Fatty acid composition of brain glycolipids in Alzheimer's disease, senile dementia, and cerebrocortical atrophy

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ABSTRACT Quantitative fatty acid compositions are reported for human cerebral gray matter glycolipids (gangliosides, cerebrosides, and cerebroside sulfates) in Alzheimer's disease (presenile dementia) and related disorders of the central nervous system. Although the fatty acid compositions were generally similar to those of the controls, some significant differences were noted in Alzheimer's disease which were not found in the single samples of senile dementia and of senile cerebrocortical atrophy.

In the ganglioside fatty acids, the percentage of palmitic acid was higher in all three cases of Alzheimer's disease than in the controls. In cerebrosides, the proportion of 2-hydroxy to nonhydroxy acids was slightly lower in Alzheimer's disease than in controls and the relative proportions of 2-hydroxystearic acid and 2-hydroxytricosenoic acid were slightly higher. In cerebroside sulfates, monounsaturated 2-hydroxy acids were lower in two of the samples with Alzheimer's disease. The results indicate possible effects of the degenerative changes in this disease on glycolipid metabolism.

KEY WORDS Alzheimer's disease · senile dementia · cerebrocortical atrophy · glycolipids · gangliosides · cerebrosides · cerebroside sulfates · fatty acids · composition · hydroxy acids · quantitative analysis

PREVIOUS STUDIES IN OUR LABORATORIES (1) on the quantitative estimation of glycolipids (gangliosides, cerebrosides, and cerebroside sulfates) from human cerebral gray matter in Alzheimer's disease (2) have shown that these classes of lipids may be involved in the degenerative process that is characteristic of this disease. The determinations of fatty acid composition of these lipids in the normal and diseased brains may further contribute to the knowledge of the metabolism of these lipids in this disease. This report is therefore concerned with the quantitative determinations of the fatty acid compositions of human cerebral gray matter glycolipids in Alzheimer's disease (presenile dementia), senile dementia, senile cerebrocortical atrophy, and normal controls. A preliminary report of some of this work has been presented (3).

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography; DEAE, diethylaminoethyl; NFA, nonhydroxy fatty acids; HFA, hydroxy fatty acids.

MATERIALS AND METHODS

Materials

Cerebral gray matter from three patients with Alzheimer's disease [the same patients as those described previously (1)], one with senile dementia (89 yr old female, A-34-66), one with cerebrocortical atrophy (91 yr old male, A-35-66), and four controls varying in age from 47 to 91 yr, were used. All the samples (4.5–50 g) except A-35-66 were taken from nonspecific areas of the cerebral cortex, excluding the hippocampus. From sample A-35-66 the frontal lobe gray matter was used. Some of the samples could not be separated into well-defined areas, as only limited portions were available.

Two of the brain samples in the Alzheimer's disease group (50 yr old female, 13755, and 72 yr old female, 12045) were available only after they had been fixed in 10% formalin for 2 and 5 months, respectively. In order to determine the effect of formalin on the fatty acid composition, we isolated samples of cerebroside sulfates from one control brain (13270) before and after fixation in formalin for 3.5 months. The fatty acid compositions of the fresh and formalin-fixed samples were essentially the same (unpublished results). Menkes, Philippart, and Concone have reported (4) that the fatty acid composition

of cerebrosides and sulfatides derived from formalinized brains did not differ significantly from that of fresh brains. The brain from the third patient with Alzheimer's disease (56 yr old male, Lo) was obtained at autopsy within 10–12 hr after death, brought to the laboratory in Dry Ice, and kept at −18°C until used. Two other diseased samples (A-34-66 and A-35-66) were obtained at autopsy within 12–14 hr after death and preserved at −18°C until used. The diagnoses for the first four cases (13755, 12045, and Lo, Alzheimer's disease; A-34-66, senile dementia) were established on the basis of clinical and histophathological findings. In the last patient (A-35-66), the brain revealed gross atrophy of the frontal lobes. Microscopic examination indicated loss of ganglion cells, pigmentary degeneration, and suggestion of neurofibrillary changes. However, no senile plaques or granulovacuolar degenerations were detected.

