J. Neurol., 216, 57—65 (1977)

Journal of
Neurology

The Fatty Acid Composition of Sphingomyelin from Adult Human Cerebral White Matter and Changes in Childhood, Senium and Unspecific Brain Damage

R. Heipertz*, H. Pilz, and W. Scholz

Departments of Neurology and Neuropathology, University of Göttingen, v. Siebold-Str. 5, D-3400 Göttingen, Federal Republic of Germany

Summary. A micromethod for the investigation of the fatty acid composition of sphingomyelin is presented. In the cerebral white matter of 17 normal adult brains, analyzed for reference, the predominant fatty acids are C 18:0 and C 24:1. Our results are in agreement with those of other authors. Short chained fatty acids are relatively increased in young children; this shift is typical of "immature" myelin. Similar changes are described here in old persons and cases of non-specific brain damage associated with demyelination (autolysis, chronic uremia, juvenile chorea). Sphingomyelin fatty acid composition can be considered a sensitive measure of both disturbed myelination and demyelination.

Key words: Cerebral white matter – Sphingomyelin – Fatty acid composition – Demyelination.

Zusammenfassung. Es wird eine Mikromethode zur Untersuchung der Fettsäurezusammensetzung von Sphingomyelin beschrieben. In der Marksubstanz von 17 normalen erwachsenen Vergleichshirnen sind die wichtigsten Fettsäuren C 18:0 und C 24:1. Unsere Ergebnisse stimmen gut mit den Ergebnissen anderer Autoren überein. Bei Kindern findet sich eine relative Vermehrung kurzkettiger Fettsäuren, diese Verschiebung ist typisch für „unreifes" Myelin. Ähnliche Veränderungen werden hier auch bei Greisen und Fällen mit unspezifischem Hirnschaden (Autolyse), chronischer Urämie und juveniler Chorea Huntington (alle in Verbindung mit diffuser Entmarkung) beschrieben. Man kann davon ausgehen, daß die Fettsäurezusammensetzung von Sphingomyelin einen empfindlichen Maßstab sowohl für eine gestörte Myelinisierung wie auch für Demyelinisierung darstellt.

Sphingomyelin, especially the fraction containing longer chained fatty acids, has been considered a typical myelin component [8] and numerous authors have associated alterations in the fatty acid composition of sphingomyelin with

* *Present address:* Department of Neurology, University of Hamburg, Martinistr. 52, D-2000 Hamburg 20, Federal Republic of Germany

demyelinating diseases [6, 8—10, 12, 14]. We have adapted a simple micromethod, originally developed by us for the analysis of cerebroside and sulfatide fatty acid composition [3], to the determination of the sphingomyelin fatty acid composition from small amounts of human cerebral white matter in normal individuals and pathological cases with unspecific brain damage, chronic uremia and juvenile chorea.

Material and Methods

A block of material weighing approximately 1 g was excised from the right frontal white matter of 17 formalin fixed brains of patients of both sexes aged from 17 to 52 years who had died of a non-neurological condition. The brain tissue was examined histologically to ensure that there were no pathological changes. In a similar way material was obtained from the brains of children aged 1.2, 3.0, 3.0, 3.2, 4.4 years as well as 3 old persons aged 65, 79 and 85 years. In all cases there was no evidence of pathological changes in the brain.

Pathological material was derived from four patients (aged 18, 27, 38 and 44 years) with marked autolytic change of the brain as a result of prolonged artificial respiration after brain trauma and diffuse brain swelling. Brain tissue was also obtained from a case of juvenile Huntington's chorea (aged 21) in an advanced stage of mental and motor deterioration and from a case of chronic uremia (aged 37) that had died in uremic coma with generalized cerebral convulsions. All these brain samples showed diffuse reduction of myelin with the Heidenhain-Woelcke stain which was most marked in the case of juvenile chorea and in the case of chronic uremia.

