The 2-hydroxy fatty acids in white matter of infant and adult brains

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SUMMARY White matter from five adult and five infant brains was analyzed for total fatty acids, galactolipids, and 2-hydroxy fatty acids. The distribution of 2-hydroxy fatty acids was determined quantitatively for each sample by gas–liquid chromatography of their methyl esters without masking their hydroxyl groups.

The galactolipid values in a case of multiple sclerosis and in the temporal areas of two other cases were about 25% lower than that reported for normal brains by other authors, but the content and composition of the 2-hydroxy fatty acids did not correlate with galactolipid contents and were similar for all adult samples.

The total fatty acid, galactolipid, and 2-hydroxy fatty acid concentrations in the infant brains were small compared with those of adults. The composition of the 2-hydroxy fatty acids was also different.

KEY WORDS brain · white matter · human · infant · adult · fatty acids · galactolipids · 2-hydroxy fatty acids · gas–liquid chromatography · multiple sclerosis

RADIN AND AKAHORI (1) demonstrated that cerebrosides of adult human brain contain large amounts of 2-hydroxy fatty acids (HFA). Cerebrosides are more prevalent in white than in gray matter and rapidly increase during the postnatal period concomitantly with formation of myelin in both the human (2) and in experimental animals (3). The rate of C^{14} incorporation into cerebrosides is greatest at the time of myelin deposition (4). These findings have been taken as evidence that cerebrosides are constituents of myelin. Indeed, cerebrosides were depleted in areas of early and advanced demyelination in the brains of three cases of multiple sclerosis (MS) studied by Cumings (5). Consequently, the HFA became of interest from two points of view: namely, whether synthesis and deposition of the HFA correspond to those of the cerebrosides; and whether differences in HFA composition and total quantity are to be found between the brains of patients with MS and those of controls.

MATERIALS AND METHODS

Brain tissue, obtained at autopsy and not exposed to any fixative, was extracted for lipids with chloroform–methanol 2:1 (v/v), as reported previously (6). The MS brain samples analyzed were taken from areas of intact white matter and identifiable plaques were avoided. The procedures for determining the water, total fatty acid, and carboxyl ester contents have been described earlier (7). Galactolipids[1] were estimated by the method of Radin et al. (8).

The HFA methyl esters were isolated from methanolysates of total lipid extracts by thin-layer chromatography (TLC) (9). Gas–liquid chromatography (GLC) of the HFA methyl esters was done on a Barber-Colman, Model 10 instrument equipped with an argon ionization chamber (1 cm diode). Liquid phases used were SE-30 (Analytical Engineering Laboratories, Inc., Hamden, Conn.) and Reoplex 400 (Geigy Pharmaceuticals, Division of Geigy Chemical Corp., Ardsley, N. Y.). The Reoplex 400 was made more thermally stable by chromatographing it on an Amberlite-400 column (10). Preliminary treatment of glass columns and glass wool packing with a 5% solution of dichlorodimethylsilane in chloroform considerably reduced tailing of peaks. Good resolution of the HFA methyl esters was achieved

[1] *Galactolipid* applies in this article to cerebroside, sulfatide, and galactosyl diglyceride but not to ganglioside.

