ULTRASTRUCTURAL FINDINGS OF PERIPHERAL NERVE IN A
PRECLINICAL CASE OF ADULT METACHROMATIC
LEUKODYSTROPHY

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ABSTRACT

In a 13-year-old neurologically healthy boy from a family with adult-onset of metachromatic leukodystrophy (MLD) showing arylsulfatase A-deficiency in the adult, sural nerve biopsy probably was performed 2–3 decades before clinical manifestation of the disease could be expected. Ultrastructurally 4 basic types of inclusion bodies in Schwann cells could be demonstrated (pleomorphic “zebra body”-like inclusions, double-lamellated inclusions, “tuffstone”-like inclusions, granular osmiophilic inclusions). Additionally, endoplasmatic reticulum, mitochondria and lysosomes showed marked alterations. Advanced damage of myelin was only rarely seen, but initial segmental demyelination was a common finding. These early pathological changes in chronic MLD are thought to represent a subcellular metabolic insufficiency of Schwann cells in this disease.

INTRODUCTION

Metachromatic leukodystrophy is a familial progressive demyelinating disorder of the nervous system caused by a cerebrosidesulfatase- or arylsulfatase A-deficiency in the catabolism of sulfatides (4, 22). Metachromatic granules and demyelination in the central and peripheral nervous system are prominent morphological features. Jatzkewitz (18) and Austin et al. (4) could confirm that the metachromatic particles represent sulfated cerebrosides derived from ordinary myelin sheaths.

Since metachromatic granules can be detected in peripheral nerves, first reported by Jacobi (17), sural nerve biopsy is a routine procedure today in confirming morphologically the clinical diagnosis of MLD.

Previously, morphological findings in patients with the late infantile, juvenile and adult type of MLD were described, who had already shown manifest neurological signs (ref. see Pilz, 31).

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Multidisciplinary investigations of a family (case 2) with adult-onset MLD (27) prompt us to report on the electron microscopical findings of a sural nerve biopsy obtained from a 13-year-old boy of this family prior to the appearance of neurological deficits.

**CLINICAL DATA**

At the age of 13 years this boy appeared clinically normal based on detailed neurological examination of his mental status and the motor and sensory functions of his peripheral nervous system. EEG, EMG and ENG also revealed normal findings.

On grounds of the arylsulfatase A-deficiency in his leukocytes (28) and urine (family B) as well as his massive urinary excretion of sulfatides (30), he was considered to carry the homozygous trait of MLD. The boy's mother also revealed a defect of arylsulfatase A in leukocytes and urine, already disclosed in a preclinical state (29). However, she has now, at the age of 44 years, developed neurological symptoms, and manifest MLD was confirmed by nerve and muscle biopsy (13). The boy's father is a heterozygous carrier based on the degree of arylsulfatase A-deficiency in his leukocytes.

**MATERIALS AND METHODS**

At biopsy, the sural nerve was portioned into several fragments for histological, histochemical and electron microscopical studies. 1–2 mm sized blocks were immediately fixed in 2.5% phosphate-buffered glutaraldehyde, washed in buffer and postfixed in OsO₄. After dehydration in increasing concentrations of ethanol, the blocks were embedded in Epon and after thin sectioning were stained with uranyl acetate and lead citrate. Formalin-fixed specimens were cut frozen to document presence of metachromatic granules by the acid cresyl violet (15), acriflavine (16) and toluidine blue techniques. Paraffin-embedded material was stained with routine methods for light microscopy.

**RESULTS**

*Light microscopy.* By light microscopy, metachromatic granules (Fig. 1) were noted in Schwann cells and in a few macrophages. No other abnormalities of myelin sheaths, axons, interstitial connective tissue and vessels were observed.

*Electron microscopy.* The chief ultrastructural findings consisted of pleomorphic inclusion bodies located in the Schwann cell cytoplasm of myelinated and unmyelinated nerve fibers.

Several types of residual bodies could be distinguished. Firstly, oval, round or polygonal cytosomes measuring up to 2 μ which were generally separated by a unit membrane from the remaining Schwann cell cytoplasm (Fig. 2). Occasionally, a distinct boundary membrane could not be ascertained.