The fatty acid analyses were performed essentially as previously described [3]. The lipids were extracted with chloroform/methanol 2:1 and 5 mg of total lipid extract was subjected to column chromatography on Florisil. After elution of fraction I with chloroform/methanol 2:1, fraction II containing the phospholipids was eluted with 12 volumes of chloroform/methanol/water/ammonia 63:40:7:1.

For thin layer chromatography 200 µl of a solution of fraction II dissolved 5:1 in chloroform/methanol 2:1 was applied to the plates covered with a 300µm layer of Kieselgel G (Merck) with a Hamilton microlitre syringe; from each brain two adjacent tracks were used. The plates were developed in chloroform/methanol/water 73:28:4.5 with chamber saturation. After drying, the plates were sprayed with 0.1% Rhodamin B (Merck) and viewed under ultraviolet light at 264 nm (Camag) as spraying with water did not show up the sphingomyelin spots. The sphingomyelins were identified by theirs R_F values compared to the R_F values of commercial brain sphingomyelin (Serva) run in parallel and outlined with a needle. With the thin layer system used here sphingomyelin separated into two spots of which the one with the higher $R_F$value contained mainly longer chained fatty acids, whereas the one with the lower R_F value contained shorter chained fatty acids [8].

The two sphingomyelin spots on each track were scraped off together and the sphingomyelins from the two adjacent tracks (i.e. deriving from the same brain extract) were united. Transmethylation was carried out with 5% sulfuric acid in methanol (v/v) by heating in an oil bath for 12 h at 75°C. The fatty acid methyl esters (FAME) were extracted into hexane and, after drying, taken up in 10 µl carbon disulfide for gas chromatography.

Gas-liquid chromatography was performed with a Pye Unicam model 104 gas chromatograph fitted with double FID. The silanized glass columns were 5 ft long, 2 mm internal diameter and filled with 3% OV 1 on 100/120 Gas Chrom Q (Serva). Carrier gas was purified nitrogen at a flow rate of 70 ml/min. Temperature programming for the column oven was started at 195°C for 3 min, then raised 3°C/min to 255°C final temperature. Injection point increment was set at 3 and detector temperature was kept constant at 300°C. Peaks were recorded with a dual channel recorder (W + W 1200) and identified by their retention times compared to standard samples of FAME (Serva). Peak area was measured by electronic integration (Autolab System I) and expressed as a relative percent of the total sum of peak areas. To further assist identification the samples were also run on 5 ft columns filled with 3% Silicone Apolar 5 CP (50% cyano, 50% phenyl, Applied Science Laboratories).

Table 1. Fatty acid composition of sphingomyelin from frontal lobe white matter of 17 normal adult human brains, aged 17—52 years. All values as relative percent, mean value ± standard deviation. Fatty acid methyl esters (FAME) denoted by chain length and double bond

FAME	$\bar{x} \pm s$	Range
14:0	0.6 ± 0.2	0.1— 0.9
15:0	0.5 ± 0.1	0.2— 0.8
16:1	0.3 ± 0.2	0.0— 0.8
16:0	3.1 ± 0.7	1.8— 4.0
17:1	0.8 ± 0.5	6.3— 1.6
17:0	0.5 ± 0.2	0.3— 0.9
18:1	5.0 ± 1.1	2.9— 7.3
18:0	22.5 ± 2.5	16.6—27.2
19:1	0.3 ± 0.3	0.0— 1.4
19:0	0.9 ± 0.3	0.3— 1.5
20:1	1.2 ± 0.3	0.5— 1.9
20:0	1.2 ± 0.3	0.5— 1.5
21:1	0.6 ± 0.5	0.0— 1.3
21:0	1.3 ± 0.8	0.3— 3.2
Sum 14:0—21:0	38.7 ± 3.6	32.5—44.0
22:1	0.7 ± 0.2	0.2— 1.0
22:0	2.0 ± 0.5	1.2— 2.8
23:1	1.1 ± 0.2	0.7— 1.5
23:0	2.8 ± 0.4	2.2— 3.4
24:1	30.1 ± 2.3	25.6—34.7
24:0	8.5 ± 1.6	5.3—10.9
25:1	6.2 ± 1.1	4.7— 8.4
25:0	4.4 ± 1.6	2.4— 8.1
26:1	4.7 ± 0.7	3.6— 6.5
26:0	0.9 ± 0.8	0.2— 3.0
Sum 22:1—26:0	61.3 ± 3.6	56.0—67.6