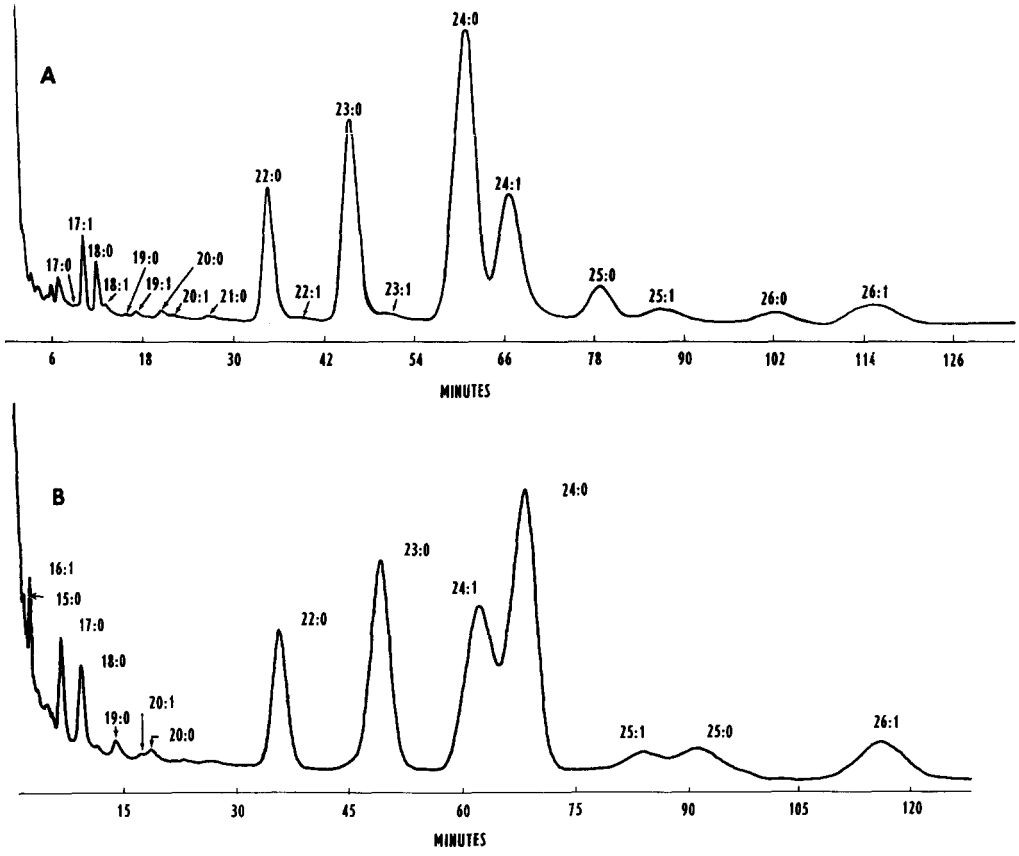

FIG. 1. Gas chromatographic tracings of HFA methyl esters from adult human brain white matter. Chromatographic conditions: *A*, 6 ft × ⅛ inch (i.d.) glass column; 10% Reoplex 400 (w/w) on 80–90 mesh Anakrom ABS; column temperature, 193°; inlet pressure, 28 psi. *B*, 6 ft × ⅛ inch (i.d.) glass column; 10% SE-30 (w/w) on 60–70 mesh Anakrom ABS; column temperature, 217°; inlet pressure, 31 psi.

with both types of columns (Fig. 1). Individual HFA methyl esters were identified by comparing retention data with those of purified acids[2] and by using the equivalent chain length method of Miwa et al. (11).

For the purpose of calibration, 18h:0, 20h:0, 22h:0 and 24h:0 acids[3] were prepared from pure saturated fatty acids according to Stumpf and James (12). The methyl esters of the synthesized HFA and of 26h:0 (California Biochemical Co., Los Angeles, Calif.) were prepared by the method of Radin et al. (13), purified by TLC (9), and tested for purity by GLC and infrared (IR) spectrometry. GLC of the synthetic and C_{26} HFA indicated purities of approximately 95%. The IR patterns of these HFA methyl esters revealed the absorption bands characteristic for such compounds (14). Three mixtures containing different proportions of 18h:0, 20h:0, 22h:0, 24h:0, and 26h:0 methyl esters were subjected to GLC in quintuplicate (Table 1). Peak areas determined by triangulation agreed within 1% with those found by electronic integration. Detector response was linear for the quantities of HFA methyl esters tested, and the percentages found were generally within 1% of the theoretical values (Table 1). All chromatographic analyses were done in duplicate and results differing by more than 5% were discarded. Averages of the duplicate results are reported.

