ Yr		AB 211 F Ref. 8 29 Yr	CB 251 M Ref. 9 12 Yr	CB 252 M 5 Yr	AB 251 F 40 Yr	11 Yr†	
	Total Lobe	Cerebral Cortex	White Matter	Cerebral Cortex	White Matter	Cerebral Cortex	White Matter	Total Lobe	Total Lobe	Cerebral Cortex	White Matter	Cerebral Cortex	White Matter	Total Lobe	White Matter	Total Lobe	Total Lobe	Cerebral Cortex	White Matter
14, 15	0.1	—	0.1	0.9	1.3	0.1	0.1	0.1	0.3	—	0.1	0.2	0.2	0.3	0.2	0.6	0.5	1.3	1.0
16:0	7.1	3.8	9.7	2.7	5.6	2.6	3.5	6.3	4.2	4.2	6.6	3.9	6.5	6.4	5.7	4.3	3.3	17.0	10.1*
17:0	0.1	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.3	—	0.1		
18:0	81.4	88.1	60.2	88.9	72.7	88.2	47.8	68.6	67.4	84.8	66.1	80.3	60.6	59.5	63.5	50.9	40.8	72.0	73.8
18:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.4	2.9	1.2
19:0	0.1	0.1	0.3	0.1	0.1	0.1	0.2	0.3	0.2	0.3	0.2	0.3	0.4	0.3	0.3	0.4	0.2	—	—
20:0	1.9	2.1	2.1	1.9	2.0	2.3	1.9	1.8	2.8	1.8	1.3	2.8	1.7	3.3	2.0	1.7	1.8	2.0	1.2
20:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.1	—	—	Tr.	Tr.
21:0	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	—	—
22:0	1.6	1.0	2.9	0.8	2.3	1.2	2.4	2.1	1.6	1.7	1.7	2.0	2.2	1.8	2.9	0.8	1.6	0.7	0.8
22:1	0.2	0.1	0.6	0.1	0.6	0.2	0.8	0.6	0.5	0.3	0.5	0.2	0.5	0.4	0.6	0.8	0.7	Tr.	—
23:0	0.6	0.3	1.6	0.2	1.2	0.3	1.9	1.4	1.0	0.5	1.4	0.5	2.2	1.4	1.1	1.9	2.0	Tr.	0.6
23:1	0.2	0.1	0.4	0.1	0.3	0.1	0.8	0.7	0.6	0.1	0.4	0.2	0.6	0.6	0.5	0.8	0.7	Tr.	Tr.
24:0	1.9	1.0	3.9	0.7	2.3	0.9	6.9	2.9	2.8	1.8	4.0	2.3	3.2	2.9	3.0	7.2	5.1	1.0	1.7
24:1	3.8	2.2	15.7	2.3	8.5	3.0	21.7	10.2	14.1	3.8	13.2	5.8	16.1	17.0	17.1	20.3	30.0	1.7	7.2
25:0	0.1	0.1	0.4	0.1	0.5	0.2	1.8	1.0	0.7	0.1	0.7	0.2	0.8	0.9	0.5	2.2	1.8	Tr.	Tr.
25:1	0.4	0.1	0.5	0.1	0.6	0.1	2.6	1.1	1.3	0.1	0.1	0.4	2.0	2.5	0.7	2.9	6.0	Tr.	0.8
26:0	0.1	Tr.	0.4	Tr.	0.2	0.2	0.9	—	0.3	Tr.	0.4	Tr.	Tr.	0.2	0.1	0.7	0.2	Tr.	Tr.
26:1	0.3	0.9	0.8	0.6	1.2	0.2	6.3	2.5	2.2	0.2	2.0	0.6	2.6	2.1	1.0	4.3	4.2	Tr.	1.0
22–26	9.2	5.8	27.2	5.0	17.7	6.4	46.1	22.5	25.1	8.6	25.4	12.2	30.2	28.7	27.5	41.9	52.5	6.4	12.4

* 16:1 = 0.3%, 16:0 = 9.8%. † O'Brien (30).

