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Morphological and Biochemical Observations in the Jimpy Spinal Cord*

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Summary. The cervical spinal cords from Jimpy and normal mice were examined by light and electron microscopy at 2 to 29 days after birth. By 2 days after birth the number of myelinated fibres present in the Jimpy spinal cord was significant less than the number present in normal spinal cords. This made it possible to identify the Jimpy mice before onset of the clinical symptoms. The paucity in myelin was also demonstrated by biochemical criteria. Astroglia cell sappeared normal in the Jimpy spinal cord but there was an apparent disturbance in the development of the oligodendroglial cell line, resulting in the appearance of abnormal cells containing voluminous lipid inclusions and/or membranous tubes. The probable significance of these observations and their relation to the myelin defect in the Jimpy mouse is discussed in the light of pathogenetic theories of human leukodystrophies.

Key words: Oligodendrocyte — Lipid Inclusion — Myelin Sheath — Jimpy Mouse — Leukodystrophy.

Introduction

The Jimpy mouse, first described by Philips in 1954, contains an X-linked recessive mutation which in males (jp/Y), results in a myelin deficiency in the central nervous system (Sidman *et al.*, 1964). It is of interest as an animal model for human leukodystrophy. The clinical symptoms begin approximately at the 10th day after birth with hind leg tremors, followed a week later by convulsions. The animals usually die by 30 days after birth, apparently from hypoxia, after a series of status-like convulsions.

Previous morphological studies of the Jimpy mouse have shown an almost complete lack of myelinated fibres in the central nervous system (Sidman *et al.*, 1964). Many investigators have observed cells in the Jimpy CNS which contain inclusions staining positively for neutral fat (Sidman *et al.*, 1964; Hirano *et al.*, 1969; Torii *et al.*, 1971; Farkas-Bargeton *et al.*, 1972; Privat *et al.*, 1972), however their interpretations of these cells have differed.

In this study we have used the fact that myelination occurs quite early in the mouse spinal cord to identify young Jimpy mice before onset of clinical symptoms (by the relative absence of myelin in their spinal cords) and to study the development of the oligodendroglial cell line in these animals over the entire post-natal life of the animals.

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Material and Methods

Males from the C57 BL/6 J-A^{W-J} strain, which originated at the Jackson Laboratories, Bar Habor, Maine, U.S.A., were used as controls. Males hemizygous for the Jimpy locus (Jp$^+$/Y) came from the same strain. Tentative identification was done at birth by determining the sex and by counting the supraorbital vibrissae according to Wolf and Holden (1969).

Upper cervical spinal cords of both Jimpy and control animals at 2, 4, 6, 10, 11, 15, 17, 22, 23 and 29 days after birth were examined. Fixation was either performed *in situ* by perfusion of a phosphate buffered mixture of paraformaldehyde and glutaraldehyde according to Karnowsky (1961) followed by postfixation in 2% osmiumtetroxide for 4 h, or, after removal of the spinal cord and slicing by immersion in 2% phosphate buffered osmiumtetroxide for 4 h. After aceton dehydration the material was embedded in araldite. For light microscopy 1 micron semi thin cross sections of the whole spinal cord were stained with Methylene blue. For electron microscopy thin sections of the ventral and ventro-lateral tract regions were stained with uranyl acetate and lead citrate.

For biochemical investigation the spinal cords were frozen and kept at $-90°$C until being used for biochemical analysis. Lipids were extracted as described by Folch *et al.* (1957) and separated by thin layer chromatography as described by Herschkowitz *et al.* (1968a). The amounts of cerebroside and sulfatide were estimated by gas-liquid chromatography (Herschkowitz *et al.*, 1968b).

Results

I. Myelination

In both normal and Jimpy animals myelin sheaths are first apparent in the ventral and lateral tracts of the spinal cord. In the Jimpy animals subsequent myelination occurs primarily in the tracts that were initially unmyelinated so that the eventual distribution of myelinated fibers in the Jimpy spinal cord is approximately uniform. The absolute number of myelinated axons in the Jimpy spinal cord was determined at different ages by counting directly in 1 micron sections at 400× magnification (Table 1). By 2 days after birth there was a significant difference in the number of myelinated fibers present in the Jimpy and in the normal spinal cord. The paucity of myelinated fibers in the Jimpy spinal cord at this age could therefore be used as an absolute criteria to identify the animals before any clinical symptoms were apparent (Fig. 1).

In the normal spinal cord, above a minimum diameter of about 1 micron, there was a correlation between axon size and extent of myelination, whereas in the

Table 1. Numbers of myelinated axons in the upper cervical spinal cord (myelinated axons were counted at 400× magnification in 1 micron semi thin sections stained with methylene blue, $n = 3$)

Jimpy-mouse Age/days	Number of myelinated axons	
2	10—15	(10, 13, 15)
6	ca. 200	(162, 204, 212)
11	ca. 250	(216, 264, 282)
17	ca. 300	(272, 312, 314)
23	ca. 350	(328, 352, 384)
29	ca. 350	(306, 372, 382)

In the control there were about 500 myelinated axons by the 2nd day after birth and by 6 days after birth there were several thousands.