The control brains were obtained at autopsy from patients with no known neurological diseases and were preserved at −18°C until used. Histological examination confirmed the absence of neurological diseases in these brains. They included the following samples: 47-63, 47 yr old male; 12428, 67 yr old female; 13270, 71 yr old male; and 14563, 91 yr old female.

All solvents were redistilled unless they were analytical reagents. All solvent ratios given in the test are on a volume basis.

Extraction of Lipids from Brain

Gangliosides and the "total lipids" (excluding proteolipids, gangliosides, and other water-soluble components) containing cerebrosides and cerebroside sulfates were prepared as reported previously (1). Care was taken to reduce oxidation to a minimum by conducting all evaporation under an atmosphere of high-purity dry nitrogen.

The gangliosides obtained after dialysis and lyophilization (1) were further purified by chromatography on 80–200 mesh alumina (Fisher Scientific Company, Chicago, Ill.) essentially by the method of Sambasivarao and McCluer (5). 1 g of adsorbent was used for 50 mg of gangliosides. The sample was applied to the adsorbent (packed in a 1 cm I.D. column) in a small volume of pyridine. For 1–2 g of adsorbent, 75 ml of pyridine followed by 150 ml of methanol were used for elution. The combined eluates were concentrated at room temperature in a flash evaporator, transferred with water, and lyophilized. Lyophilization yields almost white, fluffy samples, but quantitative recoveries were seldom achieved as there was some loss during this last process. In order to check whether the gangliosides were recovered quantitatively after chromatography on alumina we transferred the concentrated solutions with methanol from the flash evaporator into weighted flasks and dried them under nitrogen. In four such experiments 6.9, 30.0, 31.2, and 43.9 mg of gangliosides from samples Lo, A-35-66, 14653, and 13755, respectively, were chromatographed and 6.8, 28.0, 29.6, and 42.0 mg were recovered. The phosphorus analysis (6) of such samples indicated a concentration of less than 0.02%.

A mixture of cerebrosides and cerebroside sulfates was isolated from the "total lipid" fraction by chromatography on 60–100 mesh Florisil (Floridin Co., Tallahassee, Fla.) essentially by the method of O'Brien, Fillerup, and Mead (7). The cerebrosides and cerebroside sulfates were then separated by chromatography (8) on DEAE-cellulose (Whatman) [washed according to the method of Rouser, Kritchevsky, Heller and Lieber (9)]. In all cases involving this procedure, over 95% of the glycolipids were recovered. In some instances partial purification was achieved by chromatography on alumina (10) before chromatography on Florisil.

A few samples, particularly cerebrosides and cerebroside sulfates from formalin-fixed samples of brain, were further purified by preparative TLC on a mixture of 90% silica gel (Research Specialties Co., Richmond, Calif.) and 10% magnesium silicate (Allegheny Industrial Chemical Co., Butler, N.J.) in butanol–acetic acid–water 60:20:20 and in chloroform–methanol–water 65:25:4 (11). The plates were dried in air and sprayed with water or 2′,7′-dichlorofluorescein for detection of the spots. The spots were scraped off the plates, extracted several times with chloroform–methanol 2:1, and filtered through sintered glass filters. In each instance the purity of the sample was checked by TLC in chloroform–methanol–water 65:25:4 before isolation of the fatty acids.

Isolation of Fatty Acid Methyl Esters

Fatty acid methyl esters were isolated by a modification of the method used by Radin, Hajra, and Akahori for the methylation of free fatty acids (12). In this method, the glycolipid was refluxed with methanol–concd HCl–2,2-dimethoxypropane 8:1:1 for 5–6 hr and the solution was cooled and extracted thrice with petroleum ether. The fatty acid methyl esters from gangliosides were then purified either by chromatography on 100 mesh silica gel (Mallinckrodt Chemical Works, St. Louis, Mo.) or on Florisil (13). The fatty acid methyl esters from cerebrosides and cerebroside sulfates were separated into NFA methyl esters and HFA methyl esters by chromatography either on Florisil according to the method of Kishimoto and Radin (14) or on deactivated Florisil (7 ml of water + 100 g of Florisil) in petroleum ether–benzene 90:10 or 85:15 and petroleum ether–diethyl ether 85:15.

