Morphological and neurochemical study of Pelizaeus-Merzbacher disease¹

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Of the various types of demyelinating disorder, Pelizaeus-Merzbacher disease (Pelizaeus, 1885; Merzbacher, 1910) is probably the rarest and least understood form. For while its genetic, clinical, and patho-anatomical features have been fairly well delineated, the underlying biochemical disturbance thus far has been only incompletely analysed. Consequently the pathogenesis of the disorder has remained unclear and controversial.

Three main hypotheses have been advanced as to the manner in which the changes are produced, viz., through destruction or removal of myelin, similar to that in multiple sclerosis (Spielmeyer, 1923); through faulty or incomplete formation of myelin that undergoes progressive degeneration and thus is considered to be a form of leucodystrophy (Bielschowsky and Henneberg, 1928; Poser and van Bogaert, 1956; Seitelberger, 1963); and through arrest of myelination at an early age (Blackwood and Cumings, 1954; Zeman, Demyer, and Falls, 1964).

The purpose of this investigation is to report the patho-anatomical findings in a case of Pelizaeus-Merzbacher disease, and through a detailed chemical analysis of the fixed brain tissue, contribute to the chemical pathology of the disease.

CASE REPORT

HISTORY R.F., a 13-year-old white boy, had a normal birth with the exception of bilateral congenital cataracts. His birth weight was 6 lb. 2 oz. and his height was 20 inches. Up to the age of 6 months his development proceeded normally and by that time he more than doubled his birth weight, weighing 14 lb. 12 oz. But from the age of 6 months on he showed signs of increasing physical and mental retardation. His body weight and height remained virtually stationary so that by the age of 3½ years his weight was only 17 lb. and height 29 inches. There was a marked degree of microcephaly. He displayed no signs of intelligence or interest in his surroundings, had no sense of balance, did not learn to walk or talk, and gradually

developed marked contractures of the lower extremities (further details of his clinical course were lacking). He suffered frequently from upper respiratory infections and ultimately expired of bronchopneumonia.

The family history showed that an older brother suffered from the same condition and died at the age of 22 years (there is, however, no record of a necropsy having been performed). A sister, age 15, is living and is apparently normal.

POST-MORTEM FINDINGS The general necropsy findings were bilateral bronchopneumonia and pulmonary oedema; hypoplasia of the thyroid and pituitary glands; undescended atrophic testes, and general underdevelopment of the skeleto-muscular system.

The brain weighed 500 g., being reduced by more than 50% of normal size (Fig. 1). The cerebral hemispheres were symmetrical and their convolational pattern was normally outlined, showing no undue evidence of atrophy. The brain-stem was proportionately small but the cerebellum was diffusely and severely atrophied. The optic nerves were also bilaterally atrophic, especially the one on the right.

Coronal sections (Fig. 2) disclosed a normal amount of grey but a marked reduction of the white matter which exhibited a mottled greyish-white appearance. Both the central and gyral white matter was bilaterally and equally involved throughout all the lobes. There was also atrophy of the corpus callosum, anterior commissure, internal and external capsules, cerebral peduncles, the base of the pons, and the medullary pyramids. By contrast, the basal ganglia appeared normal with the exception of moderate atrophy of the thalamus and brownish pigmentation of the globus pallidus. In the brain-stem, the normal appearance of the tecto-tegmental structures contrasted with atrophy of the base. The most severe and widespread atrophy was encountered in the cerebellum, where both the grey and white matter was equally involved. The entire ventricular system was widely and symmetrically dilated. (The spinal cord was not available for examination.)

Microscopically, the grey matter of the entire brain showed a normal cytoarchitecture and, with the exception of terminal oedema, there were no significant alterations in either the nerve cells, myelin content, or glial elements. The only abnormalities in the grey matter were severe and widespread degeneration of the Purkinje

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FIG. 1. External appearance, showing small size of brain, and severe atrophy of cerebellum as well as of optic nerves, especially on the right side.

FIG. 2. Coronal section, showing diffuse reduction of all parts of the white matter that has a vaguely mottled appearance.

FIG. 3. Section of cerebrum, showing tigroid pattern of demyelination because of preserved islands of myelin: Luxol fast blue-periodic-acid Schiff stain.