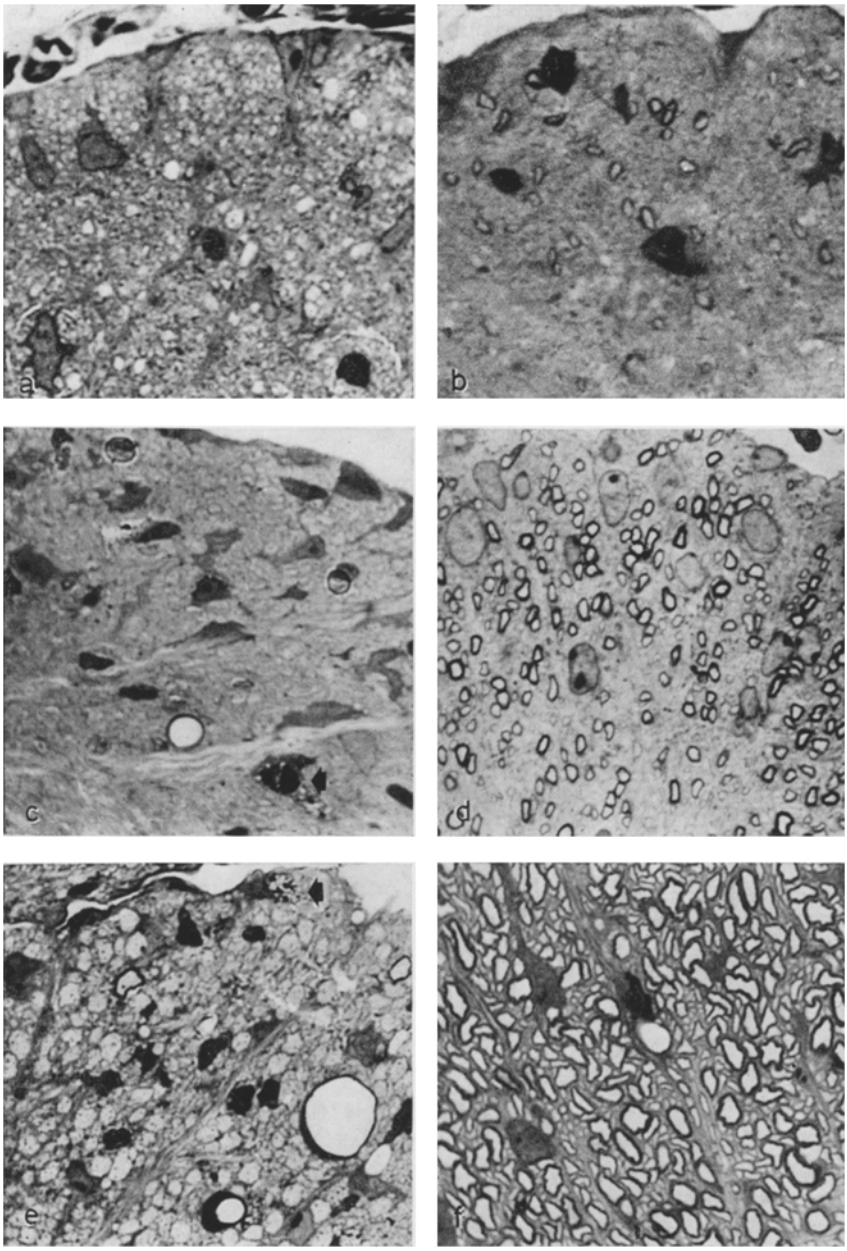

Fig. 1 a—f. Semi thin section of the ventral tracts of the spinal cord showing the extend of myelination in 2, 6, and 23 days old Jimpy (a, c, e) and normal mice (b, d, f). Cells containing lipid inclusions are indicated by arrows in plates c and e. (Methylene blue stain. Original magnification × 500, oil immersion)

Table 2. Numbers of lamellae in the best myelinated axons

Age/days	Number of lamellae	
	Jimpy	Control
2	6— 7	7— 8
6	9—10	15—18
10	10—12	18—22
16	12—14	30—40
22	12—14	40—50
29	12—15	40—50

Lamellae were counted in electron micrographs at 123000 and 177000× magnification.

Jimpy spinal cord, the myelinated axons, while always attaining the normal minimal diameter, were often adjacent to much larger, unmyelinated axons. Furthermore, the number of lamellae in the thickest myelin sheaths present in the Jimpy spinal cord at any one age was always less than the number in the thickest sheaths present in the normal spinal cord of the same age (Table 2). In the young Jimpy mice most of the axons in the spinal cord were completely nude. However in older animals many of the axons were surrounded by thin glial processes occasionally with, but more often without, typical mesaxon formation. In all ages examined, we found individual axons surrounded by several loose, non-compact lamellae, similar in appearance to the early stages of normal myelin development. The axons surrounded by compact myelin were often found in groups of 2—6 as if they had been myelinated by a single cell or by a group of related cells. All the normal structural stages of myelination were observed in the Jimpy spinal cord, indicating that the few myelin sheaths which appear are formed by a normal process of myelination. Serial sections at intervals of 5 microns over a distance of 150 microns showed that the pattern of myelinated axons changed along the length of the Jimpy spinal cord. This observation indicates that the myelin sheaths observed in the Jimpy spinal cord do not extend over the length of the axons, but arise through the random appearance of functional oligodendroglial cells.

