A MOLECULAR DEFECT OF MYELINATION

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A molecular defect has been found in metachromatic leucodystrophy (MLD), a rare hereditary disorder of myelin formation in which cerebroside sulfates accumulate. In normal white matter, cerebrosides and sphingomyelins contain large proportions of very long chain fatty acids (21-26 carbons) but in MLD these lipids are nearly depleted of these fatty acids. In this report these findings are documented and a working hypothesis is presented to partially explain the biochemical defect and the pathogenesis of MLD.

MATERIALS AND METHODS

Frozen tissue from the frontal lobes from two cases of MLD were manually separated into grey and white matter, and extracted with chloroform-methanol 2:1 as described previously (1). The lipid extracts from each tissue were separated into cholesterol, ceramides, cerebrosides, cerebroside sulfates, sphingomyelins, gangliosides, and ethanolamine phosphatides, serine phosphatides, and choline phosphatides using a combination of a Florisil column with a diethylaminoethyl (DEAE) cellulose column (1) and a combination of a DEAE cellulose column with a silicic acid column or with a silicic acid-silicate column (2,3). The quantity of each lipid was determined by weighing on a analytical balance. Paper chromatography (1,3,4) was used to evaluate the purity of each fraction as were analyses of phosphorus, glycerol, fatty acid, hexose and sulfate content. The results of these analyses corresponded closely with the theoretical values indicating that the fractions were uncontaminated and that abnormal compounds were absent or undetectable. The fatty acid compositions of the three glycerolphosphatides were determined by gas-liquid chromatography (GLC) of fatty acid methyl esters and aldehyde dimethylacetals after the latter had been selectively isolated by mild alkaline hydrolysis (3,5). The fatty acid compositions of the sphingolipids were determined by GLC (6).

RESULTS

The lipid compositions of grey matter from each patient compared with a normal control of a similar age are given in Table 1. The quantities of total lipid, cholesterol, choline phosphatides, sphingomyelins, and gangliosides were somewhat diminished. In Case 1 cerebrosides were increased while in Case 2 they were decreased. Cerebroside sulfates were increased 3-4 fold in both cases and ceramides were moderately increased.

TABLE 1

LIPID COMPOSITION OF FRONTAL LOBE

	GREY MATTER			WHITE MATTER		
	Case 1	Case 2	Normal	Case 1	Case 2	Normal
Age	9	11	9	9	11	9
Total lipid	34.80	39.35	47.80	53.50	50.30	74.13
Protein	65.20	60.65	52.20	46.50	49.70	25.87
Water	83.34*	84.88	85.79	79.91*	82.05	77.35
Cholesterol	7.01	5.94	7.21	10.00	8.10	13.24
Ethanolamine Phosphatides	*	9.05	9.61	*	9.10	12.11
Serine Phosphatides	*	2.64	2.68	*	2.87	5.13
Choline Phosphatides	6.91	8.15	8.99	7.70	7.60	8.84
Sphingomyelins	2.37	2.09	2.77	2.94	2.41	4.93
Cerebrosides	3.50	1.26	1.91	6.41	2.62	10.67
Cerebroside Sulfates	1.60	1.42	0.41	12.60	5.33	3.95
Ceramides	0.63	0.75	0.48	0.70	1.26	0.50
Gangliosides	*	0.28	0.40	*	0.20	0.44

All values except water are expressed as percent of dry weight.
*values affected by formalin fixation

In white matter total lipid, cholesterol, all three glycerolphosphatides, sphingomyelin, and cerebrosides were markedly decreased while cerebroside sulfates were increased three-fold in Case 1 and 1.4 fold in Case 2. Cerebrosides were diminished to 25% of normal in Case 2 while ceramides were moderately increased.

In MLD, the fatty acid and fatty aldehyde compositons of the grey matter glycerolphosphatides were normal as were those of the grey matter sphingolipids. In white matter the glycerolphosphatides contained somewhat larger proportions of polyunsaturated fatty acids and smaller proportions of aldehydes than normal. These changes were small and probably reflect a dilution of myelin in MLD white matter by glial cells. There was a striking diminution (7-10 fold) of cerebrosides and sphingomyelins containing fatty acids with 21-26 carbons (Table 2) involving both unsubstituted acids and alpha hydroxy acids*. The defect was specific for the longer chain fatty acids since sphingomyelins and cerebrosides containing fatty acids with 14-20 carbons were normal or increased (Tables 2 and 3). The diminution was proportional to the chain length of the fatty acids; the longer the fatty acid, the greater being the diminution (Table 3). Ceramides also showed a striking diminution in the proportion of long chain fatty acids. Cerebroside sulfates from MLD white matter also showed a shift in fatty acid composition toward larger proportions of shorter chain acids. However, this shift was small compared to cerebrosides or sphingomyelins (Table 3).