FIG. 4. Histology of demyelinating process showing demyelinated area (above), containing a few oligodendroglia and scattered fibrillary astrocytes contrasting with normal myelin island (below) in which the normal number of oligodendroglia is present. Luxol fast blue-periodic acid Schiff stain $\times 150$.

and granular cells of the cerebellum with secondary degeneration of mild to moderate degree in the inferior olives, dentate nuclei, and thalamus. The globus pallidus contained moderate amounts of calcareous deposits in the walls of blood vessels.

The principal histological change was noted in the white matter (Fig. 3). It took the form of irregularly alternating foci of demyelination and normal myelination, the latter inconstantly related to blood vessels, resulting in a striking tigroid appearance. This was most conspicuous in the white substance of the cerebral hemispheres of all lobes, but was also present in the corpus callosum, anterior commissure and fornix; the capsules and cerebral peduncles; the more densely myelinated parts of the basal ganglia, such as the thalamus and globus pallidus (Fig. 4); the brain-stem and cerebellum, where there were often admixtures of the primary changes with secondary tract degeneration.

The demyelinating foci (Fig. 5) were characterized histologically by paucity of oligodendroglia and mild proliferation of fibrillary astrocytes in cresyl violet preparations, by dense gliosis in Holzer preparations, and by relative preservation of axis cylinders with the Bielschowsky method. Microglial and gitter cell reaction was minimal and inflammatory response was lacking.

The staining affinities of the myelin breakdown products were as follows: Sudan IV showed rare sudanophilic droplets, diffusely scattered in the demyelinating foci and

in perivascular spaces of both the latter and the myelinated areas. Sudan black gave a negative reaction. With P.A.S. there was a positive reaction in granules of the few gitter cells and reacting astrocytes. Alcian blue gave a negative reaction, as did metachromasia.

SUMMARY OF CLINICOPATHOLOGICAL FINDINGS

Clinically, the case reported here presented the classic features of the disease, despite the meagre data, namely, the familial background and the apparent sex-linked inheritance, occurring in two male siblings; the early onset at the age of 6 months; and the chronic course. In addition, the clinical signs of microcephaly, dwarfism, mental retardation, and progressive development of contractures, although in themselves not pathognomonic, have been reported in other cases.

The case exhibited the characteristic pathology of Pelizaeus-Merzbacher disease, namely, the widespread involvement of the white matter, the tigroid pattern of demyelination, the absence of abnormal decomposition products, the sudanophilia, though mild, of myelin breakdown, the intense gliosis and the preservation of axis cylinders. The only significant degeneration of grey matter was noted in the cerebellum, a finding common to many of the leukodystrophies and most commonly attributed to the associated convulsive disorder, not known in this case because of the incomplete history.

NEUROCHEMICAL STUDIES

MATERIALS AND METHODS The brain tissue had been fixed in formalin for one year. The most important formalin-induced chemical changes are the loss of phospholipids, particularly plasmalogens, and an increase in water content (Davison and Wajda, 1962; Rodnight, 1957). Plasmalogens, therefore, were not estimated in this investigation.

Grey and white matter were dissected carefully. In view of the smallness of the myelinated areas in the atrophic white matter, it was not feasible to separate grossly myelinated from non-myelinated areas. Extraction of formalin-fixed tissues by the Folch method is not as complete as with fresh material. The Folch extraction (Folch, Lees, and Sloane Stanley, 1957), therefore, was followed by a Soxhlet extraction with Bloor's mixture and subsequently with chloroform-methanol, each for six hours. This accomplished near complete removal of lipids as evidenced by the additional yield of only 0.12-0.15% lipid phosphorus on further Soxhlet treatment of specimens of control no. 1 and the Pelizaeus-Merzbacher brain.