Occasionally, in both normal and Jimpy spinal cords, there was evidence of abortive myelination, i.e. bundles of myelin lamellae next to nude axons. There was no clear—cut evidence for myelin breakdown in either the Jimpy or the normal spinal cord at any of the ages examined. However in the osmium fixed material from older animals, we occasionally observed vesicularisation of the axolemma and inner myelin lamellae in the Jimpy spinal cord. This is probably an artefact of fixation (Rosenbluth, 1963; Thormey, 1964); nevertheless, since it was seldom observed in identical material from normal spinal cords, it may indicate a decreased stability of the Jimpy membrane.

II. Biochemistry

In the 12 day old Jimpy spinal cord, there was a pronounced decrease in the amount of galactose cerebroside relative to the normal spinal cord (Table 3). This confirms the morphological observation of decreased amounts of myelin in the spinal cord, since galactose cerebroside is found almost exclusively in myelin.

Table 3. Cerebroside and sulfatide in the spinal cord of 12 day old normal and Jimpy mice (mg lipid/g wet weight). Lipids are extracted and estimated as described in methods

	Control	Jimpy
Gal.-Cerebroside	3.10	0.53
Gal.-Sulfatide	0.84	0.57

Galactose sulfatide, on the other hand, while present in myelin, is also found in other membranes. The same was found in the brain of the Jimpy mouse (Matthieu, 1973). It should be noted that in the sciatic nerve neither morphological nor decreased amounts of cerebroside was found (Herschkowitz *et al.*, 1971).

III. Glial Cells[1]

In 2 days old animals almost half the glial cell population in the ventral tracts of both Jimpy and normal spinal cords could be clearly identified as astroglia by topographical and cytological criteria. Processes from these cells contained fibrils, glycogen granules and occasionally homogeneous, weakly osmiophilic lipid droplets, and formed the glia limitans adjacent to the meninges and the vessels in the spinal cord. In cross sections of the spinal cord, many of the astroglial processes were oriented radially, dividing the axons into different-sized bundles or fascicles.

The remaining glial cells could be separated, on the basis of nuclear and cytoplasmic appearance, into two groups, active oligodendrocytes and undifferentiated cells (glioblasts and glial precursors). The organelle-rich processes of the active oligodendrocytes were often associated with large axons. The majority of the undifferentiated cells appeared, on the basis of their evenly dispersed chromatin, occasional nucleoli and multiple cytoplasmic organelles, to be large glial precursors rather than glioblasts. Some glioblasts with condensed chromatin and sparse cytoplasmic organelles, were present around the vessels and meninges, and occasionally in the midst of axons.

In normal animals the amount of undifferentiated cells decreased with increasing age, while the active oligodendrocyte population increased. There was also an apparent decrease in the relative numbers of astroglial cells during development. By 20 days after birth, some of the oligodendrocytes had assumed the appearance of mature interfascicular oligodendrocytes with condensed cytoplasm and nucleoplasm and smaller perikarya.

In the Jimpy spinal cord the development of the oligodendroglial cell was different. At 2 days after birth, we found cells with irregularly shaped nuclei, aggregates of condensed chromatin around the nuclear membrane or the nucleoplasm, and no nucleoli (Fig. 2). The cytoplasm of these cells contained a Golgi apparatus, mitochondria with dense matrices, vesicles, and evenly distributed free ribosomes. The rough endoplasmic reticulum consisted of short irregular tubes. Stubby processes extending from these cells were often closely apposed to, and partially engulfing, large axons or groups of smaller axons. Some of the cells contained lipid inclusions in greater amounts than normally found in glioblasts.