The weights of the fatty acid methyl esters obtained after chromatography were compared to the theoretical

values. Gangliosides yielded 13.9–17.4% by weight of methyl esters. This compares favorably with the 14–16% yield reported by Rosenberg and Stern (15). From cerebrosides and cerebroside sulfates the recovery of fatty acid methyl esters was more than 95% of the theoretical values, on the assumptions of an average molecular weight of 835 for cerebrosides (16) and 900 for cerebroside sulfates (1). The purity of each group of esters was tested by TLC in petroleum ether–diethyl ether–acetic acid 70:30:1. In most cases HFA methyl esters were further converted to their acetoxy derivatives by treatment with 1–2 ml of acetic anhydride–pyridine 1:1 containing 1–2 mg of *p*-toluenesulfonic acid overnight at room temperature. The mixture was then cooled in ice and diluted with ice-cold water. This step was to prevent over-heating of the mixture due to the exothermic reaction between acetic anhydride and water. The diluted mixture was then extracted several times with ether. Completeness of acetylation was checked by TLC.

All the fatty acid methyl esters were dissolved in redistilled hexane and kept at −18°C until used.

GLC of Methyl Esters

All the methyl esters were analyzed by GLC in a Barber-Colman model 15 gas chromatograph provided with an ionization detector (radium source). A glass U-tube column (6 ft × 0.25 inch) containing 15% diethylene glycol succinate polyester (stabilized) on Anakrom ABS 60–70 mesh (Analabs, Inc., Hamden, Conn.) was used for most of the analyses. The column was maintained at 185 or 200°C for the analyses of NFA esters with an argon pressure of 20 psi. The acetoxy fatty acid methyl esters and, in later runs, the HFA methyl esters were chromatographed at 200°C with an external argon gas pressure of 30 psi. A nonpolar column (15% Apiezon L, on 100–120 mesh Gas-Chrom Q; Applied Science Laboratories Inc., State College, Pa.) was used in some instances as a check on the accuracy of results obtained with the polyester column. Percentages of fatty acids were determined by the cutting out and weighing of peaks. Fatty acids were identified by their retention times and by comparison with known standards. When standards were not available, peaks were identified by carbon number (17). Quantitative results with the National Heart Institute fatty acid methyl ester standard F agreed with the stated composition data within ±0.5% for minor components (<10%) and ±1.5% for the major components (>10%).

For the acetoxy or hydroxy esters, 2-acetoxy- or hydroxy-myristic, -palmitic, -stearic,[1] -behenic,[1] and -cerotic acid methyl esters (Aldrich Chemical Co., Inc., Milwaukee, Wis.) were used as standards. The detector response was lower for the acetoxy or hydroxy series than for the NFA series; however, this decrease was for the entire series and hence quantification was not affected in any one series (18, 19).

All analyses were done at least in duplicate and the average values are reported. Unsaturated fatty acid methyl esters were further identified by rechromatography after reduction of their solutions in absolute ethanol with hydrogen in the presence of either palladium chloride or platinum oxide.

RESULTS

Table 1 shows the fatty acid composition of gray matter gangliosides. In all cases, stearic acid was the principal fatty acid, accounting for 69–86% of the total fatty acids. However, all the samples contained[2] 18:1 and small amounts of fatty acids with more than 20 carbon atoms. In general, the percentage of 16:0 was higher in the samples with Alzheimer's disease than in the controls. No hydroxy acids were detected in the ganglioside fatty acids.

In cerebrosides from control brains, HFA accounted for about 73% (Table 2) of the total fatty acids, while in cerebrosides from brains of patients with Alzheimer's disease, they constituted only 55–66% of the total fatty acids. In one case each of senile dementia and cerebrocortical atrophy, the percentages of the HFA were close to those of the controls. In cerebroside sulfates only about 47% of the total fatty acids of controls were HFA; in the diseased brains, the percentage ranged more widely (40–54%) but had the same mean. The higher proportions of NFA in cerebroside sulfates than in cerebrosides is in agreement with the known differences in the metabolism of these two classes of glycolipids.

It must be mentioned here that the percentages calculated by weighing small amounts of fatty acid methyl esters (0.9–5 mg) are, at best, only approximate. However, the majority of the values in the Table 2 are accurate.