Water content, lipid phosphorus, carboxyl esters, total fatty acids, and cholesterol were estimated as in previous investigations (Gerstl, Tavaststjerna, Hayman, Smith, and Eng, 1963) and galactolipids and sulphatides by the methods of Radin, Brown, and Lavin (1956) and Davison and Gregson (1962), respectively. The procedures for

separation and quantification of hydroxy fatty acids have been reported in detail elsewhere (Eng, Lee, Hayman, and Gerstl, 1964). A relatively large amount of free fatty acids was present in all formalin-fixed samples, as found on thin-layer chromatography. These were extracted with petroleum-ether:diethylether 60:40 and, for the purpose of gas liquid chromatography, methylated. Identification and quantification of individual fatty acids was accomplished by gas liquid chromatography as described earlier (Gerstl *et al.*, 1963). Brain proteins were estimated by determination of total amino-acids with ninhydrin after hydrolysis with 6N HCl according to the method of Rosen (1957).

Regrettably, a control brain of similar age could not be obtained. Thus, two brains of patients, aged 52 and 62, which also had been kept in formalin for one year, were used as controls. For the purpose of comparison, chemical data on two unfixed brains from normal children, 18 and 48 months of age, were also obtained.

RESULTS Lipid data on various areas of cortex of the Pelizaeus-Merzbacher brain, two adult and two children's brains, are given in Table I. The water content of the formalin-fixed was slightly higher than that of fresh material, but was similar in the Pelizaeus-Merzbacher and the control brains. Likewise, lipid phosphorus, carboxyl esters, and total cholesterol did not exhibit any difference between the Pelizaeus-Merzbacher and the adult control brains. The total lipids, including the free fatty acids, in the Pelizaeus-Merzbacher case, were slightly less than in the controls. The amount of cerebrosides, however, on the average, was less than one-half of that in the adult control brains. Since it has been established that cerebrosides and cholesterol are not altered during formalin fixation over a period of two years (Rodnight, 1957), the data for cerebrosides represent true values. The total hydroxy fatty acids in two samples of the Pelizaeus-Merzbacher grey matter were about

one-half of the amount found in the adult controls; in the third specimen, even less. This corresponds to the low cerebroside content.

The amounts of lipid phosphorus, carboxyl esters, and total fatty acids found in the grey matter of the two unfixed children's brains were greater than those in the adult and Pelizaeus-Merzbacher brains, which may be due to absence of fixation. As to compounds not affected by formalin, the cerebrosides and cholesterol in these three brains were present in similar concentrations. Sulphatide levels in the Pelizaeus-Merzbacher brain and in the 18-month-old child's brain were similar and one-half that in the cortex of the adult brain no. 1.

In the white matter of the Pelizaeus-Merzbacher brain, the water content was higher (Table II) and the amount of lipid phosphorus, cholesterol, carboxyl esters, and total fatty acids was about one-third of that in the adult controls; that of the cerebrosides, including the sulphatides, and of the total hydroxy fatty acids, however, was only one-fifth, or less. The concentration of cholesterol, cerebrosides, and sulpholipids was also diminished when compared with that found in the two children's brains.

The analysis by gas liquid chromatography of the regular fatty acids of the grey matter (Table III) revealed little if any difference between the Pelizaeus-Merzbacher and the control brains as far as the saturated acids are concerned. The amounts of mono-unsaturated acids of chain length C₂₀ to C₂₆ were, however, one-half the amount found in the controls.

In the white matter (Table III) all types of fatty acids were at levels lower than in the two controls, and this was particularly marked in the instance of the long chain mono- and polyunsaturated acids.

TABLE I

LIPIDS OF GREY MATTER¹

Brain	Water (%)	Lipid Phosphorus	Carboxyl Esters	Fatty Acids	Cholesterol	Galactose	Sulphatides	Total 2-Hydroxy Fatty Acids
<i>Pelizaeus-Merzbacher</i>								
Frontal lobe	87.0	2.74	3.54	5.06	2.05	0.12	0.039	0.08
Parietal lobe	85.9	2.94	3.73	6.16	2.32	0.23	0.074	0.21
Occipital lobe	85.5	2.76	3.94	5.79	2.21	0.20		0.18
<i>Control no. 1</i>								
Frontal lobe	84.8	3.17	4.72	7.78	2.33	0.52	0.13	0.35
Parietal lobe	84.8	3.25	4.79	8.10	2.61	0.54		0.40
<i>Control no. 2</i>								
Frontal lobe	86.2	2.42	3.31	6.26	1.91	0.43	—	—
Temporal lobe	84.0	2.77	4.14	7.40	2.50	0.88	—	0.33
Occipital lobe	84.6	2.92	3.78	7.43	2.38	0.65	—	0.48
<i>17 months</i>	83.4	4.51	6.79	8.20	2.58	0.37	0.62	—
<i>48 months</i>								
Frontal lobe	86.2	4.00	6.96	8.48	2.08	0.18	—	—
Temporal-parietal lobe	84.5	4.03	6.97	8.30	2.12	0.13	—	—
Occipital lobe	85.0	4.25	7.18	9.29	2.30	0.16	—	—

