Peripheral neuropathy of metachromatic leucodystrophy: observations on segmental demyelination and remyelination and the intracellular distribution of sulphatide

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Peripheral nerve abnormalities in metachromatic leucodystrophy have been noted many times since Jacobi (1947) first reported metachromatic material in the sciatic nerve of her patient. The finding of metachromatic granules in a peripheral nerve biopsy, usually the sural nerve, has been recommended as a method of diagnosing this disease (Thieffry and Lyon, 1959; Hagberg, Sourander, and Thorén, 1962). The occurrence of granules, mainly in Schwann cells, and also in macrophages, has been thought to be due to the same metabolic anomaly affecting the cells responsible for myelin formation: oligodendroglia in the central nervous system, and Schwann cells in the peripheral nervous system. Phase-contrast and electron microscope studies (Webster, 1962) and electrophysiological observations (Fullerton, 1964) have suggested that the peripheral nerve lesion is that due to a Schwann cell disorder, segmental demyelination. In the case described below, the presence of this specific lesion was confirmed by examining single nerve fibres isolated by teasing, and, unexpectedly, extensive remyelination was also seen. No correlation was found between the presence of metachromatic material in a Schwann cell and integrity or disease of its myelin segment.

MATERIAL AND METHODS

Samples of the peripheral nerves of one patient with metachromatic leucodystrophy were obtained at necropsy and fixed in 10% neutral formal saline. Paraffin sections were stained by conventional methods. Frozen sections, 20 μ thick, were stained to show metachromasia by Feyrter's enclosure method, and with cresyl violet 1%, adjusted to pH 3-6 with acetic acid, by the technique of Hirsch and Peiffer (1955).

NERVE TEASING This was done by a method modified from Thomas's (1955) account. Formalin-fixed nerves were washed in water and placed in 1% aqueous osmic acid for 24 hours. After further washing, they were placed in 60% glycerol in water until required. Under a dissecting microscope, at a magnification of ×25 or ×50, single nerve fibres were teased out freehand from fascicles, using fine sewing needles for dissection. Individual fibres were placed on clean slides in batches of about 20, straightened, dried in air at 37°C, and mounted in glycerine jelly. Some specimens of nerves after fixation were stained in bulk by the Hirsch-Peiffer method, by immersion in acidified cresyl violet for 24 hours, and then macerated in 60% glycerine before being teased.

CASE REPORT

A brief summary only will be given. A 15-year-old girl (General Hospital number 221787) died after an illness lasting five years. She had appeared to develop normally until the age of 10 years when her schoolwork began to deteriorate, and she became increasingly clumsy. Aged 13 years, she was noted to be of feeble intellect and to have generalized spasticity. All the tendon jerks were present and brisk, except for the ankle jerks which were absent. The cerebrospinal fluid contained protein 60 mg/100 ml. There was a large amount of intracellular metachromatic material in the urine. Her condition deteriorated steadily, and, at the age of 15 years, she was in a permanent stupor and had flexion contractures of all four limbs. She died from a recurrent respiratory infection.

NECROPSY The body was of an emaciated young woman with severe flexion contractures of both legs. Apart from bronchopneumonia the viscera appeared normal. Metachromatic material (red by Feyrter's technique and brown with acidified cresyl violet) was found in renal tubular epithelial cells and ganglion cells of the myenteric plexus of the intestines. Macroscopically, the brain (weight 1,300 g) showed an irregular greyness of the centrum ovale except for white cortical U fibres. Histologically there was gross demyelination throughout the white
matter. Many neurones in the cortex and basal ganglia and macrophages in the centrum ovale stained metachromatically red by Feyrter's method, and brown with acidified cresyl violet. Part of one frontal lobe was frozen at necropsy, and later analysed by Professor J. N. Cumings. The results, which are typical of metachromatic leucodystrophy, are shown in Table I.

| TABLE I |
|---|---|
| ANALYSIS OF BRAIN (FROZEN) | |
| **White Matter** | Cortex |
| Total phospholipid | 16-6 | 25-61 |
| Total cholesterol | 7-8 | 5-47 |
| Esterified cholesterol | 0-45 | 0-13 |
| Cerebroside | 8-30 | — |
| Sulphatide | 13-71 | — |
| Total hexosamine | 0-56 | 0-74 |
| N-acetyl-neuraminic acid | — | 0-30 |
| Water (%) | 78-2 | 85-4 |

1All results in g./100 g. dry tissue.