Results and Discussion

The fatty acid composition of sphingomyelin from adult normal white matter as determined by our method is despicted in Table 1. Our results show a marked preponderance of C 18:0 and C 24:1 fatty acids amounting to 22.5 and 30.1 relative %, respectively. This predominance could be expected from earlier thin layer chromatographic results [8]. Long chained fatty acids (C 22:1 to C 26:0) as a group amount to 61.3 relative %. Essentially similar results were found by other authors for human adult cerebral white matter [1, 2, 5, 7, 9, 10, 12, 15] and myelin [11] as listed in Table 2, but these values show quite considerable variation. Comparing our results to the results of these authors we are in agreement with O'Brien [7] and Woelck [15] for C 18:0 and C 24:1 as well as for total long chained fatty acids. Some of the differences to the results of other investigators can be explained by the fact that these quantitated less fatty acids so that each represented a higher proportion of the total.

It had been established previously that the proportion of C 18 and C 24 sphingomyelin changes during brain maturation [6—8, 10, 12, 13]. Our values for five normal children aged 1.2 to 4.4 years are given in Table 3. As can be seen

Table 2. Fatty acid composition of sphingomyelin, adult cerebral white matter—as quoted from the literature

FAME	[5]	[7]	[2]	[15]	[1]	[12]			[11](myelin)	[10]					[9]	
						a	b	c		a	b	c	d	e	a	b
14:0	0.3	tr	0.6	0.1	—	—	—	—	—	0.1	0.1	0.3	0.1	0.1	—	—
16:1	0.3	—	0.4	—	—	—	—	—	—	—	—	—	—	—	1.4	0.5
16:0	3.6	10.2	4.0	9.8±0.3	2.5±0.5	2.3	2.3	2.4	2.3±0.1	1.2	2.1	2.1	2.1	2.1	6.4	6.8
17:0	—	—	—	—	—	0.1	0.2	0.1	—	tr	0.1	0.2	0.1	0.1	—	—
18:1	1.7	6.5	1.2	5.6±0.8	—	—	—	—	—	—	—	—	—	—	3.2	—
18:0	27.0	20.1	33.8	23.3±1.7	26.0±2.2	39.9	38.8	34.6	28.2±2.2	25.2	28.1	29.7	26.0	23.3	47.0	45.8
19:0	—	—	—	—	—	0.2	0.3	0.3	—	tr	0.2	0.3	0.2	0.2	—	—
20:0	1.3	1.1	0.9	0.6±0.1	1.1±0.3	1.4	1.6	1.5	—	1.1	1.2	1.2	0.9	1.1	1.0	1.5
21:1	—	—	1.1	—	—	—	—	—	—	—	—	—	—	—	—	—
21:0	—	—	—	—	—	0.1	0.3	0.2	—	0.2	0.5	0.5	0.1	0.1	—	—
22:1	0.8	1.3	1.3	1.5±0.2	—	0.8	0.6	0.6	—	0.7	0.9	0.7	0.6	0.9	1.9	—
22:0	1.7	1.7	1.9	1.1±0.4	1.8±0.2	1.6	1.6	1.8	1.9±0.2	2.0	1.8	1.8	1.8	2.0	3.6	2.4
23:1	1.1	1.3	1.6	0.9±0.5	—	1.1	1.0	1.2	—	1.2	1.3	1.0	1.2	1.5	—	—
23:0	2.3	2.8	4.1	1.5±0.3	2.6±0.3	1.8	2.1	2.1	2.4±0.2	2.3	2.2	2.3	2.5	3.0	—	3.1
24:1	32.8	30.2	30.7	32.8±2.1	38.5±2.0	33.1	31.7	37.2	41.6±2.4	42.3	38.2	35.5	41.2	38.8	30.9	31.7
24:0	7.5	6.9	9.6	4.8±0.1	8.1±0.8	4.9	4.3	5.6	8.8±0.7	7.0	6.9	7.9	8.1	8.1	4.5	6.7
25:1	6.3	8.3	4.2	7.9±0.7	6.8±0.7	5.3	6.4	6.4	6.2±0.8	7.4	7.1	6.1	6.8	7.8	—	1.6
25:0	2.8	3.4	1.2	2.9±0.4	3.0±0.4	2.6	3.7	1.4	2.3±0.8	3.3	3.5	2.9	2.6	3.3	—	—
26:1	5.0	5.5	3.4	6.7±0.2	6.0±0.7	4.3	4.9	4.1	4.4±0.8	6.0	5.3	5.8	5.0	6.9	—	—
26:0	0.6	tr	—	0.2±0.1	0.9±0.4	0.4	0.2	0.6	—	—	0.4	1.8	0.7	0.7	—	—