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TABLE 7 Fatty Acid Composition of Sphingomyelins from Pathological Human Brain
Weight percentages

	Amaurotic Infantile Idiocy	Amaurotic Juvenile Idiocy	Gaucher Disease	Niemann-Pick Disease	Pachygyria	Werdnig-Hoffman	Gargoylism	Alzheimer Disease
	CB 221 M Ref. 10 21 Months Total Lobe	CB 222 M Ref. 11 6 Yr Total Lobe	CB 231 F Ref. 1 7 Months Total Lobe	CB 241 F Ref. 12 10 Months Total Lobe	CB 261 M 10 Months Total Lobe	CB 262 M 10 Months Total Lobe	CB 263 F 9 Months Total Lobe	AB 271 F 72 Yr Total Lobe
14, 15	0.1	0.1	0.1	0.1	0.5	0.2	0.2	0.1
16:0	3.2	2.9	3.5	3.1	11.3	3.6	3.6	2.5
17:0	0.1	0.1	0.1	0.2	—	0.1	0.2	0.2
18:0	63.6	42.8	84.7	82.5	71.5	73.4	78.6	41.7
18:1	—	—	—	—	—	—	—	—
19:0	0.3	0.2	—	0.2	—	Tr.	0.2	0.2
20:0	2.2	1.7	1.9	2.1	1.8	1.7	2.2	1.5
20:1	—	—	0.1	—	—	—	—	—
21:0	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.3
22:0	1.8	1.7	1.4	1.5	1.2	1.7	1.4	1.5
22:1	0.7	0.4	0.3	0.6	0.4	1.0	0.7	0.7
23:0	1.2	2.0	0.5	0.5	0.6	1.0	0.5	1.7
23:1	0.5	0.7	0.2	0.3	0.3	0.5	0.3	1.0
24:0	3.9	7.8	2.1	2.1	2.5	2.2	1.9	5.3
24:1	16.1	23.0	2.6	4.2	5.9	11.0	8.4	29.2
25:0	1.2	3.4	—	0.7	1.2	0.8	0.4	2.0
25:1	1.6	4.7	1.2	0.9	0.9	0.9	0.3	6.5
26:0	0.8	1.4	Tr.	Tr.	0.3	Tr.	0.2	1.1
26:1	2.7	7.2	1.2	0.9	1.4	Tr.	1.0	4.4
22–26	30.5	52.3	9.5	11.7	14.7	19.1	15.1	53.4

In five patients with leukodystrophy (two of the globoid cell type and three of the metachromatic type) sphingomyelins were isolated from cerebral cortex and white matter. In the cerebral cortex 18:0 constituted 80–89% and 24:1 only 2–6% of the fatty acids. In white matter deviations from the normal pattern were very pronounced. The fatty acid composition was rather similar to that of normal cerebral cortex. The results also indicate that the deviation from the normal pattern increases with the progress of the disease. It was most accentuated in the two patients, CB 203 and CB 213, in whom the demyelination was most pronounced. The fatty acid pattern of sphingomyelins from white matter in a case of subchronic leukoencephalitis (CB 251) was very similar to that observed in leukodystrophy. A normal fatty acid composition was found in the one case of Alzheimer disease studied.

DISCUSSION

It is evident from the present data that the fatty acid composition of sphingomyelins from gray matter is quite different from that of white. Before myelination 18:0 is the predominating acid in both gray and white matter. The proportion of very long-chain acids (C_{22}–C_{26}) in white matter increases with age and reaches about 70% when the process of myelination is complete. This indicates that sphingomyelins that participate in myelin formation contain mainly very long-chain acids. This tallies with the fact that the content of C_{22}–C_{26} acids was low in white matter from patients who had been suffering from inherited disturbances of the myelin formation (dysmyelination) and from leukoencephalitis with severe demyelination.

The cerebral white matter is composed not only of myelin sheaths but also of glial cells and axons. Brante (27) arrived at figures of about 50% for myelin in white matter; recently Autilio et al. (28) found the content to be only 40%. The data given by Brante (27) indicate that the axons have a lipid composition similar to that of the nerve cell bodies and of the dendrites, which means that the lipids of white matter are mixed with typical gray matter material. Similarly, the cerebral cortex always contains some myelin sheaths, the amount of which increases as the subjacent white matter is approached. This tallies with the results of Robins et al. (29), who studied the distribution of lipids in cortical layers of the motor and visual cortices of the monkey. In one of our cases (CB 107) only the outer 1–2 mm of the outer cerebral cortex was used for the analysis. The content of C_{22}–C_{26} acids of sphingomyelins from cerebral cortex was very low in this case (Table 5).

In the cases of leukodystrophy studied, the sphingomyelins of cerebral white matter were diminished and contained a much lower concentration of very long-chain fatty acids than in normal individuals of the same

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age. However, the cerebrosides and sulfatides, the two lipids which are most characteristic for myelin, and the total amount of which is increased in metachromatic leukodystrophy, have the same concentration of very long-chain fatty acids as found in normal individuals of the same age.[1]

In view of this result it is difficult to accept the conclusion of O'Brien (30) that the biochemical defect in metachromatic leukodystrophy is a failure to elongate the fatty acids of sphingolipids beyond 18 carbon atoms via the "chain elongation system" (31–33). In our opinion there is no evidence for a primary failure in elongation of the fatty acids in the cases of leukodystrophy. The changed quotient between fatty acids with 14–20 carbon atoms and those with 22–26 carbon atoms can be completely explained by the changed ratio between axoplasmic tissue and myelin.

