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Peripheral Nerve Changes in the Diagnosis of Metachromatic Leucodystrophy

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Progressive brain disorders of degenerative type are often impossible to differentiate clinically during infancy and early childhood. As some of these diseases are caused by genetically determined inborn errors of metabolism but others are not, an early correct diagnosis has not only theoretical but also practical importance, especially for future family planning.

One of the leucodystrophies, the late infantile metachromatic type, now more adequately called sulphatide lipidosis, is possible to diagnose during life due to the fact that sulphatides are also accumulated in organs outside the central nervous system. Changes in urine and cerebrospinal fluid and at cholecystographic examination have been used in diagnostic tests [2, 7, 9, 10] but some are non-specific and others laborious to perform. Alternative diagnostic procedures, which can be used are histological examinations of biopsy specimens for the occurrence of accumulated metachromatic substances. In this paper a series of peripheral nerve studies made on 25 infants and children with different diffuse progressive neurologic disorders will be presented and discussed.

Material and Methods

Clinical material. The nerve specimens examined derive from 25 infants and children aged 5 months to 10½ years of age (Table I). Three of them were previously healthy and had died a sudden death due to suffocation (Cases 22 and 23) or road accident (Case 24). Case 25 also died a sudden death, was regarded as neurologically normal and is acceptable as a control. Of the other 21 not less than 8 were verified cases of metachromatic leucodystrophy. Cases 1, 2, 3, 4, 5 and 7 were verified at autopsy and Cases 6 and 8 were clinically diagnosed with characteristic urinary findings. In addition Case 6 is the younger sister of Case 5. Four cases of globoid cell leucodystrophy (type Krabbe) are included. Two of them, Cases 9 and 10, were verified at autopsy. Cases 11 and 12 are still alive. Each has had one elder sibling with the same type of disease, the diagnosis verified at autopsy as Krabbe's disease in the sibling of Case 11. The series also includes one case with a clinical diagnosis of Pelizaeus-Merzbacher's disease and one other patient still living (Case 14) probably also belonging to the leucodystrophy group. Cases 15 and 16 represent the spinal muscular atrophy of Werdnig-Hoffman. Case 15 was verified at autopsy; Case 16 was diagnosed clinically and showed characteristic electromyographic and conclusive muscular changes at biopsy. Two further cases (17 and 18) probably belong to the group of infantile

TABLE 1. *Clinical material.*

Case no.	Name	Sex and age (years)	Diagnosis	Autopsy	Heredity	Duration of disease (years)	Specimen examined	References (Case reports)
1	R. O.	♂ 3 1/4	Metachromatic leucodystrophy	+	+	2	Spinal roots	7, 8
2	K. E.	♂ 4		+	+	2 1/2		7, 8
3	A. L.	♀ 3 3/4		+	−	2 1/2	*n. suralis* + *ischiadicus*	7, 10
4	I. J.	♀ 3		+	−	1 1/4	Spinal roots	7
5	E. M.	♀ 6		+	+	5		7
6	I. M.	♀ 6		−	+	4	*n. suralis*	7, 10
7	R. A.	♂ 4		+	−	2 3/4	*n. suralis* +	7, 10
8	K. Ö.	♂ 5 3/4		−	−	4 1/2	*ischiadicus*	7
9	A. W.	♀ 5/12		+	−	2 1/2		
10	E. M-L.	♀ 1 3/4	Globoid cell leucodystrophy (Krabbe)	+	+	1 1/2		
11	M. S.	♀ 1 1/2		−	+	1 1/4	*n. suralis*	
12	C. J.	♀ 7/12		−	+	3/12		
13	L-H. L.	♂ 10 1/2	Chronic leucodystrophy (Pelizaeus–Merzbacher ?)	−	−	9		
14	M. Å.	♀ 2	Leucodystrophy (type ?)	−	+	3/4		
15	M. D.	♀ 9/12	Infantile spinal muscular atrophy (Werdnig–Hoffman)	+	−	4/12	*n. ischiadicus*	
16	L. L.	♀ 9		−	−	8		
17	T. B.	♂ 5 3/4	Infantile dementia of Heller (?)	−	−	3 1/4		
18	A-M. W.	♀ 3		−	−	2	*n. suralis*	
19	P-O. S.	♂ 8	Subacute encephalitis (?)	−	−	5/12		
20	K. W.	♂ 3 1/4		−	−	3/4		
21	K. J.	♀ 1 1/4	Postinfectious encephalopathy	−	−	5/12		
22	A-K. W.	♀ 4	Control	+		—	*n. ischiadicus*	
23	I. W.	♂ 2		+		—		
24	M. J.	♀ 8		+		—	*n. suralis*	
25	B. A.	♂ 1/12		+		—	*n. ischiadicus*	

dementia of Heller. As they are still alive, it has not yet been possible to differentiate them more accurately. Two other cases (19 and 20) presented clinical symptoms and signs and EEG-changes compatible with subacute encephalitis and Case 21 developed a severe encephalopathy related to an acute infection with repeated severe convulsions at 11 months of age.