All values as relative percent of total fatty acids. Fatty acid methyl esters (FAME) denoted by chain length and double bond. Numbers heading columns refer to references. 5: Mean values, n = 6, 7: 1 case, 55 yrs, 2: 1 case, 69 yrs, 15: Mean values + SD, n = 11, 1: Mean values + SD, n = 9, 12: Single cases, a = 16 yrs, b = 33 yrs, c = 64 yrs, 11: Mean values + SD, n = 4, myelin fraction. 10: Single cases, a = 15 yrs, b = 16 yrs, c = 32 yrs, d = 33 yrs, e = 77 yrs, 9: Single adult cases, age not specified

Table 3. Fatty acid composition of sphingomyelin in white matter of children and old persons. Values in relative percent

FAME	Age in years							
	1.2	3.0	3.0	3.2	4.4	65	79	85
14:0	2.4	0.7	—	1.4	0.8	1.0	0.9	2.0
15:0	1.1	0.5	—	1.5	0.8	0.9	0.6	1.9
16:1	0.7	0.5	0.2	1.4	0.4	0.5	0.5	1.9
16:0	5.9	4.6	5.6	5.6	3.7	4.1	4.5	7.0
17:1	1.4	1.1	—	1.8	1.0	1.4	1.1	2.9
17:0	1.0	0.6	0.1	—	0.6	0.7	0.7	—
18:1	5.8	5.0	5.7	3.9	6.2	7.1	7.2	7.3
18:0	36.1	34.6	41.2	31.2	31.2	27.5	29.5	30.5
19:1	—	—	—	—	—	1.1	0.9	—
19:0	—	0.3	0.4	1.0	0.9	1.0	1.1	—
20:1	0.5	0.5	0.6	1.8	0.4	2.1	1.7	0.9
20:0	1.4	0.9	1.2	1.8	0.8	1.5	1.4	1.0
21:1	—	—	0.2	3.7	1.3	1.2	1.4	1.2
21:0	0.3	0.7	1.4	5.8	2.3	3.6	1.3	3.7
Sum 14:0—21:0	56.7	50.0	56.6	60.9	50.2	53.7	52.8	60.3
22:1	1.1	0.8	0.9	1.2	0.3	0.8	1.0	—
22:0	2.6	1.9	2.2	3.3	1.7	2.1	2.1	1.0
23:1	0.7	0.9	0.8	1.1	0.6	0.7	0.9	0.5
23:0	2.2	2.0	2.1	2.7	2.2	1.9	2.0	1.3
24:1	21.2	26.7	23.0	17.8	22.9	22.9	25.1	23.8
24:0	6.8	7.9	7.5	6.1	9.3	6.8	5.5	4.7
25:1	2.2	3.1	2.2	2.5	3.2	4.0	5.0	4.5
25:0	3.5	2.5	2.1	2.0	2.8	2.4	2.4	1.4
26:1	2.8	4.3	2.6	2.3	4.4	3.3	2.3	1.8
26:0	—	0.2	—	0.2	2.3	1.4	0.8	0.9
Sum 22:1—26:0	43.1	50.3	43.4	39.2	49.7	46.3	47.1	39.9