RESULTS

The concentrations of total galactolipids in 8 specimens of 4 adult brains (cases 1–4, Table 2) were within the range given by Brante (15), Davison and Wadja (16), Plum[4], and O'Brien et al. (17) for white matter, when their gram values were converted to mmoles on the basis of a molecular weight of 835. The specimens 3b and 4b

[2] Kindly made available by Dr. J. F. Mead, Department of Biophysics and Nuclear Medicine, University of California School of Medicine at Los Angeles, Calif.; also purchased from Applied Science Laboratories, State College, Pa.

[3] Fatty acids are identified by their chain length and number of double bonds; h indicates the presence of a 2-hydroxy group.

[4] Personal communication, Dr. C. M. Plum, The Biochemical Research Laboratory, Kolonien Filadelfia, Dianalund, Denmark.

and the frontal lobe and temporal-parietal lobe areas from the case of MS (5a and 5b) revealed galactolipid levels about 25% lower than the others. The HFA amounted to 2.3–3.8 mmoles in cases 1–4, and 2.3–2.6 in the white matter from case 5 (MS), thus failing to suggest a difference between the two groups. The concentration of total fatty acids (Table 2) was, however, distinctly lower in the MS brain.

The distribution of the HFA (Table 3) in cases 1–4 corroborates previous reports (1, 18) that these acids are predominantly of the long-chain type, the acids with 19 carbons or more making up 73–98% in cases 1–4, and 79–94% in case 5. The unsaturated HFA totaled 18–33%; in the case of MS, 17–29%. Thus, the case of MS appears not to differ from the controls in regard to composition of HFA.

The concentrations of galactolipids and of total fatty acids in infant brains (cases 6–10, Table 2) were small compared with those of adults. The ratio of HFA to total fatty acids in the galactolipids of premature infants was less than one-tenth, and in early childhood only one-half of that found in adults (Table 2). The two prenatal specimens (cases 6d and 7, Table 3) contained higher percentages of C_{15} to C_{21} HFA than the adult samples. The increase of 23h:0 and 25h:0 with age is in agreement with that found in rats (19, 20). The change in the HFA distribution from shorter to longer chain length with increasing age is similar to that found for the nonhydroxy fatty acids of sphingomyelin (18).

TABLE 1 Quantification of Hydroxy Fatty Acid Methyl Esters by GLC*

	True Weight	Average Weight†	Relative Error
	%	%	%
Mixture A			
18h:0	20.0	20.8	+4.0
20h:0	20.8	20.9	+0.5
22h:0	18.8	18.9	+0.5
24h:0	21.4	20.1	−6.1
26h:0	19.0	19.5	+2.6
Mixture B			
18h:0	5.2	5.1	−1.9
20h:0	5.5	5.7	+3.6
22h:0	13.6	13.5	−0.7
24h:0	66.4	65.9	−0.8
26h:0	9.3	9.7	+4.3
Mixture C			
18h:0	2.0	2.2	+10.0
20h:0	4.1	4.7	+14.6
22h:0	9.7	10.2	+5.2
24h:0	46.8	47.2	+0.9
26h:0	37.5	35.7	−4.8

* Reoplex 400 (10%) on Anakrom ABS.
† Average of quintuplicate analyses of each mixture.

DISCUSSION

The variation of total galactolipid concentration between various areas of white matter of adult brains was less in