1 The criteria described by Vaughn (1969) were used to classify the stages in glial cell development.

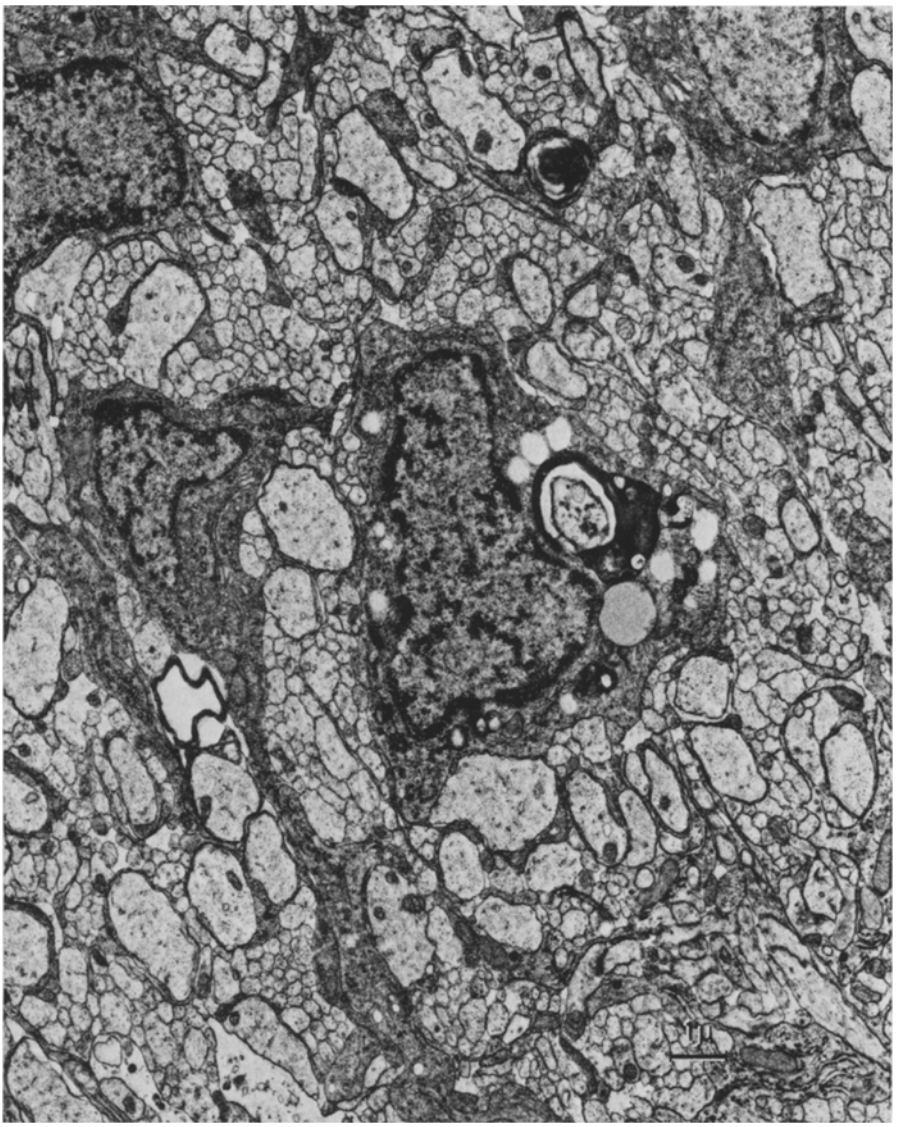

Fig. 2. Abnormal oligodendroglial cells in the ventral tract of a 2 day old Jimpy mouse. The large cell in the center of the field contains lipid inclusions. (Paraformaldehyde/glutaraldehyde fixation)

Because of their characteristic topography and cytological appearance, these cells were tentatively identified as abnormal oligodendroglial cells. Typical glioblasts were also present, in greater than normal amounts, in the 2 days old Jimpy spinal cord, but large glial precursors were seldom seen.

In later stages (5, 10, 15 days) the relative number of typical glioblasts decreased while the number of cells which, according to their size, nuclear structure,