We are greatly indebted to Mrs. Gunilla Westerdahl and Mr. Hans Thorin for skillful assistance and to Dr. Ragnar Ryhage for mass spectrometric analyses. The work has been supported by grants from the Swedish Medical Research Council.

Manuscript received July 30, 1964*; accepted October* 6, 1964.

References

1. Svennerholm, L. In *Brain Lipids and Lipoproteins and the Leucodystrophies*, edited by J. Folch-Pi and H. Bauer. Elsevier Publishing Co., Amsterdam, 1963, pp. 104–119.
2. Merz, W. *Z. Physiol. Chem.* **193:** 59, 1930.
3. Rennkamp, F. *Z. Physiol. Chem.* **284:** 215, 1949.
4. Thannhauser, S. J., and N. F. Boncoddo. *J. Biol. Chem.* **172:** 141, 1948.
5. Bernhard, K., and P. Lesch. *Helv. Chim. Acta* **46:** 1798, 1963.
6. Svennerholm, L. *J. Neurochem.*, in press.
7. Hagberg, B., P. Sourander, and L. Svennerholm. *J. Neurol. Neurosurg. Psychiat.* **26:** 195, 1963.
8. Sourander, P., and L. Svennerholm. *Acta Neuropathol.* **1:** 384, 1962.
9. Hagne, I., P. Sourander, and L. Svennerholm. *Acta Paediat.*, in press.
10. Svennerholm, L., and L. Zettergren. *Acta Pathol. Microbiol. Scand.* **41:** 127, 1957.
11. Svennerholm, L. *J. Neurochem.* **10:** 613, 1963.
12. Ivemark, B. I., L. Svennerholm, C. Thorén, and R. Tunell. *Acta Paediat.* **52:** 391, 1963.
13. Svennerholm, L., and H. Thorin. *J. Lipid Res.* **3:** 483, 1962.
14. Lowry, O. H., N. R. Roberts, K. Y. Leiner, M.-L. Wu, and A. L. Farr. *J. Biol. Chem.* **207:** 1, 1954.
15. Svanborg, A., and L. Svennerholm. *Acta Med. Scand.* **169:** 43, 1961.
16. Strid, L. *Acta Chem. Scand.* **15:** 1423, 1961.
17. Beiss, U., and O. Armbruster. *Z. Naturforsch.* **13b:** 79, 1958.
18. Wagner, H., L. Hörhammer, and P. Wolff. *Biochem. Z.* **334:** 175, 1961.
19. Rhodes, D. N., and C. H. Lea. *Biochem. J.* **65:** 526, 1957.
20. Orr, C. H., and J. E. Callen. *J. Am. Chem. Soc.* **80:** 249, 1958.
21. Craig, B. M., and N. L. Murty, *Can. J. Chem.* **36:** 1297, 1958.
22. Lipsky, S. R., R. A. Landowne, and M. R. Godet. *Biochim. Biophys. Acta* **31:** 336, 1959.
23. VandenHeuvel, W. J. A., B. G. Creech, and E. C. Horning. *Anal. Biochem.* **4:** 191, 1962.
24. Horning, E. C., E. H. Ahrens, Jr., S. R. Lipsky, F. H. Mattson, J. F. Mead, D. A. Turner, and W. H. Goldwater. *J. Lipid Res.* **5:** 20, 1964.
25. Radin, N. S., and Y. Akahori. *J. Lipid Res.* **2:** 335, 1961.
26. Gonatas, N. K., R. D. Terry, R. Winkler, S. R. Korey, C. J. Gomez, and A. Stein. *J. Neuropath. Exptl. Neurol.* **22:** 557, 1963.
27. Brante, G. *Acta Physiol. Scand.* **18:** *Suppl.* 63, 1949.
28. Autilio, L. A., W. T. Norton, and R. D. Terry. *J. Neurochem.* **11:** 17, 1964.
29. Robins, E., D. E. Smith, and K. M. Eydt. *J. Neurochem.* **1:** 54, 1956.
30. O'Brien, J. S. *Biochem. Biophys. Res. Commun.* **15:** 484, 1964.
31. Wakil, S. J. *J. Lipid Res.* **2:** 1, 1961.
32. Fulco, A. J., and J. F. Mead. *J. Biol. Chem.* **236:** 2416, 1961.
33. Hajra, A. K., and N. S. Radin. *J. Lipid Res.* **4:** 270, 1963

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