The percentage compositions of NFA from cerebrosides and cerebroside sulfates are given in Tables 3 and 4, respectively. The major fatty acid is 24:1 (31–48%) in both cerebrosides and cerebroside sulfates; monounsaturated acids accounted for 50–72% of the total NFA in cerebrosides and 57–77% in cerebroside sulfates. In one patient with Alzheimer's disease (12045) the ratios of 24:1/24:0 and 25:1/25:0 and the percentages of total monounsaturated acids are lower than in all other sam-

[1] Generous gifts from Dr. K. K. Carroll, University of Edmonton, London, Ontario, Canada.

[2] Fatty acids are denoted by chain length:number of double bonds. Hydroxy acids are denoted by the additional letter "h" after the chain length.

TABLE 1 PERCENTAGE COMPOSITION OF GANGLIOSIDE FATTY ACIDS FROM GRAY MATTER OF HUMAN BRAIN

	Controls			Alzheimer's Disease			Senile Dementia	Cortical Atrophy
	47-63	13270	14653	Lo	13755	12045	A-34-66	A-35-66
14:0*	tr.	tr.	tr.	tr.	tr.	tr.	tr.	0.2
14:1				tr.		tr.		tr.
15:0	tr.	tr.	tr.	tr.	tr.	tr.	tr.	tr.
16:0	2.3	0.4	4.4	7.0	8.9	7.8	2.8	3.4
16:1			0.9	tr.		tr.	tr.	0.4
17:0	tr.		tr.	tr.		tr.	0.5	tr.
18:0	79.9	86.3	69.6	76.8	72.5	77.3	79.8	85.4
18:1	2.0	tr.	11.2	3.9	7.9	3.9	2.0	2.0
18:2			0.9	tr.		0.3	tr.	
19:0	0.8	0.6	tr.	tr.		tr.	0.4	0.5
20:0	10.9	9.0	7.0	8.0	6.4	7.4	10.7	6.0
20:1	tr.	tr.	0.4	tr.	tr.	0.2	0.5	tr.
21:0			tr.	tr.		tr.	tr.	tr.
22:0	1.2	1.2	0.4	1.2		0.7	1.3	0.6
22:1			1.7	tr.			0.7	
23:0	0.4	0.7	0.2	1.0	0.6	0.6	tr.	0.3
23:1			0.6	tr.		tr.	tr.	tr.
24:0	0.3		0.7	0.5	0.4	0.3		0.2
24:1	1.4	0.9	1.8	1.6	2.1	1.2	0.5	0.5
25:0			0.2	tr.			tr.	
25:1	1.0	1.0	0.2	tr.	1.3	0.3	0.8	0.4

* Chain length:number of double bonds.

TABLE 2 PERCENTAGES OF HYDROXY FATTY ACIDS IN TOTAL FATTY ACIDS FROM CEREBROSIDES AND CEREBROSIDE SULFATES

Sample	Cerebrosides		Cerebroside Sulfates	
	Total FA* Isolated	HFA† as % of Total FA*	Total FA* Isolated	HFA† as % of Total FA*
	mg		*mg*	
Controls				
12428	19.7	72	—	—
13270	34.9	73	6.7	48
14653	4.1	73	2.8	46
Disease group				
(*a*) Alzheimer's disease				
13755	2.0	55	15.9	54
12045	42.3	66	10.0	50
Lo	6.4	66	5.0	40
(*b*) Senile dementia				
A-34-66	8.8	74	4.6	50
(*c*) Cortical atrophy				
A-35-66	14.9	70	6.7	51

* FA, fatty acids.
† HFA, hydroxy fatty acids.

ples (Tables 3 and 4). Samples 13755 and 12045 gave rise to two minor unidentified peaks with retention times close to those of 14:1 and 15:0, but these peaks disappeared upon hydrogenation of the samples. They appear to represent unsaturated fatty acids containing less than 14 carbon atoms.

The compositions of the cerebroside and cerebroside sulfate HFA are given in Tables 5 and 6. In this class of fatty acids, 2-hydroxylignoceric acid (24h:0) was the major fatty acid (25–36%) in both cerebrosides and cerebroside sulfates. Saturated fatty acids accounted for more than one-half (57–72%) of the hydroxy acids in both classes of lipids. This is in contrast to the NFA, in which the monounsaturated fatty acids constituted the major portion (50–77%).