there is a considerable interindividual variation and, although the group as a whole contains less C 24 sphingomyelin than adults, there is no definite relationship between the increase of long chained fatty acids and age. Similarly the already known findings [4, 6, 7, 10, 12, 14] listed in Table 4 shown even less agreement than the results for adults. We assume that the stages of sphingomyelin development are fairly variable in younger children. This factor makes the results obtained by comparison of a pathological brain with a normal control of the same age for altered sphingomyelin fatty acid composition doubtful over a wide range.

Table 3 also shows the fatty acid composition of sphingomyelin obtained from three old persons. In these cases it must be noted that there was no histological evidence of demyelination and the fatty acid composition of cerebrosides and sulfatides was within the normal range. There is a noticeable diminution of long chained fatty acids (especially C 24:1) and a concomitant increase of short

Table 4. Fatty acid composition of sphingomyelin, cerebral white matter of children—as quoted from the literature

FAME	[6]	[14]	[7]		[12]					[4]	[10]	
			a	b	d	e	f	g	h		a	b
14:0	—	0.9	0.7	0.8	—	—	—	—	—	1.1	—	0.2
16:1	0.5	1.1	—	—	—	—	—	—	—	0.7	—	—
16:0	6.5	5.0	9.0	12.0	3.2	4.3	2.5	8.0	2.2	4.4	2.9	3.1
17:0	—	—	—	—	—	—	0.2	0.4	0.1	0.3	0.1	—
18:1	4.1	1.8	3.6	4.3	—	—	—	—	—	1.7	—	—
18:0	79.6	30.6	35.7	44.5	63.2	50.9	48.3	47.3	41.1	56.2	49.5	34.9
19:0	—	—	—	—	—	0.4	0.2	0.2	0.2	—	0.1	0.2
20:1	1.2	—	—	—	—	—	—	—	—	—	—	—
20:0	1.2	1.4	0.7	0.9	1.8	1.7	1.8	1.5	1.8	1.4	2.2	1.7
21:0	—	—	—	—	0.2	0.2	0.2	0.1	0.3	—	0.1	0.3
22:1	1.3	0.9	0.6	0.4	0.7	0.8	0.8	0.6	1.0	1.0	2.2	1.3
22:0	2.2	1.6	1.6	1.4	1.9	0.8	1.8	2.0	1.9	2.5	2.7	2.7
23:1	—	—	0.7	0.5	0.5	0.8	1.0	0.8	1.1	0.3	0.9	0.9
23:0	—	2.1	1.4	1.6	1.0	1.9	1.6	2.0	2.0	1.0	1.1	1.7
24:1	1.9	35.7	27.1	23.1	15.5	20.3	25.2	18.6	29.0	21.9	28.3	30.2
24:0	1.6	6.6	7.3	3.5	4.1	7.2	4.8	8.0	4.7	5.1	4.6	9.7
25:1	—	5.7	3.4	2.2	2.3	2.9	3.7	2.3	5.7	0.4	1.5	3.4
25:0	—	2.7	1.8	1.4	1.4	2.2	2.9	3.1	2.8	—	0.3	2.7
26:1	—	3.3	4.0	2.0	3.1	4.3	4.5	3.1	5.8	1.2	3.4	6.0
26:0	—	—	1.1	1.0	0.9	0.7	0.4	1.3	0.2	—	0.2	1.5

All values as relative percent of total fatty acids. Fatty acid methyl esters (FAME) denoted by chain length and double bond. Numbers heading columns refer to references. 6: 1 case, 0.5 yrs, 14: 1 case, 9 yrs, 7: 2 cases, a = 6 yrs, b = 9 yrs, 12: 5 cases, d = 1.5 yrs, e = 3 yrs, f = 4 yrs, g = 5 yrs, h = 8 yrs. 4: Mean values, 6 cases aged 1 yr, 10: Single cases, a = 0.8 yrs, b = 1.5 yrs