Biopsy technique. The biopsy procedure ought to be carried out under general anesthesia to avoid local exogenous changes around the nerve, which should be examined. We have found the distal part of the sural nerve very suitable. It is easy to reach, contains only sensory nerve fibers and is large enough for appropriate studies. A longitudinal incision is made in the distal third of the calf in the dorsal median line, where the nerve is to be found along the lesser saphenous vein. Dissection is cautiously performed with preservation of the perineurium and adjacent structures. All traumatic handling of the nerve must be carefully avoided. Three to four cm of the nerve is then resected and cautiously removed without using forceps or other instruments. The specimen is fixed with two pin needles (preferably stainless) on the under-side of a piece of cork in a large pot with 10% neutral formalin.

Histological technique. Ten to fifteen micron-thick formalinfixed and frozen sections were cut longitudinally from the nerve specimens and stained by the following methods: cresyl violet—1% acetic acid according to v. Hirsch & Peiffer [11]—for metachromasia, the periodic acid-Schiff

TABLE 2. *Histological examination in 25 cases.*

Clinical diagnosis	Number of cases	Age in years	Specimen	Diffuse red meta-chromasia	Brown meta-chromatic deposits	Scarlet red positive
Metachromatic leuco-dystrophy	4	3 3/4–7	periph. nerve	3	4	0
	4	3–5	spinal roots	2	4	0
Globoid cell leuco-dystrophy	4	5/12–1 3/4	periph. nerve	4	0	0
Chronic leucodystrophy (Pelizaeus–Merzbacher?)	1	10 1/2	periph. nerve	1	0	0
Other leucodystrophy (?)	1	2	periph. nerve	1	0	0
Infantile spinal muscular atrophy (Werdnig–Hoffman)	2	3/4; 9	periph. nerve	0	0	0
Infantile dementia of Heller (?)	2	5 3/4; 9	periph. nerve	1	0	0
Other encephalopathies	3	1 1/4–8	periph. nerve	2	0	0
Normal infants and children	4	1/12–8	periph. nerve	4	0	0

(PAS) technique of McManus [20] for carbohydrates and Scarlet red for simple lipids. The first two methods were used on adjacent sections stained before and after extraction with methanol–chloroform (3:1) for two hours at 60°C. In a few cases unstained frozen sections were examined with polarization microscopy for demonstration of double refractile lipids. Additional stains used were Spielmeyers myelin method on frozen sections and on paraffin sections the Klüver-Barrera stain for myelin and Palmgren's silver technique for axons.

Results

The most important results of the histologic examinations are summarized in Table 2. Red metachromasia of the myelin sheaths was found in the four controls as well as in most of the cases with neurological disorders. However, brown metachromatic deposits were revealed exclusively in all cases with sulphatide lipidosis. The brown metachromatic, granular material was localized mainly to Schwann cells and phagocytic histiocytes and accumulated in clusters around capillaries of the endo- and perineurium. In one of the cases (Case 8, Fig. 1 and 2) this perivascular reaction was particularly pronounced. Occasionally brown metachromatic substances were also seen extracellularly, replacing degenerated myelin sheaths. After extraction with methanol-chloroform both the red and the brown metachromasia completely disappeared. This was in contradistinction to the purple metachromasia of the numerous mast cells, which was never affected by the extraction procedure. The granular material of the phagocytes stained faintly pink with Scarlet red. No Scarlet red positive material (triglycerides and cholesterol-esters) was detected in any of the nerves examined. In the phagocytes the periodic acid Schiff reaction according to McManus was positive before lipid extraction (Fig. 3) but had almost vanished after this procedure. Part of the granules enclosed in the phagocytes were double refractile when examined in