Thin layer chromatography White matter showed a normal phospholipid pattern. There was a marked increase in the sulphatide fraction and a decrease in the cerebroside fraction. The cortex showed no abnormal pattern of phospholipids or of gangliosides.

RESULTS

All peripheral nerves examined showed similar evidence of a neuropathy. In paraffin sections, and Sudan black-stained frozen sections (Fig. 1), there were fewer myelinated fibres than normal and many globules of breaking-down myelin were present, scattered in a random fashion along the fibres. In silver impregnations damaged axons were not seen although there was an unusually large number of fine nerve fibres.

Metachromatic material was only shown in frozen sections. In preparations stained with thionin by Feyrter's method, reddish purple-coloured droplets were present in moderately large numbers between and along fibres. Surviving myelin sheaths were rose coloured. By Hirsch and Peiffer's technique, metachromatic, golden-brown debris appeared in much the same distribution and amount as in Feyrter-stained preparations (Fig. 2). Some metachromatic material appeared to lie in macrophages, a few of which were clustered around small blood vessels. In sections and teased preparations stained with cresyl violet and examined by polarized light (Fig. 8a and b), there was yellow-green dichroism of all material which appeared golden brown by ordinary transmitted light. The dichroism seems to be specific for sulphatides (Dayan, 1967).

TEASED PREPARATIONS AFTER POST FIXATION WITH OSMIC ACID About one hundred fibres of all diameters were examined from both motor and sensory nerves and similar changes were found in all. The only lesions seen were those of segmental demyelination and remyelination, the former being more common. The pathological processes appeared the same as in other Schwann cell diseases as noted below, and ranged from widening of the internodal gap at a node of Ranvier to complete segmental demyelination and subsequent remyelination (Figs. 3 and 4). New segments were shorter than unaffected internodes and several new ones would replace a single segment which had been destroyed. At the end stage of remyelination there were one or more newly formed nodes of Ranvier separating...
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FIG. 3. Isolated fibre showing demyelinated segment on right (Sudan black × 400).

FIG. 4. Remyelination of short, intercalated segment (osmic acid, × 240).

FIG. 5. Osmiophilic myelin debris have accumulated at one end of a remyelinated segment and may represent demyelination (osmic acid, × 100).

segments which were shorter and sometimes thinner or less densely stained than normal. Very occasionally, demyelination was seen of a segment which appeared shorter than normal and was bounded by well-formed segments of normal length (Fig. 5). This was interpreted as demyelination of a remyelinated segment. A few Schwann cells in remyelinating segments contained minute, osmiophilic cytoplasmic granules in the paranuclear position.

Of 100 fibres examined, about 80% had one or more abnormalities. Recent demyelination, shown by widening of the internodal gap, was seen in 5%; more extensive or complete demyelination of a segment in 50%; and varying stages of repair or complete remyelination in the remainder. Loss of myelin from remyelinated segments was seen only twice. The lengths of individual segments were measured and correlated graphically with fibre diameter (Fig. 6) by the method of Fullerton, Gilliatt, Lascelles, and Morgan-Hughes (1965). The many abnormally short segments present are typical of a demyelinating neuropathy (Thomas and Lascelles, 1966).

TEASED ACIDIFIED CRESYL VIOLET-STAINED FIBRES

Technically, these preparations were less easy to tease because the majority of fibres were almost invisible under the dissecting microscope. Some compensation was afforded by the greater ease of dissection due to softening of the collagen of the

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nerve bundle by the acidity of the staining solution. In all, 45 fibres were analysed.