— Not done

¹Expressed in mM/100 g. wet weight

TABLE II

Brain	Water (%)	LIPIDS OF WHITE MATTER ¹					Galactose	Sulphatides	Total 2-Hydroxy Fatty Acids
		Lipid Phosphorus	Carboxyl Esters	Fatty Acids	Cholesterol				
<i>Pelizaeus-Merzbacher</i>									
Frontal lobe	86.6	2.66	3.75	6.12	2.53	0.79	—		0.36
Temporal lobe	84.7	3.08	4.27	7.69	3.44	1.29	0.21		0.54
Occipital lobe	84.4	2.96	4.36	7.07	3.07	0.83			0.45
<i>Control no. 1</i>									
Frontal lobe	71.5	8.11	9.90	20.20	8.59	5.19	0.97		2.18
Parietal lobe	71.3	8.21	9.98	20.09	9.99	4.87	—		2.45
<i>Control no. 2</i>									
Frontal lobe	70.8	8.18	9.95	22.90	10.33	5.63	1.07		2.66
Temporal lobe	71.2	8.01	9.68	22.55	10.35	5.32	1.05		2.59
Occipital lobe	70.7	7.32	9.21	21.50	9.14	5.58	1.14		2.88
<i>18 months</i>	71.4	8.98	11.60	19.86	9.39	3.59	0.33		0.98
<i>48 months</i>									
Frontal lobe	77.3	8.08	11.88	17.02	8.34	3.17	—		1.50
Temporal-parietal lobe	74.8	9.54	13.14	20.65	10.03	5.07	0.63		1.68
Occipital lobe	75.3	8.86	12.73	18.69	9.18	3.72	—		1.66
— Not done									

¹Expressed in mM/100 g. wet weight

TABLE III

Brain	FATTY ACIDS IN WHITE AND GREY MATTER ¹					PUFA	Odd Total
	12:0—18:0	20:0—26:0	14:1—18:1	20:1—26:1			
<i>Grey matter</i>							
<i>Pelizaeus-Merzbacher</i>							
Frontal lobe	3.19	0.02	1.39	0.03	0.29		0.03
Temporal lobe	3.90	0.03	1.60	0.07	0.29		0.06
Occipital lobe	3.59	0.03	1.55	0.07	0.26		0.10
<i>Control no. 1</i>							
Frontal lobe	4.29	0.05	2.15	0.18	0.88		0.09
Parietal lobe	4.21	0.06	2.13	0.16	0.77		0.06
<i>Control no. 2</i>							
Frontal lobe	3.46	0.03	1.32	0.11	0.15		0.10
Parietal lobe	4.57	0.04	2.03	0.12	0.23		0.11
Occipital lobe	4.73	0.06	1.59	0.15	0.31		0.10
<i>White matter</i>							
<i>Pelizaeus-Merzbacher</i>							
Frontal lobe	3.95	0.08	1.79	0.18	0.28		0.07
Temporal lobe	3.70	0.24	2.04	0.55	0.42		0.19
Occipital lobe	3.42	0.08	1.83	0.24	0.24		0.10
<i>Control no. 1</i>							
Frontal lobe	6.90	0.52	6.14	2.56	1.57		0.38
Temporal lobe	7.19	0.41	6.93	2.49	1.88		0.72
<i>Control no. 2</i>							
Frontal lobe	7.81	0.51	7.15	2.65	1.66		0.52
Temporal lobe	8.08	0.60	6.88	2.47	1.22		0.64
Occipital lobe	8.26	0.45	6.35	2.38	0.69		0.54