Table 5. All values in relative percent of total fatty acids. Fatty acid methyl esters (FAME) denoted by chain length and double bond

Sphingomyelin. Frontal lobe white matter						
FAME	Chronic uremia 37 y	Autolysis				Juv. chorea 21 y
		18 y	38 y	44 y	27 y	
14:0	1.0	0.8	0.8	0.7	0.7	0.8
15:0	1.0	0.9	0.7	0.4	0.6	0.8
16:1	0.6	0.7	—	0.4	0.4	0.7
16:0	4.7	4.7	4.2	3.3	3.7	4.2
17:1	1.4	1.6	1.2	0.9	0.9	0.9
17:0	0.6	—	0.8	0.5	0.6	0.7
18:1	5.7	3.6	9.4	8.9	7.9	5.5
18:0	28.2	31.4	31.0	29.6	25.0	34.6
19:1	—	0.8	—	—	2.3	0.8
19:0	1.1	0.8	1.1	2.8	1.0	1.0
20:1	2.1	—	1.3	1.1	1.1	1.2
20:0	2.3	1.1	0.6	0.7	1.9	1.4
21:1	2.2	0.2	0.7	—	0.5	1.1
21:0	4.8	2.4	0.2	1.4	1.4	2.3
Sum 14:0—21:0	55.7	49.0	52.0	50.7	48.0	56.0
22:1	1.0	0.2	0.3	0.2	0.8	1.1
22:0	2.1	1.4	1.0	1.2	2.1	3.1
23:1	1.1	0.6	0.7	0.7	1.0	0.8
23:0	2.1	2.2	1.5	2.2	2.5	2.3
24:1	21.3	23.7	29.2	27.4	24.3	20.2
24:0	5.2	8.8	5.8	6.3	6.6	7.0
25:1	3.9	4.1	4.2	4.8	4.9	3.7
25:0	2.2	4.4	1.9	2.6	4.3	2.6
26:1	3.1	3.6	3.5	3.5	3.8	2.8
26:0	2.3	1.7	—	0.3	1.8	0.9
Sum 22:1—26:0	44.3	50.7	48.0	49.2	52.1	44.5

chained fatty acids (especially C 18:0). We interpret these findings as indication of demyelination on the biochemical level not yet associated with structural alterations.

In Table 5 we see values from young adults with non-specific brain damage (four cases of autolysis), chronic uremia and juvenile Huntington's chorea. All these brains show diffuse demyelination of varying degree on histological examination and in all cases the fatty acid composition of cerebrosides and sulfatides is within the normal range. There is a general decrease of long chained fatty acids accompanied by a relative increase of short chained fatty acids which is most marked in the cases of chronic uremia and juvenile chorea, both with longlasting cerebral involvement. The extent of histological demyelination did not show any correlation to the amount of alteration in sphingomyelin fatty acid composition.

We conclude that during childhood myelin maturation manifests itself by an increase in long chained fatty acids (particularly C 24:1) in the sphingomyelin fraction [8, 10, 12, 13]. During old age there appears to be a reversal with a relative increase of short chained fatty acids, the sphingomyelin fatty acid pattern seeming more "immature". Possibly this change arises without histological myelin loss. Our results demonstrate that alterations of sphingomyelin fatty acid pattern with a shift to more short chained fatty acids can be seen in non-specific brain damage with histological demyelination such as autolysis as well as in cases of juvenile chorea and chronic uremia. Svennerholm et al. [13] maintain that an altered sphingomyelin fatty acid pattern is a sensitive measure of disturbed myelination. In contrast to the fatty acid composition of cerebrosides and sulfatides which was normal in all these cases, alterations in sphingomyelin fatty acid composition can be considered a sensitive biochemical measure of demyelination [8]. In this respect it is of interest to note that various authors have reported that there is no such shift in the fatty acid pattern of sphingomyelin from apparently normal white matter of multiple sclerosis [10, 11, 15]; only Alling et al. [1] have described a moderate diminution of the sum of long chained fatty acids.