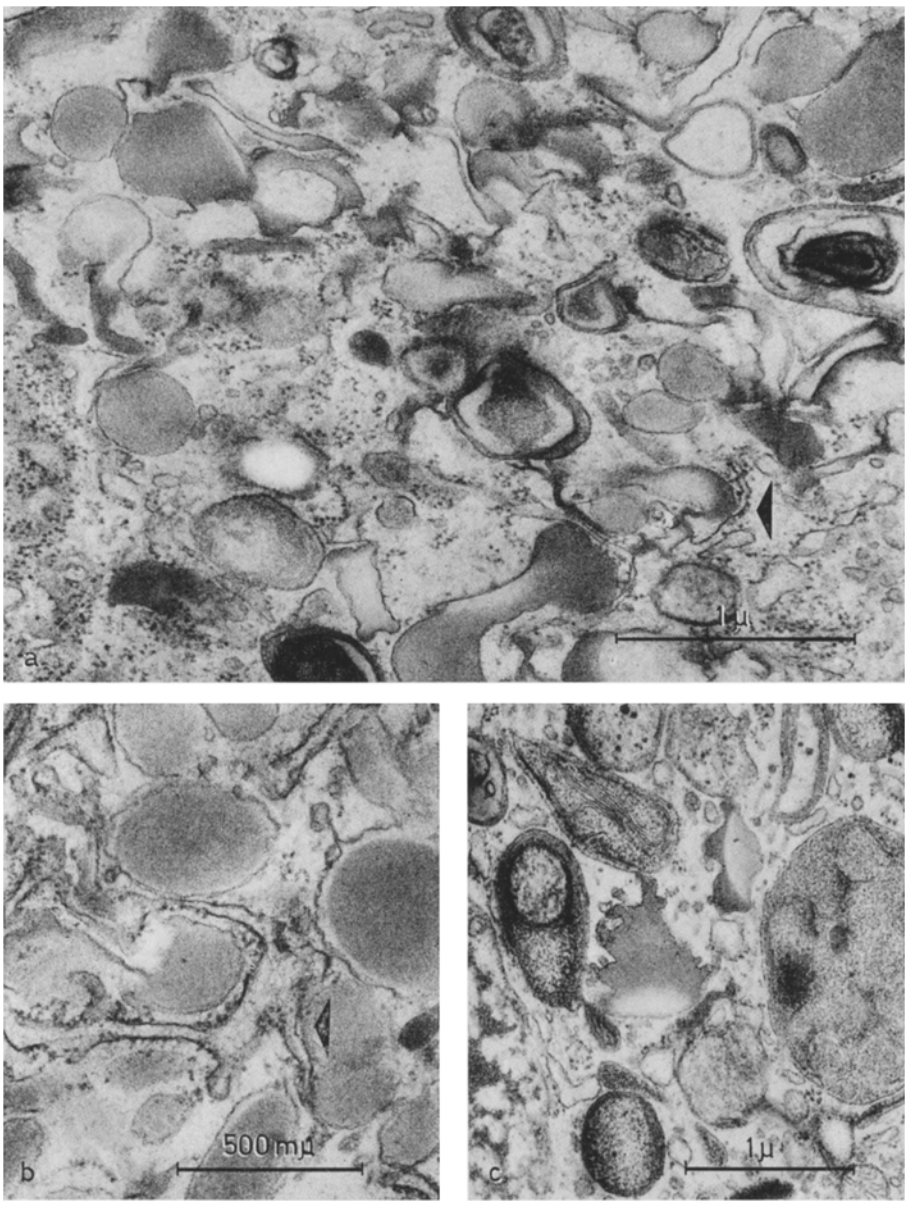

Fig. 3. Polymorphous lipid inclusions found in abnormal oligodendroglial cells in the Jimpy spinal cord. Arrows in plates a and b indicate apparent associations of the inclusions with the E.R. In plate c laminar lipid inclusions are shown. (Osmium immersion fixation)

and axon-associated processes, appeared to be oligodendrocytes, increased. However these cells now contained polymorphous lipid inclusions, usually membrane bound, which often occupied a large proportion of the perikarya and processes, and sometimes appeared to displace normal cell organelles (Fig. 3). These inclusions

were usually round or ovoid, homogeneous, and weakly osmiophilic, with diameters of 0.5—1 micron. Sometimes the lipid inclusions were connected, via small isthmuses, to other inclusions or to empty vesicles which may have been derived from the Golgi apparatus or the smooth endoplasmic reticulum. They also occasionally appeared contiguous with the cytoplasm through funnel-like openings in their surrounding membrane. In older animals, some of the inclusions had a lamellar structure with a periodicity of about 200 Å, and there were also intermediate forms with a lamellar shell and a homogeneous or granular core. In the inclusion-free areas of the cytoplasm of these cells, the Golgi apparatus and the endoplasmic reticulum were less prominent than in normal active oligodendrocytes and there was an almost total absence of microtubules. There were also small, undifferentiated membrane vesicles present in the cytoplasm, similar to those found in normal glioblasts. The mitochondria in these cells, as in normal oligodendrocytes, were small, round or ovoid, cristatype, with dense matrices. Besides the lipid-containing cells in the Jimpy spinal cord, there were many examples of partially matured oligodendroglial cells, the most advanced of which still contained less prominent Golgi apparatus, endoplasmic reticulum and microtubules than comparable cells from normal spinal cord. In older animals both the lipid-containing cells and the undifferentiated cells often contained long membraneous tubes running the entire length of the perikarya and often into the processes (Fig. 5a, b, c). These tubes which could appear straight or serpentine, and which were often formed of several layers of membrane, contained cytoplasm and different cell organelles (mitochondria, endoplasmic reticulum, lipid inclusions). There were indications that the tubes may have originated by invagination of the plasma membrane by a process similar to myelination. These tubes which have not to our knowledge been previously reported in either normal or pathological material will be discussed in more detail in a subsequent publication. Both the lipid inclusion containing cells and the undifferentiated oligodendroglial cells were found to have axons engulfed in their cytoplasm with typical mesaxon formation (Fig. 4). This was taken to be *prima facie* evidence that they were, in fact, myelination glia.

At all ages in the Jimpy spinal cord we found lipid-containing and lipid-free cells in the process of necrosis, usually surrounded by astrocytic processes (Fig. 6). The astrocytes appeared normal except in the vicinity of these necrotizing cells, where the astrocytic processes often contained lipid inclusions and phagocytosed cell debris.