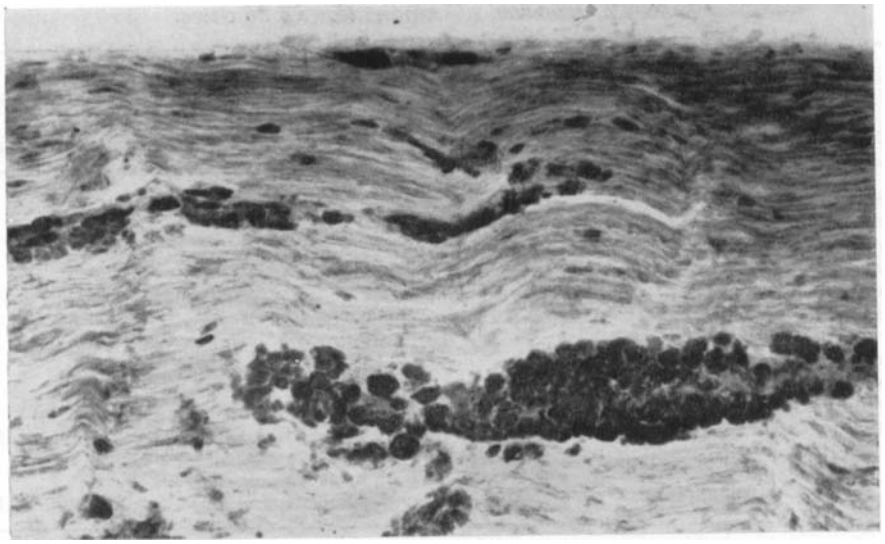

Fig. 1. Sural nerve preparation from a case of metachromatic leucodystrophy (Case 8). Metachromatic deposits are seen in Schwann cells and in numerous phagocytes. Cresyl violet–acetic acid. Low power view.

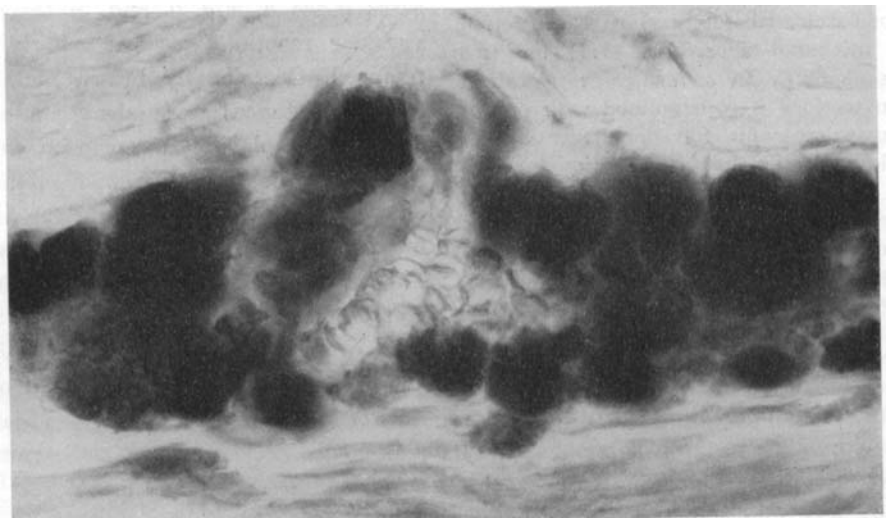

Fig. 2. The same preparation as in Fig. 1, demonstrating pericapillary accumulation of phagocytes loaded with metachromatic granular substance. Cresyl violet–acetic acid. High power view.

polarized light (Fig. 4). Marked demyelination (or defect myelination) was seen only in one of the four cases of metachromatic leucodystrophy (Case 3, Fig. 5) where biopsy specimens from the sural nerve had been obtained. On the whole, the axons were well preserved.

