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ABSENCE OF THE MAJOR DENSE LINE IN MYELIN OF THE MUTANT MOUSE 'SHIVERER'

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SUMMARY

The myelin of the central nervous system (CNS) of the mutant mouse Shiverer is characterized by the absence of the major dense line (MDL). The intraperiod line, as seen in conventional electron micrographs and in freeze-fractured replicas, appears normal. Peripheral myelin, as seen in ventral and dorsal roots of spinal cord, is unaffected by the mutation. During the period of active myelination, the cytoplasm of most oligodendrocytes (ODs) is packed with electron-lucent vacuoles in continuity with the Golgi apparatus and with bundles of microtubules. It is concluded that a metabolic pathway possibly involving the Golgi apparatus, and contributing to the formation of the MDL is selectively affected in this mutant.

One of the most intriguing processes in membrane biology is the close apposition of plasma membranes by their two faces occurring during myelination. Ultrastructural studies [9,21] established the 'jellyroll' theory of myelin formation in the peripheral nervous system. This led to the classical concept of the unit membrane, similar to that proposed on other grounds by Davson and Danielli [6]. Later, it was shown [5,15] that central nervous system (CNS) myelin was produced by oligodendrocyte (OD) processes spiraling around axons, and the identity of ODs at the ultrastructural level was firmly established [14]. Recently, replicas of freeze-fractured myelin have disclosed a great variety of particle arrays, both in the nodal and internodal region of the myelin sheath, and given further details on the initial steps of myelinogenesis [17,18,19,22].

However, much about the intimate mechanism of myelination is still unknown, both morphologically and biochemically. A fruitful approach has

proven to be the use of mutant dysmyelinic animals. Jimpy mouse [16] was found to lack myelin in the CNS at all ages studied (23), which we [20] correlated with defective maturation of ODs. Quaking mice presented a less severe dysmyelination that Jimpy, plus a dystrophy of ODs [3,13].

Here, we describe the morphological characteristics of a new mutation, Shiverer, in which the major dense line (MDL) of myelin is lacking*. The biochemical defects are reported in a companion paper [8].

Shi/Shi is a recessive autosomal mutation isolated by Bird et al. [2]. Animals provided by Dr. Bird were raised in our laboratory (U. 134 INSERM). For morphological examination, mutants 18, 38 and 90 days old, together with controls of the same age, were perfused through the heart with a mixture of 1.5% glutaraldehyde and 1.5% paraformaldehyde in a 0.1 M phosphate buffer. Blocks from cerebellum, corpus callosum, spinal cord and optic nerves were dissected out and separated in two groups. The first group was postfixed with osmic acid, stained in black with uranyl acetate and embedded in araldite. Selected areas were trimmed, semithin sections stained with toluidine blue, and ultrathin sections examined in a Siemens Elmiskop electron microscope. The second group was infiltrated for two hours with 25% glycerol, frozen in 'slush' nitrogen, and fractured in the 'cryofract' apparatus (Escaig-Reichert) (vacuum 10^{-8} Torr; temp. −150°C). Platinum replicas were examined with the electron microscope.

In semithin sections, examined with the light microscope, the corpus callosum appeared affected the most, with an almost total absence of myelin in 18-day-old animals contrasting with 50% myelination in the control. Myelin was more abundant in the white matter of the cerebellum and even more so in that of the spinal cord. There was an increase of the myelin from 18- to 38- and 90-day-old animals.

In the electron microscope, in 18- and 38-day-old mutants, many axons appeared unmyelinated in the three regions studied. Those which were myelinated had no more than ten turns of myelin, and this myelin was abnormal (Fig. 1): the MDL was absent, and the space between two intraperiod lines was occupied by an 8-nm thin sheath of OD cytoplasm. This cytoplasm was continuous with that contained in inner and outer tongues as seen in cross sections, as well as in paranodal loops. Paranodal regions appeared frequently abnormal with a profileration of paranodal loops, lacking normal contact with the axons.

In 90-day-old mutants, most axons were surrounded by thin OD processes: some of those which were myelinated had foci of normal myelin, i.e. with the formation of the MDL. However, of more than 1000 myelinated axons inspected at high power, we did not see any fully normal myelin sheath (i.e., with a continuous MDL).