IV. Axons

There was great variability in axon diameter in the ventral tract of the spinal cord; however, the size spectrum appeared to be the same in both Jimpy and normal mice, as was the increase in axonal diameter with age. There were no obvious differences in distribution or types of axoplasmic organelles between the normal and Jimpy spinal cords. In particular, we did not observe the proliferation of endoplasmic reticulum or "honey-comb structure" reported by Hirano in Jimpy cerebellum (Hirano *et al.*, 1969). No evidence of axonal degeneration was observed in either the normal or Jimpy mouse except for the previously mentioned vesicularization of the axolemma in older Jimpy spinal cords and one observation

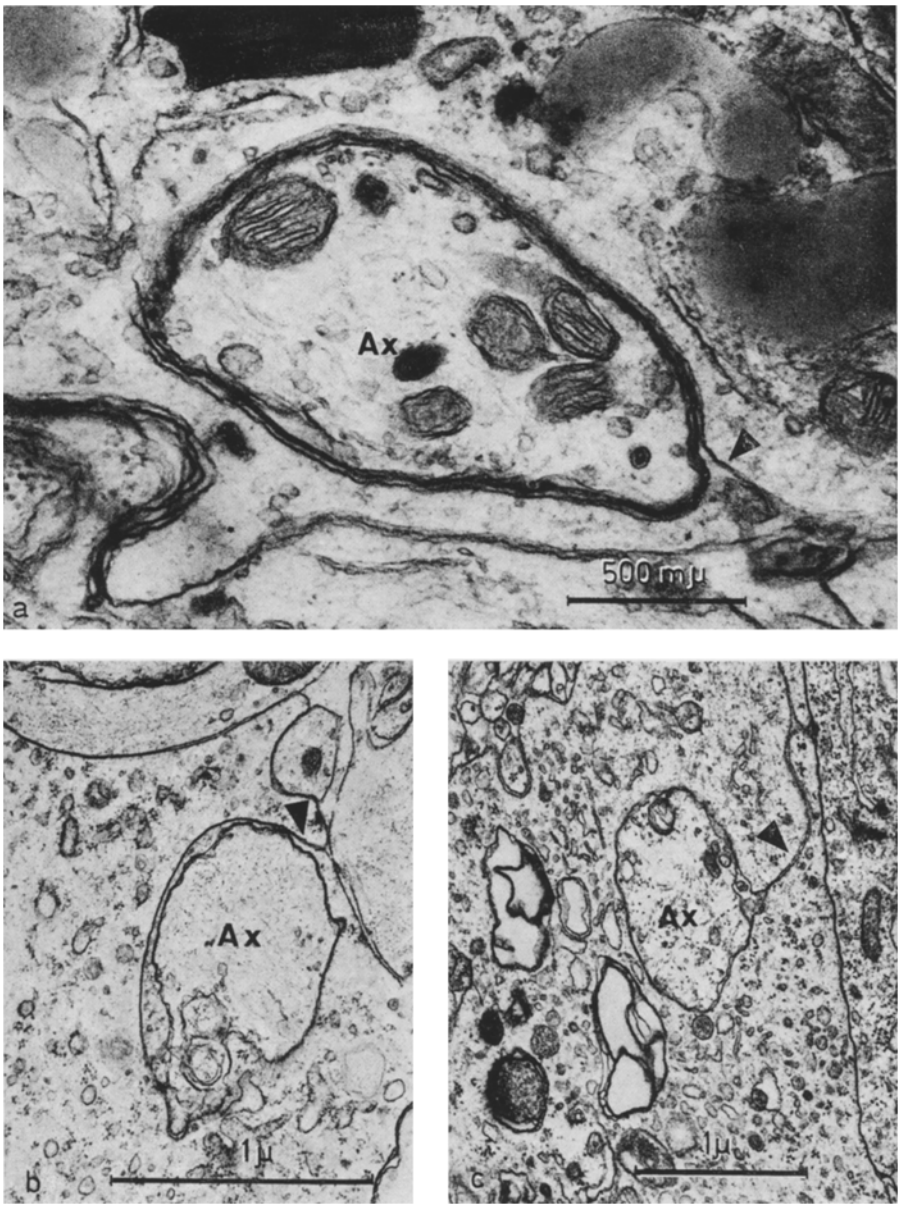

Fig. 4a—c. Axonal profiles (*ax*) showing typical mesaxon formations (arrows) in abnormal oligodendroglial cells in Jimpy spinal cords. The axon in a is surrounded by several layers of myelin. The oligodendroglial cytoplasma in a contains large lipid inclusions, in b poorly developed E.R. and in c abnormal membranous material. (Osmium immersion fixation)

in a 15 days old Jimpy mouse of a swollen axon filled with smooth membrane vesicles. This was an isolated observation however, and may be unrelated to the Jimpy mutation.