DISCUSSION

The biochemical defect in MLD appears to be a failure to elongate the fatty acids of sphingolipids beyond 18 carbons. The defect is specific for longer chain acids (21-26 carbons) and effects the fatty acids of cerebrosides, ceramides and sphingomyelins much moreso than those of cerebroside sulfates.

The nature of the defect is not completely understood. It has been shown that two systems exist in the brain for the biosynthesis of saturated fatty acids, 1) a de novo system which condenses 8 acetate units to form palmitic acid (7,8) and 2) a chain elongation system which attaches 2 acetate

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*The proportions of total hydroxy acids in cerebrosides and cerebroside sulfates from grey or white matter were equal to the control.

TABLE 2

FATTY ACIDS OF SPHINGOLIPIDS
(As percent of total unsubstituted fatty acids in each lipid)[11]
GREY MATTER

	Sphingomyelin			Cerebroside			Cerebroside Sulfate		
ACID	Case 1	Case 2	Normal	Case 1	Case 2	Normal	Case 1	Case 2	Normal
14:0	1.6	1.3	1.4	10.3	2.8	4.7	10.8	4.7	17.2
16:1	tr	tr	tr	tr	3.7	tr	0.4	0.9	2.2
16:0	10.3	17.0	9.4	20.4	26.3	23.4	17.5	18.7	20.0
18:1	3.0	2.9	2.2	23.0	23.6	21.3	1.8	8.5	5.0
18:0	76.7	72.0	78.6	31.0	29.6	34.3	16.4	24.3	13.1
20:1	tr	tr	tr	tr	tr	tr	tr	tr	0.4
20:0	2.0	1.3	1.2	0.5	0.5	tr	1.0	1.6	0.2
22:1	tr	tr	tr	tr	0.3	tr	0.3	1.1	tr
22:0	0.3	0.7	0.5	2.0	0.1	tr	2.1	1.6	0.8
23:1	tr	tr	tr	0.4	tr	tr	0.5	0.4	tr
23:0	tr	tr	tr	0.8	0.1	tr	2.1	1.4	1.2
24:1	2.9	1.7	2.9	5.0	1.0	4.4	15.0	14.3	13.0
24:0	2.0	1.0	1.5	3.5	0.6	2.1	11.5	14.3	5.3
25:1	0.9	tr	0.8	1.0	0.2	0.4	7.4	2.9	3.2
25:0	0.3	tr	tr	0.7	0.1	tr	3.0	1.2	1.3
26:1	tr	tr	tr	0.5	0.3	tr	8.2	2.6	3.8
26:0	tr	tr	--	0.3	tr	--	1.7	0.5	tr
14-20	93.6	94.5	92.8	84.8	85.6	83.7	47.9	58.7	57.9
21-26	6.4	5.5	7.2	15.2	14.4	16.3	52.1	41.3	42.1

WHITE MATTER

	Sphingomyelin			Cerebroside			Cerebroside Sulfate		
ACID	Case 1	Case 2	Normal	Case 1	Case 2	Normal	Case 1	Case 2	Normal
14:0	3.0	1.0	7.8	0.7	0.8	0.8	0.1	1.2	4.6
16:1	0.8	0.3	0.5	1.5	2.2	tr	0.9	1.0	0.4
16:0	10.3	9.8	6.7	18.1	22.8	8.3	9.4	4.3	3.5
18:1	3.8	1.2	1.3	32.8	19.0	3.3	4.7	2.0	1.5
18:0	50.0	73.8	30.5	26.4	25.9	7.9	7.2	8.2	3.1
20:1	0.1	tr	tr	1.3	0.3	0.1	tr	--	tr
20:0	0.9	1.2	0.9	0.5	0.3	0.4	1.8	1.6	0.5
22:1	0.1	--	0.4	0.1	0.3	0.4	0.8	--	tr
22:0	0.9	0.8	1.4	0.3	0.6	1.8	2.7	3.3	1.8
23:1	0.1	tr	0.5	0.3	0.2	0.8	0.9	tr	0.6
23:0	1.0	0.6	1.6	0.8	0.7	3.1	3.2	4.0	3.3
24.1	16.5	7.2	25.2	6.8	7.3	40.0	27.3	27.3	38.7
24:0	3.4	1.7	6.5	1.9	6.2	11.3	17.9	19.2	16.1
25:1	2.5	0.8	3.2	1.1	2.2	7.0	6.6	7.6	9.0
25:0	1.5	tr	1.4	0.3	0.3	3.5	5.5	3.7	5.0
26:1	3.0	1.0	2.3	1.2	0.2	7.4	9.8	9.6	9.8
26:0	tr	tr	tr	tr	0.2	1.0	0.9	2.8	1.8
14-20	72.6	87.6	57.5	84.1	78.0	22.7	25.5	22.7	13.4
21-26	27.4	12.4	42.5	15.9	22.0	77.7	74.5	77.3	86.6