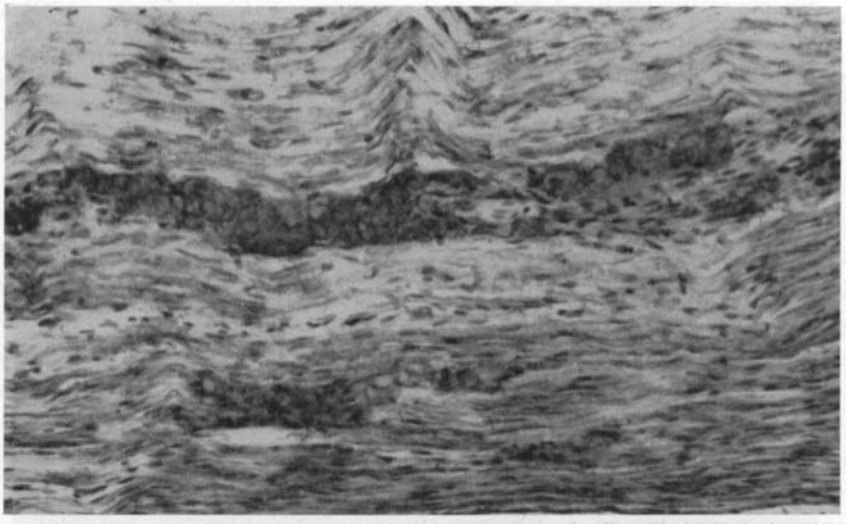

Fig. 3. PAS positive reaction of phagocytes in a case of metachromatic leucodystrophy. Low power view.

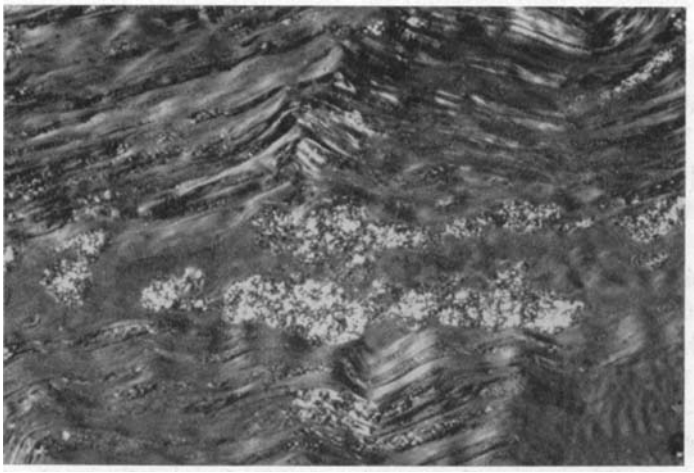

Fig. 4. Phagocytes containing double refractile granules in a case of metachromatic leucodystrophy. Polarization microscopy. Low power view.

Discussion

Histological aspects. The mere finding of demyelination (or defect myelination) of peripheral nerves cannot yield any diagnostic information of importance in metachromatic leucodystrophy. This change was found to be inconstant in our cases and probably depends on the stage of the disease in the particular case.

The demonstration of metachromatically stained lipid material in peripheral nerves is of considerable theoretical and

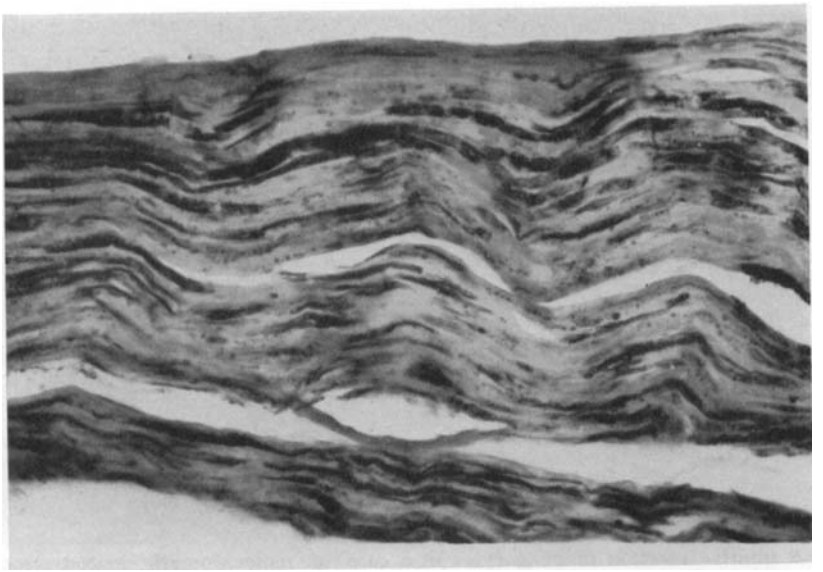

Fig. 5. Fragmentation and swelling of myelin sheaths of the sural nerve in a case of metachromatic leucodystrophy (Case 3). Spielmeyer. Low power view.

practical importance and a more thorough discussion is therefore required.

Feyrter [6] showed that frozen sections of formalin fixed normal myelin sheaths of peripheral nerves reveal an intense *red* metachromasia when stained with an aqueous mixture of thionin and tartaric acid. Using their very accurate and stable technique, v. Hirsch & Peiffer [11] demonstrated that the presence or absence of the red metachromasia of the myelin sheaths depends upon the pH of the staining solution. This is the probable explanation for the inconstant findings of diffuse red metachromasia in our series, where special precautions for a constant pH level were not found to be necessary for diagnostic purposes. In general it can be stated that diffuse red metachromasia has no diagnostic value.