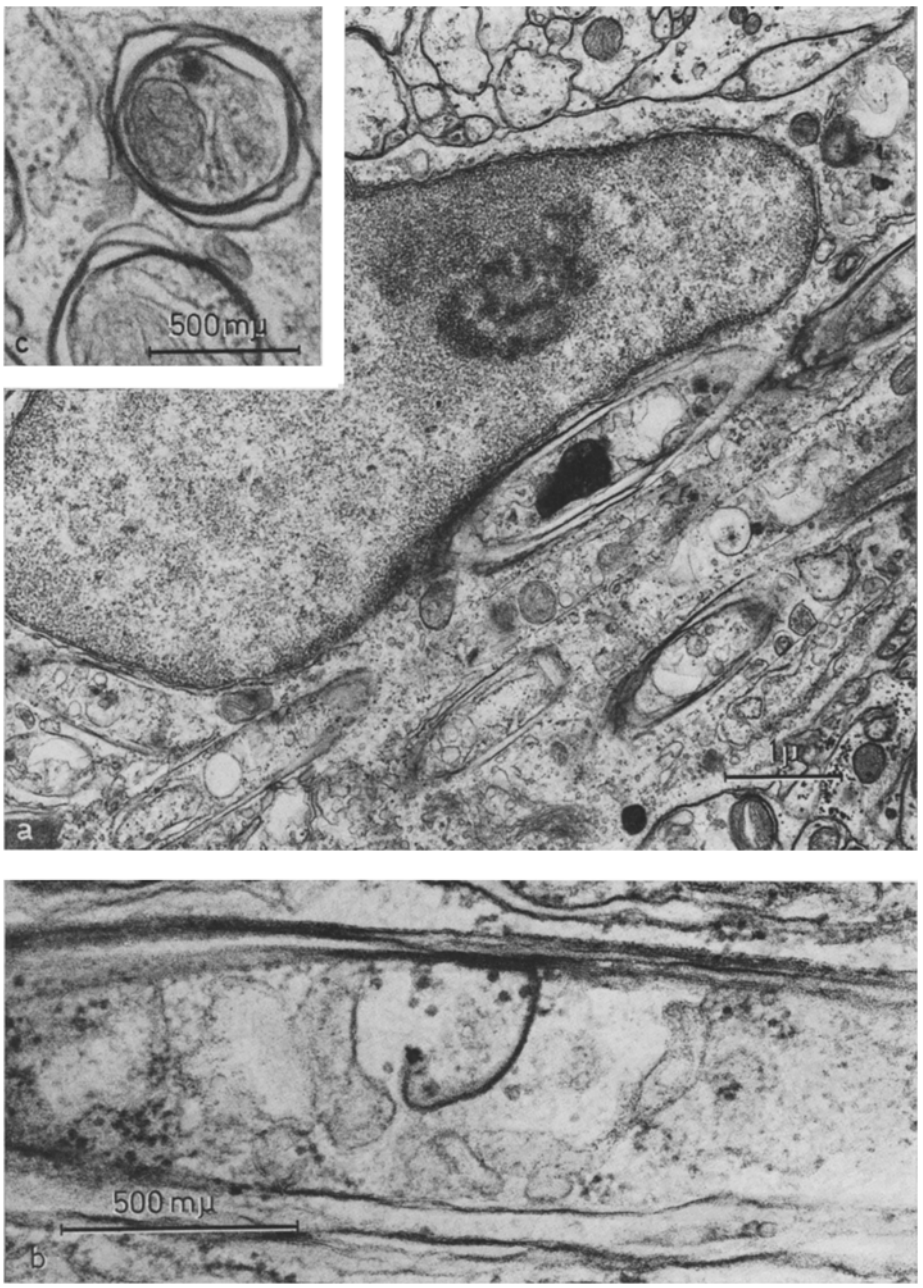

Fig. 5a–c. Abnormal oligodendroglial cell containing membranous tubes in 22 day Jimpy spinal cord. Plate a suggests the serpentine nature of the tubes and shows the engulfed cytoplasmic organelles. Plates b and c show the multilamellar structure of the tubes in longitudinal and cross section. (Osmium immersion fixation)

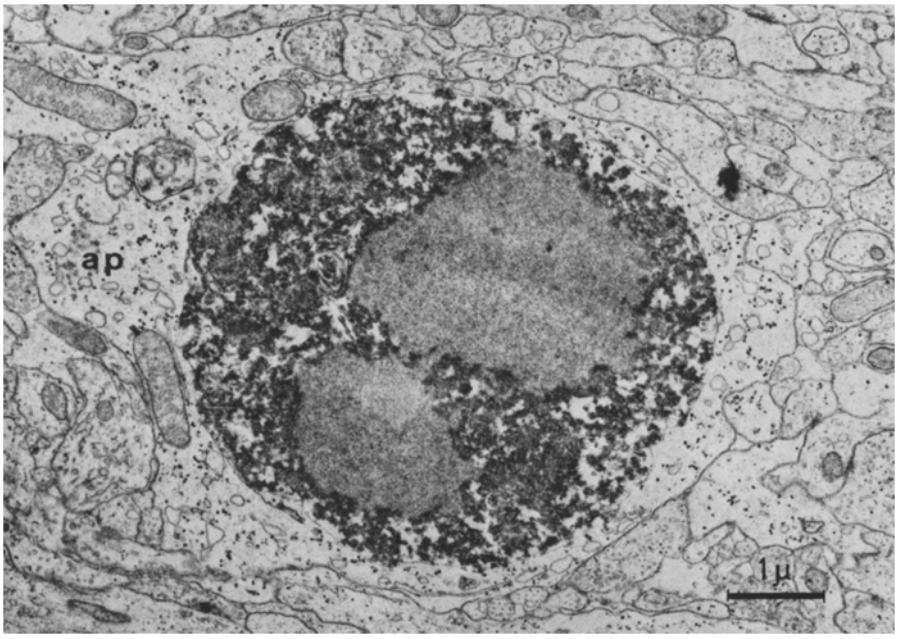

Fig. 6. Astrocytic process (*ap*) engulfing a necrotic cell. (Osmium immersion fixation)