At higher magnification, they consisted of alternating light and dark leaflets of irregular size. The electron-dense osmiophilic leaflets represented groups of layered membranes with regularly arranged lamellae. The distance between 2 osmiophilic lamellae was 37–50 Å. These "zebra body”-like inclusions lay in close spatial proximity to dilated endoplasmic reticulum of Schwann cells. Occasionally, they contained glycogen granules.

A second type of inclusion within the cytoplasm of Schwann cells were 2.5 μ sized lysosome-like bodies (Fig. 3), which were composed of a strongly osmio-
philic seemingly amorphous part and a moderately electron-dense portion. At higher magnification, both parts appeared lamellated, consisting of double lamellae. Those double lamellae measured 98.5 Å (Fig. 4) and showed a space of 59 to 68 Å between the individual lamellae, while the double lamellae of 60 Å were separated by a 26-35 Å space (Fig. 5). The clear space inside the double lamellae measured 8.5-17 Å. The vacuoles were entirely or partly filled. They were usually separated by a membrane, possibly representing lysosomes. Additional storage material floated freely within the endoplasmic reticulum (Fig. 5). Occasionally, membranes appeared isolated among osmiophilic and osmiophobic portions of such an inclusion.

A third component, present in Schwann cells and rarely in axons, consisted of several spheroid or oval structures (Fig. 6). They contained an irregularly compact matrix of moderate electron density with regional light vesicular spaces. At higher magnification, the matrix either contained circular membranes or a mosaic of regular slender lamellae with a periodicity of 27.5 Å (Fig. 6, inset).
Finally, amorphous or granular osmiophilic material was present in Schwann cells of unmyelinated axons (Fig. 7). Schwann cells contained either single, several or all of the various inclusions. In addition, normally appearing lysosomes were also found in the Schwann cell cytoplasm.

The majority of nerve fibers exhibited a normal ultrastructure. Myelin sheaths had regular major dense lines and intraperiod lines. A few myelin sheaths, however, showed widening (up to 135 Å) of individual lamellae, chiefly located in the outer part of the sheath.

An occasional myelin ovoid was also found (Fig. 8). Several such myelin ovoids were adjacent to normal or widened myelin sheaths and some had spatial contact with the external myelin ovoid-lamellae.

The ovoid lamellae had a similar periodicity of 95-135 Å as did the myelin lamellae (Fig. 8a). The myelin ovoid-lamellae were either well preserved or in the process of dissolution into an amorphous substance (Fig. 9). From external lamellae of a few myelin ovoids arcades had been separated that themselves consisted of lamellae. The lamellae of these arcades had a periodicity of 25 Å.

Some fibers showed early segmental demyelination (Fig. 10). Advanced damage of myelin was a rare event, apparently commencing with vesiculation of terminal loops at Ranvier's nodes.
The endoplasmic reticulum of many Schwann cells was considerably dilated (Fig. 11), forming vacuoles and cisternae, varying in size and form. Several appeared electron-lucent; others contained osmiophilic amorphous material or lamellae and granular inclusions. Numerous of these dilatations were surrounded by a distinct membrane and seemed to represent lysosomes. The endoplasmic reticulum of fibroblasts was also dilated. The cytoplasm of several Schwann cells was rich in glycogen which formed rosettes (Fig. 9) or

![Image](http://jnen.oxfordjournals.org/)
Fig. 4. Membrane-bound inclusion body with a strongly osmiophilic seemingly amorphous part and an electron-dense portion, both composed of double lamellae. × 101,500.

aggregates or had spread diffusely (Fig. 6, 8a). Glycogen granules also encircled the dilated endoplasmic reticulum (Fig. 11). The glycogen granules were frequently located in the paranodal regions of myelinated axons and only rarely were they found within axis cylinders where they were distributed in a diffuse manner.

Of the mitochondria of Schwann cells and axons several were aligned in
rows (Fig. 12c) or appeared forked (Fig. 12d). Their cristae were abnormally electron-dense (Fig. 12b) and were situated in the paranodal regions and in an occasional axis cylinder (Fig. 12 a and b). Others, apart from only a few cristae (Fig. 12c), contained an amorphous granular matrix with regionally
empty spaces. A few glycogen granules were also found inside mitochondria.