TABLE 2 Fatty Acids and Galactolipids of Human White Matter

Case No.	Age	Diagnosis	Total Fatty Acids	Galacto-lipids	HFA	Ratio of HFA to Total Galactolipid Fatty Acids
			mmoles/100 g wet wt			
1*a**	43	Thrombosis pulmonary artery; pulmonary emboli; well nourished	23.71	5.09	2.37	0.47
b			26.42	5.54	2.33	0.42
c			25.14	5.04	2.59	0.51
2*a*	45	Pulmonary abscess; post-operative death; well nourished	24.03	5.40	3.00	0.56
c			26.56	5.74	3.82	0.67
cc			21.34	4.86	2.65	0.55
3*b*	79	Cancer colon; emaciated	19.93	3.66	2.49	0.68
cc			26.34	4.23	3.11	0.44
4*b*	53	Latent diabetes mellitus; pulmonary emphysema; secondary polycythemia; moderately well nourished	21.17	4.08	2.50	0.61
cc			25.10	5.72	2.69	0.47
5*a*	66	MS; well nourished; gastric ulcer	21.38	4.28	2.52	0.59
b			16.70	3.81	2.32	0.61
cc			17.87	4.66	2.56	0.55
6*d*		Prematurity	6.28	0.27	0.02	0.07
e			6.45	0.40	0.15	0.37
7*d*		Prematurity	5.33	0.04	0.04	0.09
8*d*	7 weeks	Acute pneumonitis; tear of falx cerebri	7.39	0.59	0.13	0.23
			10.05	1.21	0.32	0.27
9*d*	8 months	Congenital heart disease	9.79	1.45	0.49	0.34
10*d*	17 months	Congenital heart disease	19.86	3.59	0.98	0.27

* Symbols: *a*, frontal lobe; *b*, temporal-parietal lobe; *c*, occipital lobe; *cc*, corpus callosum; *d*, random white; *e*, cerebellum white.

TABLE 3 PERCENTAGE COMPOSITION OF HFA METHYL ESTERS DETERMINED BY GLC

Fatty Acid	1			2			3		4		5			6*		7*	8		9	10
	a†	b	c	a	c	cc	b	cc	b	cc	a	b	cc	d	e	d	d	e	d	d
										mole %										
14:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14:0	—	—	—	—	—	—	—	2.5	—	—	—	—	0.4	—	—	—	—	—	—	—
15:1	—	—	—	—	—	—	—	2.8	—	—	—	—	—	—	—	—	—	—	—	—
15:0	2.8	—	—	0.8	1.0	1.8	1.4	2.2	—	1.1	—	6.4	0.5	9.2	1.8	—	—	—	—	1.1
16:1	—	—	—	—	—	—	—	—	—	—	—	—	—	16.0	3.9	7.9	—	—	—	—
16:0	8.2	2.6	5.6	0.2	—	—	0.3	2.4	—	—	1.0	8.1	—	16.0	6.9	6.4	—	—	—	1.1
17:1	—	2.5	—	0.3	—	—	0.3	—	—	—	—	—	—	4.7	0.8	9.5	—	—	—	0.6
17:0	—	—	4.2	—	1.5	3.8	0.7	10.8	2.9	4.6	—	—	7.6	1.0	—	6.1	4.3	1.2	1.2	0.5
18:1	—	—	—	0.4	—	—	0.6	—	—	—	0.5	1.9	—	3.9	2.8	—	—	—	—	0.7
18:0	1.1	2.5	3.3	1.1	1.0	2.7	1.8	6.1	3.5	2.6	4.5	4.7	3.7	3.4	4.1	8.8	6.5	4.2	3.1	1.4
19:1	—	—	—	—	—	—	—	—	—	—	1.1	—	—	1.7	1.2	—	1.6	—	0.4	—
19:0	—	—	—	0.7	0.5	1.4	—	3.7	0.7	1.4	0.3	—	2.9	0.9	1.2	5.6	3.9	2.9	—	0.7
20:1	—	—	—	0.8	—	—	0.2	—	0.2	—	0.3	—	—	—	—	—	—	—	0.5	—
20:0	—	1.4	—	0.5	0.7	0.8	0.4	0.9	0.5	0.4	0.5	0.9	0.8	—	0.8	—	2.6	2.1	1.8	0.5
21:1	—	—	—	0.6	—	—	—	0.7	—	0.3	1.3	—	—	—	—	—	3.6	1.4	0.3	0.2
21:0	—	—	—	—	—	—	0.3	1.0	—	0.1	—	—	0.7	—	0.6	—	2.2	2.7	—	0.4
22:1	—	—	—	—	—	—	0.3	—	—	—	0.6	—	—	—	—	—	0.7	—	—	—
22:0	14.1	9.4	9.9	9.0	9.5	8.1	11.9	8.1	8.2	9.2	9.5	10.0	9.0	9.7	16.7	12.5	16.1	19.7	16.8	11.7
23:1	—	—	—	—	1.1	—	0.8	—	1.1	0.7	—	—	0.5	—	—	—	0.7	—	—	0.4
23:0	19.5	19.3	15.2	18.8	18.6	15.9	15.4	13.5	18.6	17.5	21.9	12.5	18.0	3.1	5.2	4.3	4.6	5.2	6.9	13.1
24:1	19.5	9.6	18.5	22.0	22.5	17.6	19.1	8.4	21.8	22.2	18.8	13.6	11.0	—	4.0	2.3	19.2	5.4	10.4	11.5
24:0	25.6	41.0	29.3	35.3	31.5	32.5	32.8	29.0	30.8	28.5	29.9	27.4	32.7	19.5	46.4	26.7	32.1	49.1	51.4	49.1
25:1	2.2	1.5	3.6	3.0	2.5	3.2	3.8	1.4	3.4	3.5	2.8	4.3	2.6	—	—	—	—	—	—	0.9
25:0	3.3	5.7	4.9	3.8	3.3	5.7	5.9	3.9	5.7	3.9	3.4	5.7	5.2	4.6	1.2	—	0.9	1.7	2.6	3.5
26:1	3.7	4.3	5.5	3.0	6.4	6.4	3.9	2.7	2.6	3.6	3.8	4.5	3.2	6.4	1.2	9.8	1.2	2.2	4.5	2.3
26:0	—	—	—	—	—	—	—	—	—	0.7	—	—	1.3	—	1.2	—	—	2.2	—	0.6