It appears that in cerebrosides the percentage of 18h:0 is slightly increased in all three cases (Lo, 13755, and 12045) of Alzheimer's disease. In all the brains with neurological disease, 23h:1 was increased slightly, and the increase was significant in sample 12045 compared to that of the controls. In this sample there was a general decrease of all HFA that contain 24 or more carbon atoms. In samples 12428 and 12045, a peak with a retention time close to that of 19h:1, and in sample 14653 two peaks with retention times similar to, but not identical with, 14h:0 and 17h:0, have been found. These peaks have not been identified.

In cerebroside sulfates (Table 6) monounsaturated hydroxy fatty acids were lower (33% and 28%) in two of the samples with Alzheimer's disease (13755 and 12045) than in the two controls and the rest of the diseased brains (41–44%). In sample 14653 (control), one peak with a retention time close to 15h:0 has been noted. This peak disappeared on hydrogenation, which indicates that it may be an unsaturated hydroxy fatty acid that has 13 or fewer carbon atoms.

TABLE 3 PERCENTAGE COMPOSITION OF CEREBROSIDE NONHYDROXY FATTY ACIDS

	Controls			Alzheimer's Disease			Senile Dementia	Cortical Atrophy
	12428	13270	14653	Lo	13755	12045	A-34-66	A-35-66
14:0	tr.	tr.	tr.	tr.	tr.	tr.	tr.	
14:1			tr.		tr.			
15:0	tr.	tr.	tr.	tr.	tr.			
16:0	1.2	0.5	0.7	0.7	1.5	3.7	tr.	1.1
16:1			0.2		tr.	0.4		tr.
17:0	tr.	tr.			tr.			0.2
17:1						0.6		
18:0	6.2	16.5	9.4	8.9	7.6	17.8	8.6	9.0
18:1	1.0	0.9	2.5	1.5	1.9	2.5	tr.	1.4
18:2				tr.	tr.	tr.		tr.
19:0		0.2	0.3	tr.	0.1	tr.	tr.	
20:0	1.0	1.6	1.1	0.9	1.0	1.8	1.3	1.1
20:1	tr.		tr.	tr.	tr.	0.6		tr.
21:0	tr.	0.3	tr.	tr.	0.2		0.3	tr.
21:1					0.1			
22:0	2.8	3.4	3.0	1.8	2.5	3.2	2.1	3.1
22:1	tr.	0.5	0.5	0.6	0.5	0.5	0.8	0.6
23:0	3.6	5.0	3.0	3.1	3.9	4.8	3.3	3.5
23:1	0.4	0.7	0.9	0.9	1.0	0.9	1.6	1.0
24:0	10.0	12.5	8.8	9.6	10.6	13.2	10.9	9.9
24:1	48.2	40.5	44.8	44.6	41.3	30.9	45.1	41.4
25:0	3.0	3.5	3.3	3.9	4.6	4.5	3.6	4.0
25:1	13.2	9.2	13.4	13.1	13.0	7.1	13.8	13.5
26:0	tr.	0.4	1.1	0.6	0.9	1.6	0.3	0.7
26:1	9.3	4.4	7.0	9.8	9.3	5.9	8.4	9.5