Pathological changes were observed in axis cylinders and here the myelin sheaths were also severely damaged. In addition to altered mitochondria they contained glycogen granules, inclusions, membranous profiles (Fig. 13) and condensed neurofilaments which were rarely fragmented. Macrophages containing amorphous, granular or lamellar inclusions were found within the slightly thickened endoneurium.

**DISCUSSION**

Markedly reduced activities of leukocytic and urinary arylsulfatase A and a massive excretion of sulfatides at the age of 13 years led to suspicion of a preclinical stage of MLD in this neurologically healthy boy from a family with adult MLD. According to our present knowledge only one type of manifestation of this inherited disease occurs within a family. Since two family members had their first clinical symptoms not earlier than 40 years of
age, one may presume that the nerve biopsy in this case was performed 2-3 decades before clinical manifestations were to be expected. The existence of the preclinical state of adult MLD could then be confirmed by a morphological study.

On grounds of their fine structural features, residual bodies can be grouped into 4 different classes: The first one consisting of 2 μ large lamellated bodies resembles "zebra bodies". These had previously been observed in MLD by Bischoff and Ulrich (6), but were also described in Hurler's type of mucopolysaccharidoses (1). The second type of cytosomes, measuring about 2.5 μ and composed of double lamellae had also been seen in MLD (36: Fig. 6a; 9: Fig. 2c; 24: Fig. 5). Many of these cytosomes were surrounded by a membrane; others lay naked in the cytoplasmic matrix. Other structures partly filled or distended vacuoles which themselves were surrounded by membranes of varying thickness and resembled lysosomes.

These two different types of inclusions were similar to so-called π-granules (38) which give metachromatic reactions using the toluidine blue or acid cresyl violet techniques (25, 32). Phosphatides and sulfatides, present in these granules, are supposed to be responsible for this metachromasia. Acid phosphatase activity in π-granules substantiate their lysosomal character (40).

Fig. 7. Granular or amorphous osmiophilic material in Schwann cell of unmyelinated axons. × 20,000.
The third group of cytosomes resembles those lamellar inclusions described by Grégoire et al. (14): Bischoff and Ulrich (6) also called them "tuffstone" or tufaceous bodies. These inclusions were sparsely present in Schwann cells and only very rarely in myelinated axons. This type of cytosome was not found in the central and peripheral nervous and visceral tissues of another case of adult metachromatic leukodystrophy (19).

Since sulfatides could be documented biochemically in these inclusions by Suzuki et al. (34), "tuffstone"-like bodies were regarded as the "characteristic ultrastructural substrate" of MLD by several authors (7).

The fourth type of inclusion, namely osmiophilic bodies, frequently resembled lipofuscin granules and was found chiefly in Schwann cells of unmyelinated axons; they are also encountered in healthy elderly humans (33).

Schwann cells and numerous fibroblasts contained an endoplasmic reticulum (ER), that was frequently dilated and even appeared vacuolar. Most often it was empty, but sometimes it might be replete with membranous structures, similar to the inclusions described above. This dilated endoplasmic reticulum was found in Schwann cells of otherwise normal nerve fibers. Serial sections suggested that the small dilated ER-spaces first appeared
empty and then were successively filled with membranes as the process of vacuolation continued. Similar vacuolation in Schwann cells has been described previously in sensory peripheral nerves of MLD (9). In infantile MLD dilated cisternae of the ER were located in the perinuclear region of oligodendrocytes (2). Dilated ER was also observed in cortical neurons in MLD of late onset (20), in anterior horn cells in infantile MLD (24) and in pericytes of prenatal tissues in MLD (41).

Mitochondria of Schwann cells and myelinated or unmyelinated normal and damaged axons showed a variety of structural alterations. Many mitochondria contained only sparse cristae and an amorphous granular electron-
Fig. 9. Portion of a Schwann cell. The myelin ovoid lamellae are either well preserved (arrow) or in a process of dissolution into an amorphous substance (A). G = glycogen granules, M = myelin sheath. × 66,700.

dense matrix. The inner membrane was often missing. Similar mitochondrial alterations had previously been reported in peripheral nerves (10, 39), and in oligodendroglia (2) of MLD cases. These investigators pointed out the morphological similarities between several cytosomes and the altered mitochondria suggesting a transformation of such mitochondria into cytosomes. In the paranodal region of Schwann cells, mitochondria had clustered; their cristae appeared condensed in a fashion also described in MLD by Cravioto et al. (10). The mitochondria accumulating in areas of segmental demyelination, did not show condensation of their cristae however.