¹Expressed in mM/100 g. wet weight

Small amounts of free fatty acids have been found in fresh brain tissue (Rouser, Galli, Kritchevsky, Lieber, and Heller, 1964; Rowe, 1964), but the presence of large amounts of free fatty acids is apparently the result of the exposure to formalin (Jatzkewitz, 1964). They amounted to 20 to 30% of the total fatty acids in the grey matter of the Pelizaeus-Merzbacher brain, and 26 to 33% in the controls; the corresponding figures for the white matter were 17.0 to 21.5%, and 18.5 to 21.5% for the Pelizaeus-Merzbacher and control brains, respectively. Also, the percentage distribution of the individual acids did not reveal any significant difference.

Gas liquid chromatography of the hydroxy fatty acids (Table IV) revealed a higher percentage of C16:0 and C18:0 acids in the grey matter of the Pelizaeus-Merzbacher brain than in the controls, while the distribution of the longer chain acids was similar and corresponds to that given by O'Brien, Fillerup, and Mead (1964) for those of the combined cerebroside and sulphatides. No essential difference in the percentage distribution was observed for the white matter (Table V).

Quantitative estimation of proteins revealed a slightly lower protein content of the Pelizaeus-Merzbacher brain (Table VI).

TABLE IV

PERCENTAGE COMPOSITION OF HYDROXY FATTY ACIDS IN GREY MATTER¹

Fatty Acid	Adult Brain Formalin-fixed				13-Year Brain Formalin-fixed		
	Control no. 1		Control no. 2		Pelizaeus-Merzbacher		
	Total Grey	Frontal Lobe	Temporal Lobe	Occipital Lobe	Frontal Lobe	Temporal Lobe	Occipital Lobe
14:0	—	—	—	—	—	—	—
15:0	0.4	0.9	0.7	1.3	—	1.0	—
15:1	0.6	0.6	0.7	0.8	—	1.4	0.5
16:0	0.3	0.5	0.4	—	3.8	0.9	6.7
16:1	—	—	—	—	5.7	—	—
17:0	0.2	0.6	0.4	0.5	0.9	0.5	0.5
17:1	0.4	0.3	0.2	0.3	2.7	0.2	5.4
18:0	1.3	1.5	1.5	1.6	4.3	3.3	3.7
18:1	—	0.2	0.4	0.3	—	0.5	—
19:0	1.2	3.4	3.1	2.6	0.5	3.2	5.4
20:0	0.6	1.4	0.9	1.0	0.8	1.9	2.7
20:1	0.2	0.2	0.4	0.4	—	0.3	—
21:0	0.2	0.2	0.4	0.3	—	0.8	—
21:1	—	0.3	0.4	0.4	1.1	0.1	—
22:0	7.1	7.4	7.4	7.2	6.4	8.0	7.1
22:1	0.6	0.4	0.1	—	0.7	0.6	0.7
23:0	17.1	15.7	16.1	14.9	5.5	14.8	14.7
23:1	1.4	0.9	1.5	1.3	3.5	1.5	1.4
24:0	29.7	29.5	32.2	27.0	30.6	31.9	26.9
24:1	22.1	19.7	17.7	21.9	11.1	15.7	9.8
25:0	5.6	5.7	6.1	5.6	5.1	5.2	5.1
25:1	5.3	5.2	4.8	5.4	4.7	3.7	3.4
26:0	1.0	1.5	1.4	1.1	0.6	1.0	1.6
26:1	4.1	3.6	3.1	4.4	3.4	3.1	4.2