When individual fibres were separated by teasing, their overall morphology resembled that of osmic-stained preparations and various abnormalities were found scattered along their lengths. The site of the Schwann cell nucleus of each segment could be recognized sometimes by seeing the nucleus as a red-stained structure, by phase-contrast microscopy, and by its characteristic slight swelling with indentation of the myelin midway between nodes (Fig. 7). In about a third of these swellings a number of small, rounded, green-yellow dichroic, metachromatic-brown granules (approximately 1 to 2 μ diameter) lay grouped in the longitudinal plane (Figs. 7, 8, 9a, 9b, and 12) in the cytoplasm on each side of the nucleus. A few segments at the cut ends of fascicles sometimes contained very small red granules more or less concentrated in bands along the length of sheaths (probably at Schmidt-Lantermann clefts), and at the nodes of Ranvier (Figs. 10 and 11). Segments situated well inside nerve bundles did not show this which was probably an artefact due to the trauma of cutting the nerve. Occasional minute brown granules were found in the myelin sheaths of segments from the interior of fascicles. From their peripheral appearance when viewed tangentially the granules appeared to lie in some part of the myelin sheath, and were quite separate from the paranuclear granules described above. Normal demyelinated and remyelinated segments were recognized by the width of fibres and their internodal length and by several microscopical techniques (see below), and in none was any generalized meta- or orthochromatic staining seen of myelin, not even in demyelinating segments.

In isolated fibres changes were demonstrated in myelin sheaths by phase-contrast microscopy and by ordinary bright-field illumination when the condenser diaphragm was closed to pinhole size. Viewing conditions were bad in both techniques because the refractive index of the medium (glycerine jelly, r = 1.47) was too low for critical phase-contrast observations, and, by the latter technique, illumination necessarily was very poor. Normal myelin sheaths by both techniques appeared to have evenly distributed, fine granularity interrupted only by the bright bands of Schmidt-Lantermann clefts. In the paranodal regions of many sheaths, the even granularity was replaced by numbers of small, concentric, bright-margined spheres and ovoids packed closely together. No consistent differences were seen in any of these structures in sheaths which did not bear metachromatic material in either the paranuclear position or elsewhere in the sheath. Very occasionally, rounded granules with a Maltese cross pattern of birefringence were seen in a demyelinating segment. No change in or abnormal staining of axons was seen anywhere.

The present findings were made in one case only of 'juvenile' metachromatic leucodystrophy. Similar observations have been made in two examples of the infantile disease.

Control nerves were obtained from patients of about the same age who had died after road accidents (two cases), and from respiratory complications of acute infectious polyneuritis (one case). After cresyl violet staining, teased fibres from these patients showed a few red granules evenly distributed along myelin sheaths, and in the paranuclear position in Schwann cells. Occasional Schmidt-Lantermann clefts were visible, and, in a few fibres, there was also a fine dusting of red granules in paranodal regions. No brown-stained material was ever seen in these nerves.

**DISCUSSION**

Segmental demyelination, first recognized as a pathological entity by Gombault (1880), is due to disease processes which affect Schwann cells and causes the destruction of individual segments of myelin sheath without apparent damage to the axon. Should recovery occur, new short myelin segments are found 'intercalated' between segments of normal length (Stransky, 1903; Lubinska, 1958). Remyelination may require the formation of Schwann cells (Geren, 1954; Lubinska, 1958), or,
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**FIG. 7.** Node of Ranvier is present on left and a Schwann cell nucleus is present at the indentation of the myelin sheath on right (Hirsch-Peiffer, × 100).

**FIG. 8.** Paranuclear metachromatic granules in Schwann cell (Hirsch-Peiffer, × 400).

**FIG. 9a.** Isolated fibre showing brown stained paranuclear granules. (Hirsch-Peiffer, × 240.)

**FIG. 9b.** Identical field under partly crossed Polaroid filters. There is golden-yellow dichroism of metachromatic granules.