units at a time to make longer chain fatty acids (9,10). The latter system appears to be exclusively involved in MLD. It is not obvious from our knowledge of these systems however, how the fatty acids of one sphingolipid can be affected more than those of another. The fact that cerebroside sulfates have a fatty acid composition which most nearly approaches normal suggest that this lipid class accumulates in MLD via a compensatory synthesis since it is the only lipid in MLD containing appreciable proportions of fatty acids with 21-26 carbons.

TABLE 3

COMPARISON OF CONCENTRATIONS OF SPHINGOLIPIDS IN WHITE MATTER IN MLD AS A FUNCTION OF FATTY ACID CHAIN LENGTH

	SPHINGOMYELIN		CEREBROSIDE		CEREBROSIDE SULFATE	
FATTY ACID	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2
14:0	23	6	54	27	5	30
16:1	96	28	185	118	543	245
16:0	92	72	294	167	643	141
18:1	175	47	613	160	267	152
18:0	98	118	206	91	754	304
20:0	59	68	77	19	86	353
21:0	--	--	92	44	--	--
22:1	15	--	15	19	--	--
22:0	38	28	10	8	364	212
23:1	12	--	60	20	380	--
23:0	37	18	16	5	233	140
24:1	39	14	10	5	169	81
24:0	31	13	10	15	267	137
25:1	47	12	10	8	176	97
25:0	64	--	5	--	263	84
26:1	--	21	10	2	241	113
14-20	82	75	232	97	456	194
21-26	30	14	13	8	206	102

Values are expressed as percent of the normal concentration for each compound in white matter. Only unsubstituted fatty acids are included. The hydroxy acids of cerebrosides and cerebroside sulfates showed a similar decline with increasing chain length.

The pathogenesis of MLD may be explained as follows. One of the major forces involved in the cohesion of membranes is that due to carbon-carbon interactions (11). Fatty acids in complex lipids are major sources of this force since they present a large surface of available carbon atoms for interaction with adjacent molecules. Long chain fatty acids impart greater stability than short chain acids since the longer the carbon chain, the larger the number of interactions and the greater the cohesion. In addition, only saturated fatty acids with 18 carbons or more are long enough to extend across the center of the myelin bimolecular leaflet and interdigitate with the fatty acids of lipids in the opposite layer (12). This cross-linking of one side of the bimolecular leaflet with the other provided by very long chain fatty acids may help to stabilize the myelin membrane.

We postulate that myelin is unique in its requirement for sphingolipids containing very long chain fatty acids since it is a uniquely stable membrane (13,14), and that sufficient quantities of sphingomyelins and cerebrosides containing these fatty acids must be synthesized before stable myelin can be made. This hypothesis is supported by the findings that these lipids containing very long chain fatty acids are localized almost exclusively to white matter (Table 3) and more specifically to myelin isolated by ultracentrifugation from white matter (15). In MLD, deficient synthesis of cerebrosides and sphingomyelins containing very long chain fatty acids leads either to cessation of myelination or to formation of unstable myelin.*

It is our prediction that the sphingolipid fatty acid chain elongation system is also effected in other disorders of myelin formation and breakdown. In confirmation of this point we have recently found a marked deficiency of sphingolipids containing long chain fatty acids in white matter from Niemann-Picks disease.

Addendum. It has been brought to the author's attention that Svennerholm has recently reported a deficiency of long chain fatty acids from frontal lobes in two cases of MLD, one case of globoid-cell leucodystrophy and one case of infantile Gauchers disease (16).

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*Analysis of myelin isolated from MLD by ultracentrifugation (now in progress) may shed further light on this point.

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