TABLE 4 PERCENTAGE COMPOSITION OF CEREBROSIDE SULFATE NONHYDROXY FATTY ACIDS

	Controls		Alzheimer's Disease			Senile Dementia	Cortical Atrophy
	13270	14653	Lo	13755	12045	A-34-66	A-35-66
12:1				0.6	0.1		
14:0	0.5	tr.	tr.	1.1	1.0	tr.	tr.
14:1	0.2	tr.		(1.8)*	(0.7)*		tr.
15:0	tr.			(1.4)*	(0.9)*	tr.	
16:0	4.1	0.9	1.7	2.2	5.6	0.4	0.6
16:1	0.5	0.2		0.4	0.3		tr.
17:0	tr.					tr.	tr.
17:1				tr.	tr.		
18:0	6.6	3.4	4.6	3.0	8.1	2.7	2.3
18:1	5.6	1.1	1.5	1.0	3.0	0.8	1.1
18:2	0.8	tr.	tr.	tr.			
19:0	tr.	tr.	tr.	tr.		tr.	tr.
20:0	0.5	0.5	0.6	0.4	0.6	0.3	0.4
20:1	0.4		0.4	0.1	0.3		tr.
21:0	0.3			0.1	0.3	tr.	0.1
22:0	2.0	1.7	1.5	1.8	2.4	1.8	2.0
22:1	0.4	0.5	0.5	tr.	0.4	0.7	0.6
23:0	2.6	2.8	2.9	3.4	4.1	3.1	3.2
23:1	0.8	0.9	1.1	0.8	0.6	0.8	1.0
24:0	8.4	10.4	9.8	12.6	13.6	10.2	9.6
24:1	41.4	48.5	44.3	41.0	35.9	44.8	46.1
25:0	2.9	3.9	3.9	5.3	5.2	3.9	4.7
25:1	14.0	15.0	14.7	12.9	9.8	16.7	16.1
26:0	0.5	0.8	1.2	0.8	0.8	0.7	1.0
26:1	7.5	9.6	11.5	9.4	6.1	13.2	11.5
27:0					0.5		
27:1				0.2			

* Unidentified.

TABLE 5 PERCENTAGE COMPOSITION OF CEREBROSIDE HYDROXY FATTY ACIDS

	Controls			Alzheimer's Disease			Senile Dementia	Cortical Atrophy
	12428	13270	14653	Lo	13755	12045	A-34-66	A-35-66
12h:0			0.1	0.3				
13h:0				tr.	0.3	0.3	0.3	
14h:0	0.3	tr.	(1.0)*	0.1	0.2	0.4		tr.
15h:0	tr.			tr.	tr.	0.7		tr.
16h:0	tr.	tr.	0.1	0.1	0.1	0.3	tr.	0.1
16h:1				0.2			0.1	
17h:0	tr.		(0.7)*	tr.	tr.	tr.		
18h:0	0.8	1.0	0.2	1.6	2.5	4.1	0.9	0.8
18h:1				tr.	0.2	tr.		
19h:0	0.2		tr.	0.2				
19h:1	0.2			0.4	0.5	0.5		
	(0.4)*					(0.9)*		
20h:0	0.4	0.2	0.2	0.4	0.3	0.9	0.3	0.4
20h:1			0.2	0.3	0.7	0.9		
21h:0	tr.		tr.			0.6	0.1	0.2
22h:0	7.1	8.8	5.9	5.8	6.0	7.5	7.1	6.9
22h:1			0.3				1.0	0.9
23h:0	17.1	17.9	14.0	14.4	13.7	12.9	14.8	14.1
23h:1	tr.	tr.	0.7	1.2	0.9	5.1	2.6	3.0
24h:0	34.3	31.6	27.6	28.1	24.8	29.2	28.5	28.2
24h:1	20.0	24.2	27.7	25.3	24.5	18.7	28.0	27.1
25h:0	8.2	5.2	5.8	6.9	7.1	6.6	5.2	6.6
25h:1	4.5	6.4	7.4	6.2	7.2	4.4	4.9	5.1
26h:0	tr.	tr.	0.7	1.2	1.5	1.6	tr.	0.4
26h:1	6.6	4.8	7.4	7.6	9.0	4.5	6.3	6.2
27h:0					0.6			

* Unidentified.