— Not found

¹Expressed in Mole %

TABLE V

PERCENTAGE COMPOSITION OF HYDROXY FATTY ACIDS IN WHITE MATTER¹

Fatty Acid	Adult Brain		Fresh 17-Month Brain	Fresh 48-Month Brain	Pelizaeus-Merzbacher Brain Formalin-fixed
	Fresh	Formalin-fixed			
14:0	—	—	—	0.5	Trace
15:0	—	Trace	1.1	Trace	0.4
15:1	—	Trace	—	Trace	0.3
16:0	Trace	Trace	—	0.2	0.2
16:1	—	—	—	Trace	—
17:0	0.2	0.1	0.5	0.1	0.3
17:1	—	Trace	0.6	Trace	0.5
18:0	1.0	1.0	1.4	0.9	1.5
18:1	—	—	0.7	Trace	—
19:0	Trace	0.1	0.7	Trace	1.5
20:0	0.3	0.2	0.5	0.4	0.6
20:1	—	—	—	—	0.2
21:0	0.1	Trace	0.4	Trace	0.7
21:1	0.4	Trace	0.2	0.3	0.2
22:0	6.2	7.2	11.7	8.0	8.7
22:1	0.2	0.2	—	0.4	0.3
23:0	15.7	17.0	13.1	12.8	13.5
23:1	0.8	0.5	0.4	0.6	0.9
24:0	40.2	36.9	49.1	47.9	33.8
24:1	19.6	23.4	11.5	14.8	17.7
25:0	6.6	5.4	3.5	5.3	6.6
25:1	3.5	3.2	0.9	1.9	4.4
26:0	1.2	1.2	0.6	1.7	2.2
26:1	4.4	3.5	2.3	4.2	4.8

— Not found

¹Expressed in Mole %

DISCUSSION

PATHO-ANATOMICAL FEATURES For a detailed account of the historic development of the concept of Pelizaeus-Merzbacher disease, the reader is referred

to the recent paper by Zeman *et al.* (1964). However, certain questions raised by the latter deserve further comment.

Following the consideration by Bielschowsky and Henneberg (1928) of Pelizaeus-Merzbacher disease

TABLE VI
PROTEIN CONTENT OF FORMALIN-FIXED BRAIN¹

Brain	White Matter	Grey Matter
Pelizaues-Merzbacher		
Frontal lobe	9.3	—
Temporal lobe	9.6	9.1
Occipital lobe	10.3	9.6
Control no. 1	12.3	10.6
Control no. 2	10.2	11.6
— Not done		

¹Expressed in g./100 g. wet weight

as a form of leucodystrophy, implying faulty myelin metabolism, the condition has been classified as a 'neutral fat' leucodystrophy (Poser and van Bogaert, 1956) or as a 'sudanophil' leucodystrophy (Seitelberger, 1960; Norman, 1962; Peiffer, 1962). Under the latter designation, a heterogeneous collection of conditions with varied clinical, genetic, and neuropathological findings has been included. Thus, Peiffer (1962) classified four different subtypes: (1) simple orthochromatic leucodystrophy; (2) classical Pelizaues-Merzbacher disease; (3) Seitelberger type; and (4) adult type, which was first described by Löwenberg and Hill (1933). The criteria of a leucodystrophy were present in all, namely, a diffuse, non-focal degenerative familial disorder but, in contrast to other leucodystrophies, the myelin breakdown involved normal (sudanophil) decomposition products. Our case resembled the classical Pelizaues-Merzbacher form in its genetic, clinical, and morphological aspects.

Zeman *et al.* (1964) have objected to the designation of the disorder as a sudanophil leucodystrophy. In their opinion, the process is substantially a disturbance of myelinogenesis taking place early in life, probably before birth. They differ, however, from Blackwood and Cumings (1954) who proposed that myelination is arrested at a chronological age of 2 years. Relying on their findings in three cases of the Seitelberger type, dying in early childhood, Zeman *et al.* (1964) point to the complete lack of myelin stainability in their cases as evidence of faulty myelination rather than demyelination. They see in this an expression of an intense genetic defect operating in cases of younger age. By contrast, the cases dying during adult life show the characteristic patchy lack of myelin stainability as a result of a probable milder genetic defect. On the basis of these observations, the authors would exclude a number of cases reported in the literature that differ from theirs in genetic, clinical, or patho-anatomical manifestations. Such a view, in our opinion, would seem unwarranted, especially since our knowledge of the nature of the metabolic defect is still inadequate. In agreement with others, we feel that the sparse sudanophil breakdown products merely reflect the unusually slow progress of the degenerative process

and this is substantiated by the intense reactive gliosis.