Discussion

Previous light microscopic studies have indicated a defect in oligodendroglial cell maturation in the Jimpy CNS (Farkas-Bargeton *et al.*, 1972; Kraus-Ruppert *et al.*, 1973), however the ultrastructural studies have been somewhat contradictory. Hirano *et al.* (1969) made no mention of oligodendroglial cells in his investigation of the Jimpy cerebellum, while Torii *et al.* (1971) reported decreased numbers of oligodendrocytes in the Jimpy frontal lobe and cerebellum on the light microscopic level but also failed to observe any ultrastructural abnormalities in oligodendrocytes. Privat *et al.* (1972) on the other hand, found a total absence of mature oligodendrocytes, decreased numbers of immature oligodendroglial cells, and indications of oligodendrocytic cell death in the Jimpy corpus callosum. These inconsistencies may be attributable to the fact that all the investigations were carried out after the appearance of clinical symptoms in the animals (10—12 days after birth or later), which makes identification of the various cell types difficult since some may have developed abnormally by this stage, and which limits the time span of observation since the animals invariably die 10 to 20 days after the onset of clinical symptoms. The results described in this paper, on the development of glial cells and myelin in Jimpy and normal spinal cord, provide a method for positively identifying the Jimpy animals (by their relative paucity of spinal cord myelin) as early as 2 days after birth. This allowed us to follow glial cell development in the Jimpy spinal cord from early stages until the death of the animals, and to compare it with the parallel development in normal spinal cord.

In the Jimpy spinal cord we found an apparent disruption of the development of the oligodendroglial cell line such that normally developed active oligodendrocytes did not appear but were replaced by cells which by their overall appearance and topographical association resembled oligodendroglial cells, but which had more sparsly developed endoplasmic reticulum and Golgi apparatus and fewer microtubules. Some of these cells contained anomalous polymorphous lipid inclusions and/or membrane tubes. They were identified as abnormal oligodendrocytes by the occasional presence of axons embedded in their cytoplasm with typical mesaxon formation.

Previous investigators (Sidman *et al.*, 1964; Hirano *et al.*, 1969; Torii *et al.*, 1971) have suggested that the lipid containing cells in the Jimpy CNS may be microglial cells or macrophages which have phagocytosed myelin breakdown products. We feel this interpretation may be incorrect for a number of reasons. Contrary to the observations of Torii *et al.* (1971) on the Jimpy cerebellum, we found no decrease in the number of myelinated axons present in the spinal cords of older Jimpy mice; nor did we find any morphological evidence for myelin breakdown in the Jimpy spinal cord at any of the ages examined [in agreement with earlier work of Bischoff and Herschkowitz (1973) on the Jimpy optic nerve]. There was also no indication of microglial proliferation in the Jimpy spinal cord, and the lipid containing cells were uniformly distributed in the white matter and did not appear to be concentrated around the vessels. Furthermore, the lipid containing cells were already present at 2 days after birth, before there was an appreciable accumulation of myelin in the Jimpy spinal cord. The origin of the lipid inclusions within the cells was unclear, however they often appeared to be associated with endoplasmic reticulum, —either opening directly into reticular lacunae, or surrounded by reticular membranes. For the reasons described above, it seems unlikely that the lipid inclusions represent phagocytosed myelin break-down products. On the contrary, we believe, that they reflect precursors of myelin which may have failed to assemble into normal membrane and thus have accumulated in the cytoplasm of the oligodendroglial cells.

The significance of the membranous tubes found in the abnormal oligodendroglial cells in the Jimpy spinal cord is unclear. They appeared to originate as invaginations of the plasma membrane, and thus may represent dystopic myelin membrane formation.

On the basis of the results presented in this paper it may be unjustified to consider the Jimpy mouse as an analogue of human sudanophilic leukodystrophy as suggested by Sidman (1965). The Jimpy defect is probably not a demyelination, in the strictest sense, since it does not result in the accumulation of catabolic products of myelin breakdown. Rather we believe the Jimpy mouse may be an analogue of Pelizaeus-Merzbacher disease in which, according to pathogenetic theories of Merzbacher (1910) and Zeman *et al.* (1964) the myelin dysplasia results from malformation of the oligodendrocytes. This interpretation is supported by observations of Watanabe *et al.* (1969) and Schneck *et al.* (1971) who found abnormal oligodendrocytes containing lipid inclusions in the CNS material from patients with presumed Pelizaeus-Merzbacher disease. In a recent report on early lesion of Pelizaeus-Merzbacher disease Watanabe *et al.* (1973) showed "spherical inclusion bodies" in

oligodendrocytes which look very similar to the membranous tubes (in cross sections) we observed in the oligodendroglial cells of the Jimpy spinal cord.

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