TABLE 6 PERCENTAGE COMPOSITION OF CEREBROSIDE SULFATE HYDROXY FATTY ACIDS

	Controls		Alzheimer's Disease			Senile Dementia	Cortical Atrophy
	13270	14653	Lo	13755	12045	A-34-66	A-35-66
13h:0	tr.		tr.			0.4	tr.
13h:1							0.3
14h:0	0.1	0.1	0.4	0.2	tr.	tr.	tr.
15h:0	tr.	(1.6)*	0.2	tr.	0.3	tr.	0.4
16h:0	0.1		0.3	0.3	0.5	tr.	0.2
16h:1	0.3	0.2				0.1	
17h:0	tr.		tr.		tr.	tr.	
18h:0	1.2	2.9	2.5	1.3	1.3	0.7	0.8
18h:1	tr.		0.5				
19h:0			0.3	tr.	tr.		
19h:1	0.5	0.2					
20h:0	0.1	tr.	1.0	0.8	0.6	0.1	0.2
20h:1	0.4	0.1	0.4				
21h:0		tr.	tr.	0.5	0.7	tr.	tr.
22h:0	4.7	4.7	3.7	4.5	6.9	4.5	4.4
22h:1						0.8	0.5
23h:0	13.3	12.3	12.4	13.4	16.5	12.1	11.4
23h:1	0.8	0.9	0.6	tr.	tr.	2.9	2.1
24h:0	28.6	26.9	29.2	33.7	36.5	28.3	29.5
24h:1	25.6	25.5	23.5	18.2	18.5	28.1	27.3
25h:0	7.2	7.0	7.9	11.0	7.8	7.8	9.3
25h:1	8.5	6.9	6.8	5.7	3.9	6.7	5.3
26h:0	1.3	1.6	1.1	1.2	1.3	1.4	tr.
26h:1	7.3	9.2	9.3	8.1	5.2	6.1	8.4
27h:0				0.6			
27h:1				0.7			

* Unidentified.

DISCUSSION

This study establishes the fatty acid compositions of human cerebral gray matter glycolipids in a limited number of cases of human neurological diseases, particularly Alzheimer's disease. We chose the cerebral gray matter for these experiments because this part of the brain is known to be affected more by the degenerative changes that take place during this disease.

As stated earlier, two of the samples of brain (13755 and 12045) were available only after they were fixed in formalin. Because fresh samples are rare, formalin-fixed samples were used in this study. It was difficult to purify the cerebroside and cerebroside sulfate samples from these brains by column chromatography alone, but they could be purified by preparative TLC. Polyunsaturated acids have been reported (20) to be virtually absent from formalin-fixed brains. The fact that our samples of fresh brains contained very little, if any, polyunsaturated fatty acids provides evidence that their absence is not caused by formalin fixation. This is in agreement with the findings of Menkes et al. (4). The similarity in all other respects between fatty acid compositions of formalin-fixed and fresh brains makes it unlikely that formalin had a significant effect on gangliosides from these samples.

Although we took care to prevent oxidation of the unsaturated fatty acids by handling all samples under nitrogen, many steps are involved in the separation and purification of the glycolipids and isolation of fatty acid methyl esters, and oxidative changes cannot be ruled out entirely.

The relative percentage compositions of the NFA and HFA in the diseased brains are generally similar to the control values reported here and by other investigators (16, 21). However, some differences were noticeable in Alzheimer's disease. In this disease, the neurons of the cerebral cortex, which contain the major portion of the brain gangliosides (22), undergo degeneration, and the amounts of gangliosides, as determined by their *N*-acetylneuraminic acid assay, are known to decrease (1). The slight decrease in the stearic acid (the principal fatty acid) and the corresponding increase in the palmitic acid content of gangliosides may indicate a preferential breakdown of the most predominant ganglioside, namely, the ganglioside that contains stearic acid.

In most instances of Alzheimer's disease, the cerebroside and cerebroside sulfate fatty acids contained a greater number of fatty acids than the controls. Rouser, Feldman, and Galli (23) noted similar results in the sphingomyelin and lecithin fatty acids in Alzheimer's disease. This may indicate the presence of a larger variety of glycolipids and phospholipids in these brains as a result of the degenerative changes. Kishimoto and Radin (24) found slightly increasing amounts of HFA of rat brain cerebrosides with increasing age. If the same holds true for human brain cerebrosides, it is possible that in Alzheimer's disease this small, normal increase does not occur, because of an abnormal metabolism. The data for cerebroside sulfate total NFA and HFA do not indicate any definite pattern of change. Among the two classes of fatty acids of cerebrosides and cerebroside sulfates, monounsaturated acids were more abundant in the NFA and saturated acids in the HFA. The monounsaturated acids of the NFA and HFA series were decreased in both cerebrosides and cerebroside sulfates from at least one brain with Alzheimer's disease (12045). Although not conclusive, this points to the possibility that monounsaturated acids may be more affected than the saturated acids of brain gray matter cerebrosides and cerebroside sulfates in Alzheimer's disease.

In one sample each of senile dementia and senile cerebrocortical atrophy, the fatty acid compositions of the glycolipids do not appear to be different from those of the controls.

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