**FIG. 10.** Accumulation of orthochromatically stained granules at a node of Ranvier. Myelin sheath is not diffusely stained (Hirsch-Peiffer, × 400).
if damage is not too great, it may proceed from surviving parts of a segment (Gombault, 1880). As each Schwann cell appears to be responsible for the myelin sheath of one segment of a myelinated (by light microscopy) nerve fibre, it was expected that diseases of Schwann cells would cause segmental demyelination. Such lesions have been seen in various conditions, including diphtheritic neuritis (Cavanagh and Jacobs, 1964) and diabetic neuropathy (Thomas and Lascelles, 1966).

In the present disease, as the pathogenetic biochemical lesion presumably affects Schwann cells as well as neurones, and as it causes demyelination in the central nervous system, its expression in peripheral nerves was expected to be segmental demyelination. This process had been demonstrated previously in the peripheral nerves of two patients with metachromatic leucodystrophy by Webster (1962) using phase-contrast and electron microscopy. Fullerton (1964) studied six patients with this disease, and found in five of them the slowing of nerve conduction characteristic of segmental demyelination. She made no histological observations on her patients. The present observations confirm the finding of segmental demyelination in metachromatic leucodystrophy. All stages of evolution of the lesion were seen from widening of the nodal gap to complete segmental breakdown. The changes were identical morphologically with those found in other Schwann cell diseases.

In teased fibres, green-yellow dichroic, metachromatic brown granules were only found in the Schwann cell myelin sheath at two sites most prominently in the paranuclear position, and, less often as small granules scattered randomly throughout the sheath. The former type, rounded and 1 to 2 μ in diameter, is probably the same as the rounded, osmiophilic paranuclear inclusions noted by Webster (1962). That they have now been found to stain metachromatically by the Hirsch-Peiffer technique make it almost certain that they contain sulphatide. The need to exceed a minimum size before structures are visible by light microscopy, conventionally accepted as 1 μ diameter, could account perhaps for the small number of fibres seen bearing metachromatic granules as compared with Webster’s estimate of 95% affected sheaths if all electron-dense granules are counted as abnormal.

The lack of generalized metachromatic staining of myelin sheaths is noteworthy. Within the limits of sensitivity of the staining method employed (Hirsch and Peiffer, 1955), which are unknown, it
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implies that the myelin sheath itself does not contain a demonstrable excess of sulphatides, not even in the still intact areas of segments which are already breaking down. The various red droplets and particles observed in sheaths are probably the \( \mu \) granules of Reich (1903), otherwise known as Elzholz granules, a normal finding in myelinated fibres stained with aniline dyes (see illustrations of Doinikow, 1911; Ukai, 1923). Although the paranuclear brown granules lay in the same place and had the same shape as Reich's \( \tau \) or 'protagon' granules, they were not identical because the latter stained red and not brown in control specimens. There appeared to be no correlation between demyelination and the presence of metachromatic material in affected segments in either the paranuclear position or along the sheath. Webster (1962) had also noted discordance between the patchy distribution of demyelination in his biopsies and the almost universal presence of osmiophilic debris (of uncertain nature) in Schwann cell cytoplasm. Combining both sets of observations, it suggests that Schwann cells, in which the basic biochemical defect has been expressed to the extent of a structural abnormality, are still able to maintain a normal myelin sheath.

The finding of widespread remyelination, also noted by Webster, raises several problems. That it occurs at all requires the production of new myelin sheaths by Schwann cells. According to Webster, demyelination appears to be due to functional derangement rather than death of affected Schwann cells, although the present finding of entire denuded segments seems to favour the latter hypothesis. It is also uncertain therefore whether reformation of segments is due to proliferation of nearby unaffected Schwann cells, or, to repair by the originally affected cell. The former suggestion is more probable perhaps because it is unlikely that the consequences of a congenital enzyme defect become apparent and then disappear so readily. Breakdown of remyelinated segments, which was seen twice, also suggested that healing could have occurred by proliferated normal Schwann cells, to be followed by the later appearance of dysfunction.