The number of glycogen granules was frequently increased in Schwann cell cytoplasm, mitochondria and axons of myelinated and unmyelinated nerve fibers. Glycogen accumulation has also been seen in polyneuropathies of various origin, especially in polyneuropathy with hypothyroidism (12).

Most nerve fibers were morphologically intact, others displayed various degrees of demyelination. The demyelination was of the segmental type, commencing in the paranodal region; it was marked by splitting of lamellae and formation of vesicles between terminal loops of Schwann cells. Unaltered myelin sheaths showed the regular major dense and intraperiod lines with a normal periodicity. The "loose myelin" that has previously been described in peripheral nerves of MLD by Cravioto et al. (10), was not observed in our
biopsy. We cannot confirm morphologically the previously mentioned hypothesis of a primarily abnormal myelin in MLD (26). This, however, does not rule out that even morphologically normal appearing myelin may have a pathological biochemical composition. Prisma-type inclusions seen by several authors (14, 37) were not present in our material.

The loosening of external lamellae of myelin sheaths and ovoids resulting

Fig. 10. Nerve fiber, early segmental demyelination. Note the vesiculation in the nodal region. × 12,250.
in the formation of arcades may represent early damage to myelin; however, artifactual separation due to surgical as well as preparative techniques cannot be excluded completely. Myelin ovoids and whorls often containing a granular or amorphous material were present in the Schwann cell cytoplasm. The periodicity of the lamellae was well-preserved among the ovoids and appeared similar to that of normal myelin sheaths. Ovoids also occurred in Schwann cells of normal nerve fibers; its significance is unclear.

Fig. 12a-d. In this set of photographs various forms of mitochondria individually described in the text are demonstrated within the different compartments of the sural nerve. a. $\times 24,000$, b. $\times 28,000$, c. $\times 24,000$, d. $\times 14,700$. 
There were only a few instances of myelin regeneration, possibly due to the mild demyelination or the severe alteration of Schwann cells. Axonal degeneration was rare and only present in pathologically altered nerve fibers. Apart from nonspecific axonal changes, also seen in segmental demyelination of other disorders, few inclusions and membranous profiles were observed in axis cylinders. It is of interest that in prenatal tissues of MLD which are not directly comparable to those of the preclinical state, pathological changes of nerve fibers were absent (41).

As known for many years, metachromatic leukodystrophy is based on an inborn deficiency of a sulfatase, resulting in the accumulation of sulfatides, which are physiological constituents of several membranes (11).
Cerebroside sulfatase- or arylsulfatase A-activity is mainly localized in lysosomes (5). Biochemical studies (11) demonstrated that sulfatides in the microsomal and mitochondrial fractions had a higher turnover than those of myelin. Thus, accumulation of sulfatides might represent a multifocal phenomenon, involving different subcellular structures (21). On the basis of these metabolic processes the demonstrated alterations of endoplasmic reticulum, mitochondria as well as lysosomes in this early case without any clinical manifestations, might represent initial morphological changes in MLD before demyelination occurs.

The cytoplasmic cytosomes resembling \( \pi \)-granules, might represent an increased storage of sulfatides. \( \pi \)-granules usually appear after the fourth year of life and achieve a maximal size of 1 \( \mu \) (7). Since \( \pi \)-granules also contain sulfatides, the giant \( \pi \)-granules, measuring about 2 \( \mu \) in our material, might be the morphological expression of an increased storage of sulfatides. Preformed \( \pi \)-granules could be utilized in such a pathological storage process. The lipofuscin granules found in Schwann cells of unmyelinated axons were probably ordinary "wear and tear" products. Accumulation of glycogen granules were also a non-specific phenomenon.

The nature of the residual bodies described and their significance for the pathogenesis of MLD could not be clarified, since the entire gamut of cytosomes cannot be separated by either morphological or biochemical techniques into primary storage granules and secondary non-specific metabolic breakdown products. The above mentioned morphological alterations of regular cytoplasmic organelles suggest that a subcellular metabolic insufficiency of Schwann cells associated with a biochemically abnormally myelin (26) may result in a slow but continuous breakdown of myelin in late-onset MLD.

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REFERENCES