* Insufficient material for duplicate analysis.
† For case and part of brain referred to, see Table 2.

the brains of two middle-aged, well nourished individuals dying from diseases of short duration (cases 1 and 2, Table 2), and greater in an older individual and in one with prolonged diseases (cases 3 and 4). In the temporal areas of the latter two cases, galactolipid values were lower than in the first two cases and in those reported by other authors (15–17). Although cerebrosides in the adult are assumed to be metabolically nearly inert (21), the diminished galactolipid values in the temporal areas of cases 3 and 4 imply that prolonged extracranial disease or inanition may influence the metabolism of these compounds. This has been suggested also for other lipids (6, 21–23). The HFA content in cases 3 and 4, however, did not reflect the lower galactolipid values. In case 5 (MS), HFA were present in concentrations similar to those in the other cases, although total fatty acids (5b and 5cc, Table 2) and phospholipids and plasmalogens [as reported elsewhere (7)] were distinctly lower than in the controls.

It is relevant, therefore, to discuss the processes that could leave the HFA intact, notwithstanding the partial depletion of the galactolipids. Since sphingomyelin and ceramidopolysaccharides appear to contain very little or no HFA (24–26), these could not account for the differences. The galactolipid contents were determined by a procedure that measures the hexose moiety of the sphingolipid. Cleavage of the hexose–ceramide bond, or incomplete synthesis of the cerebroside resulting in ceramides, would explain the above data. The presence of a ceramide in the white matter from a case of MS has been reported by Schwarz et al. (27). Another hypothetical mechanism would be cessation of biosynthesis of the long-chain nonhydroxy fatty acids, but not of the hydroxylation reaction (28). The present results are in agreement with the recent report of Jatzkewitz (29) that, in MS, cerebrosides of the cerasine type are more depleted than the phrenosine type.

It has been known for some time (30, 31) that brains of human fetuses and of newborn infants are nearly devoid of cerebrosides. This has been confirmed on brains of infants, employing the more specific method of Radin et al. (8). Even at this early age, the minute amounts of HFA present are predominantly the long-chain members which are found in the adult brain. Whether the unknown causes leading to premature birth of infants, or the congenital heart disease in cases 9 and 10, with its associated hypoxia, influenced the formation of HFA or cerebrosides, is at present unknown.

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