Acknowledgements. We thank Miss Sabine Gratz for her technical assistance. These investigations were financially supported by the Deutsche Forschungsgemeinschaft (SFB 33).

References

1. Alling, C., Vanier, M. T., Svennerholm, L.: Lipid alterations in apparently normal white matter in multiple sclerosis. Brain Res. **35**, 325—336 (1971)
2. Bernhard, K., Lesch, P.: Ein Beitrag zur Fettsäurezusammensetzung der Cerebroside, Sphingomyeline und Lecithine aus menschlichem Hirn. Helv. Chim. Acta **46**, 1798—1801 (1963)
3. Heipertz, R., Pilz, H., Scholz, W.: The fatty acid composition of major glycosphingolipids (cerebrosides and sulfatides) in human cerebral white matter measured by a simple micromethod. J. Neurol. **213**, 47—58 (1976)
4. Lesch, P., Bernhard, K.: Die Lipide aus einjährigen Kinderhirnen. Helv. Chim. Acta **50**, 1125—1130 (1967)
5. Lesch, P., Schmidt, E., Schmidt, F. W.: Effects of chronic alcohol abuse on the fatty acid composition of major lipids in the human brain. Z. Klin. Chem. Klin. Biochem. **11**, 159—166 (1973)
6. Lou, H. C., Holmer, G. K., Reske-Nielsen, E., Vagn-Hansen, P.: Lipid composition in gray and white matter of the brain in Menkes' disease. J. Neurochem. **22**, 377—381 (1974)
7. O'Brien, J. S., Sampson, E. L.: Fatty acid and fatty aldehyde composition of the major brain lipids in normal human gray matter, white matter, and myelin. J. Lipid. Res. **6**, 545—551 (1965)
8. Pilz, H., Jatzkewitz, H.: Dünnschichtchromatographische Bestimmung von C_{18}- und C_{24}-Sphingomyelin in normalen und pathologischen Gehirnen einschließlich eines Falles von Niemann-Pick'scher Erkrankung. J. Neurochem. **11**, 603—611 (1964)
9. Rouser, G., Feldman, G., Galli, C.: Fatty acid compositions of human brain lecithin and sphingomyelin in normal individuals, senile cerebral cortical atrophy, Alzheimer's disease, metachromatic leucodystrophy, Tay-Sachs and Niemann-Pick diseases. J. Am. Oil Chemists Soc. **42**, 411—412 (1965)
10. Ställberg-Stenhagen, S., Svennerholm, L.: Fatty acid composition of human brain sphingomyelins: normal variation with age and changes during myelin disorders. J. Lipid Res. **6**, 146—155 (1965)

11. Suzuki, K., Kamoshita, S., Eto, Y., Tourtellotte, W. W., Gonatas, J. O.: Myelin in multiple sclerosis. Composition of myelin from normal-appearing white matter. Arch. Neurol. **28**, 293—297 (1973)
12. Svennerholm, L.: Some aspects of the biochemical changes in leucodystrophy. In: Brain Lipids and Lipoproteins, and the Leucodystrophies, pp. 104—119. Folch-Pi, J., Bauer, H. (eds.). Amsterdam: Elsevier 1963
13. Svennerholm, L., Vanier, M. T.: The distribution of lipids in the human nervous system. IV. Fatty acid composition of major sphingolipids of human infant brain. Brain Res. **55**, 413—423 (1973)
14. Tjiong, H. B., Seng, P. N., Debuch, H., Wiedemann, H. R.: Brain lipids of a case of juvenile Niemann-Pick disease. J. Neurochem. **21**, 1475—1485 (1973)
15. Woelk, H., Borri, P.: Glycerinphosphatide und Sphingolipide der normalen weißen Substanz bei der Multiplen Sklerose. J. Neurol. **205**, 243—256 (1973)

Received February 8, 1977