According to v. Hirsch & Peiffer [11], *brown* metachromatic deposits in the cerebral white matter were constantly found in their cases with metachromatic leucodystrophy but in no other patients or controls examined. Our results show that the same also applies to the peripheral nerves. Much evidence has been presented that these brown metachromatic deposits characterizing metachromatic leucodystrophy are identical with sulphatides [3, 8, 15, 19]. As the presence of metachromatic granules in the Schwann cells always must be assessed very carefully (red staining metachromatic π-granules normally occur in Schwann cells after 8 years of age [16]; proliferating Schwann cells may show distinct metachromasia [12]), the demonstration of brown metachromatic granular deposits localized in perivascular phagocytes and soluble in lipid solvents seems to be the most reliable

and, as far as we know, pathognomonic change in peripheral nerves in cases of metachromatic leucodystrophy.

Numerous mast cells do normally occur in the endo- and perineurium and especially perivascularly [16]. Holmgren & Wilander [13] showed that the intense red metachromasia of these cells was attributed presumably due to heparin and was resistant to extraction with alcohol. In cases of metachromatic leucodystrophy the mast cells can easily be distinguished from the perivascular phagocytes.

Clinical application. In spite of a limited clinical series of other progressive brain disorders than leucodystrophies this investigation still indicates that metachromatic leucodystrophy can be distinguished from other degenerative diseases of the central nervous system by histological examination of biopsy specimens from a peripheral nerve. No other diagnostic conclusions can be drawn from our series.

Three types of biopsies have previously been used to establish a diagnosis of sulphatide lipidosis during life. Blackwood & Cumings [5] made cortical biopsies in seven cases, all diagnosed by chemical methods but three failing with a histological technique. Austin [2] performed renal biopsy which revealed characteristic histological changes. Metachromatic deposits in a peripheral nerve was observed by Jacobi [14] in her case examined at autopsy and her findings were later confirmed in other cases also at autopsy [4, 18, 19]. Thieffry & Lyon [22] were the first who studied a peripheral nerve (*N. musculocutaneus*) from a living patient. The diagnosis was verified by cortical biopsy examination. In other neural lipidoses, Nakai & Landing [17] used rectal biopsies with histochemical examination of the myenteric plexus and found this method to be useful and safe.

Weakness and valgus deformity of the foot and unsteady gait combined with diminished or absent deep reflexes are common and early signs in sulphatide lipidosis [7, 9], so that dysfunction of the second neuron seems to be present early in the disease. The chance of a positive result from a peripheral nerve biopsy thus seems to be large even in the early stages of the disease. In addition sural nerve biopsy is easy to perform and much less serious for the child than cortical or renal biopsy. More suitable nerve specimens for examination of myelin sheaths are obtained than with rectal biopsies.

The microscopic examination of sediments according to Austin [2] is a simple screening test but not reliable enough [9, 10]. Chemical analysis of urine for sulphatides gives accurate information and seems to be specific [9, 10] but is somewhat more complicated methodologically. Alternative diagnostic methods to the urinary tests are needed in practical clinical work, which was pointed out by Dunn in a recent discussion [1]. In clinically well selected cases, sural nerve biopsy seems to be a valuable diagnostic adjunct supplying this want.

Summary

A histopathological study was made on peripheral nerves and spinal roots from biopsies and autopsies of 8 cases with metachromatic leucodystrophy (sulphatide lipidosis), 4 cases of globoid cell leucodystrophy (Krabbe's disease), 9 cases

with other progressive neurological disorders of infancy and childhood and 4 controls. The sural nerve was found to give satisfactory biopsy specimens and a suitable biopsy technique is described. At histological examination, brown metachromatic deposits were revealed by the cresyl violet-acetic acid method of v. Hirsch & Peiffer in all cases with metachromatic leucodystrophy, but were never observed in any of the other cases examined. The granular metachromatic material was localized mainly to Schwann cells and histiocytes which were accumulated in clusters around capillaries of the endo- and perineurium. For diagnostic purposes the most reliable and so far pathognomonic change in peripheral nerve biopsy specimens was considered to be the demonstration of brown metachromatic granular deposits in perivascular phagocytes. It is concluded that histological examination of peripheral nerves can serve as a useful supplementary tool in the diagnosis of this genetically transmitted degenerative disorder during life.

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