BIOCHEMICAL FEATURES In the grey matter of the Pelizaues-Merzbacher brain (Table I), the concentration of cerebrosides including sulphatides, as well as the mono-unsaturated long chain fatty acids, was reduced. This was paralleled by a deficit of hydroxy fatty acids and indicates that, in contrast to the findings in 'normal areas' of multiple sclerosis, the phrenosine-type of cerebrosides is afflicted in Pelizaues-Merzbacher disease. Blackwood and Cumings (1954) also reported a deficit of cerebrosides, but their findings of reduced concentrations of cholesterol and phospholipids could not be corroborated.

In the white matter (Table II), there was a marked deficit of all lipids; however, that of the cerebrosides exceeded the others. It should be noted that the control tissues contained approximately 15% less water so that on a dry weight basis the differences in the values would be correspondingly less, however, not detracting from the significance of the altered lipid spectrum. The findings on the white matter are at variance with those of Zeman *et al.* (1964), who stated that relatively high concentrations of cerebrosides and cholesterol are found in Pelizaues-Merzbacher white matter. They also called attention to the lack of correspondence between myelin stainability and amounts of lipids still present. This may be explained by the fact that only about 50% of the total lipids are components of myelin proper (Autilio, Norton, and Terry, 1964; Gerstl, Tavaststerna, Hayman, Eng, and Smith, 1965).

If destruction and removal of myelin were the only processes underlying the pathology of Pelizaues-Merzbacher disease, the amounts of lipids absent from the tissue should reflect their proportions in purified myelin. These have been reported as 47, 57, and 57.6%, respectively, for phospholipids, cholesterol, and galactolipids (Autilio *et al.*, 1964). A similar point can be made concerning the unsubstituted fatty acids, the long chain members being deficient to a greater extent than those of C₁₄ to C₁₈ chain length. These observations would be also difficult to reconcile with arrest of myelination as assumed by Blackwood and Cumings (1954). Seitelberger's hypothesis (1960, 1963) of an inherent disorder of glycerophosphatides could not be tested since those compounds were largely destroyed by formalin fixation.

The metabolism and synthesis of the fatty acid and galactose moieties of the cerebrosides have been fairly extensively studied. Fulco and Mead (1961) came to the conclusion that C₂₄:0 is the precursor of cerebronic acid, while Bernhard, Hany, Hausheer,

and Pedersen (1962) suggested that alpha-hydroxy acids are not formed from unsubstituted precursors. The average percentage of lignoceric acid in five control samples of white matter was 1.81, but was only 0.89 (average) in the three specimens of the Pelizaeus-Merzbacher white matter. The percentage of cerebronic acid, however, was similar in both Pelizaeus-Merzbacher and control specimens. Thus, the data presented here would support the suggestion of Bernhard *et al.* (1962).

As to the pathogenesis of Pelizaeus-Merzbacher disease, one would have to postulate that the synthesis of cerebroside, including that of both substituted and non-substituted long-chain fatty acids, as well as the incorporation of galactose into the galactolipids by microsomes (Burton, Sodd, and Brady, 1958), are impaired. The cerebroside with their long-chain fatty acids are probably essential for the intactness and cohesiveness of the myelin structure (Vandenheuevel, 1963; O'Brien, 1964). Their near absence in Pelizaeus-Merzbacher disease could well be one of the factors initiating or permitting additional changes, including removal of other lipids, *e.g.*, phospholipids and cholesterol, inadequately incorporated into the myelin structure.

SUMMARY

The clinicopathological findings in a case of Pelizaeus-Merzbacher disease are reported and the literature is discussed.

Chemical analysis of the formalin-fixed brain tissue revealed diminished concentrations of cerebroside and cerebroside sulphates in the grey matter. This was associated with a deficit of long-chain mono-unsaturated unsubstituted fatty acids. In the white matter there was a decrease of all lipids, but the depletion of the cerebroside, including sulphatides, and long-chain mono- and poly-unsaturated fatty acids exceeded that of other lipids. The amount of cerebroside in the Pelizaeus-Merzbacher brain was less than in that of the two brains of children, aged 18 and 48 months.

Pathogenetically, neither general arrest nor removal in myelin would account for all the changes. Inadequate or faulty synthesis of cerebroside as the primary chemical pathology would be the most satisfactory explanation.

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