The lack of correlation between the microscopical expression of the metabolic abnormality and damage to myelin sheaths raises the problem of what causes myelin breakdown and dysfunction of cells of the nervous system in metachromatic leucodystrophy. This question cannot yet be answered with certainty. The Schwann cell-myelin sheath depends on proper integrity of the axon for its continued existence, as shown by the effects of Wallerian degeneration. As sulphatide also accumulates in the soma of neurones, theoretically it is at least possible that the biochemical lesion in neurones and their axonal processes might secondarily cause Schwann cell (and oligodendroglial) damage. The sulphatide accumulation in white matter and Schwann cells could then be interpreted either as a disturbance of the metabolism of myelin conditioned by the axonal dysfunction or, as a manifestation of the same biochemical lesion directly affecting these cells. However, there was no morphological evidence of a lesion of axis cylinders. Recent reports of abnormal gangliosides in the white matter of some cases of metachromatic leucodystrophy by O'Brien (1964), Austin (1965), Austin, Armstrong, and Bischoel (1966), Austin, Armstrong, and Shearer (1965), Mårtensson, Percy, and Svenerholm (1966), and Menkes (1966), suggest a more widespread upset of metabolism than is likely to be due just to lack of a sulphatase. Davison and Gregson (1962) showed in the central nervous system that sulphatides were present in two distinct metabolic compartments. A small amount with a rapid turnover was found outside myelin, possibly in association with such subcellular organelles as microsomes. The larger and more stable sulphatide fraction was associated with myelin. Menkes (1966) suggested that a deficiency of sulphatase rapidly could produce damaging metabolic effects in the former compartment, and a more slowly appearing accumulation of sulphatide in the latter. Thus, cellular dysfunction or death due to disordered metabolism need not parallel the accumulation of metachromatic material in myelin which is relatively inert metabolically, and this is what has been demonstrated morphologically in this paper. Classical studies on the central nervous system in metachromatic leucodystrophy also provide supporting evidence for this observation. As summarized by, for example, Bargeton (1963) and Svennerholm (1963), sulphatide accumulates in excess in the grey and white matter of the brain before there is chemical or histological evidence of demyelination.

Recently, electron microscope studies of two peripheral nerves from a patient with metachromatic leucodystrophy have been reported by Cravioto, O'Brien, Landing, and Finck (1966), and similar observations on two other patients have been summarized by Terry, Suzuki, and Weiss (1966). In all cases, the results obtained at the ultrastructural level were comparable with those of the present study, abnormal osmiophilic complexes being found in Schwann cells which still maintained intact myelin sheaths. Further support for the two compartment hypothesis has come also from ultrastructural studies of the central nervous system in metachromatic leucodystrophy (Grégoire, Périer, and Dustin, 1966), in which inclusions were found.
in glial cells before myelin breakdown became apparent. It was suggested that sulphatide accumulated first and that organized sulphatide-lipid complexes appeared later only after the breakdown of myelin.

In conclusion, based on light and electron microscope observations and metabolic studies, a hypothesis is advanced that the metabolic defect in metachromatic leucodystrophy, presumably a sulphatase deficiency (Mehl and Jatzkewitz, 1963), acts at two subcellular sites in the nervous system. It affects myelin lipids in a rapidly turning over compartment, which probably represents the membranes of cellular organelles such as microsomes, and also the metabolically less active myelin sheaths around nerve fibres. The existence of two compartments would account for the observed lack of correlation between the presence of excess sulphatide and damage to myelin in Schwann cells, and presumably also in oligodendroglial cells.

SUMMARY

Necropsy samples of peripheral nerves were examined from a 15-year-old girl with metachromatic leucodystrophy. Segmental demyelination and remyelination were found in teased preparations of single nerve fibres after staining with osmic acid. Metachromatic brown material was found only in Schwann cells of single fibres teased from nerves stained by the Hirsch-Pfeiffer technique. There was no concordance between the presence or absence of metachromatic granules from Schwann cells and damage to the myelin sheath.

Based on these observations and other evidence a hypothesis is advanced that the pathogenetic enzyme defect in metachromatic leucodystrophy acts at two sites in the nervous system. Its more important expression may lie in damaging cellular organelles rather than in causing sulphatide accumulation in myelin which is more inert metabolically.

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