The peripheral nervous system (PNS), as seen in spinal ventral and dorsal roots, appeared normal, regarding both quality and quantity of myelin (Fig. 3). It is worth noting that the CNS-PNS junction occurred most often inside the spinal cord, a rare event in the normal animal. A detailed description of

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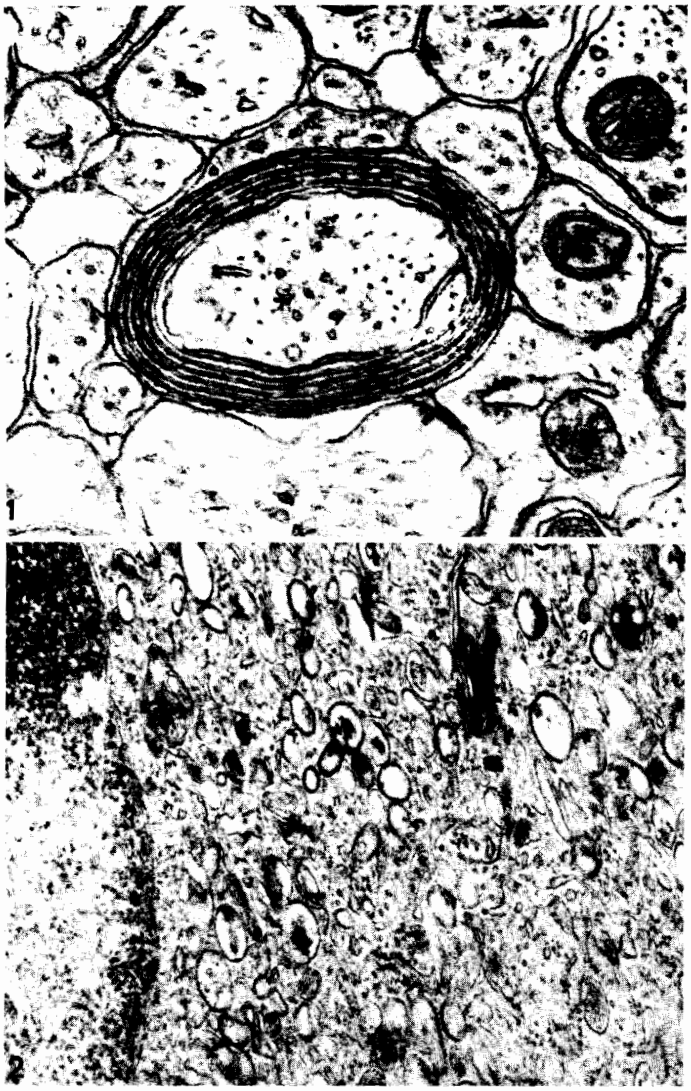

Fig. 1. 18-day-old *Shi/Shi*, cerebellar white matter. Myelinated axon showing seven turns of myelin. MDL is replaced by thin sheath of cytoplasm, continuous with inner and outer tongues. × 88,000

Fig. 2. 18-day-old *Shi/Shi*, spinal cord. Cytoplasm of most ODs is packed with electron lucent vacuoles lined with a unit membrane, some of these vacuoles have a patchy granular content. × 62,000

this region is in preparation (Private and Foncin).

Preliminary observation of freeze fracture replicas showed that the P faces of myelin had their normal content and disposition of particles, and that tight junctions [22] were present (Fig. 4). The only difference with normal myelin was the increased distance between two planes of fracture, due to the presence of the thin sheet of cytoplasm instead of the MDL.

ODs were not counted, but we had the impression that they were slightly reduced in number in 18-day-old mutants in comparison with controls of the

same age; their cytoplasm was denser and more abundant than in the control animals. At 38 days, they were clearly less numerous than in the controls. At 90 days, their number appeared slightly increased as compared to previous stages, and most of the cells were similar to mature ODs; however, less mature forms persisted.

ODs from 18-day-old *Shi/Shi* appeared markedly abnormal (Fig. 2): most of them, in the three regions studied, appeared as large cells, with a round to oval nucleus eccentrically located in the cytoplasm. The abundant cytoplasm

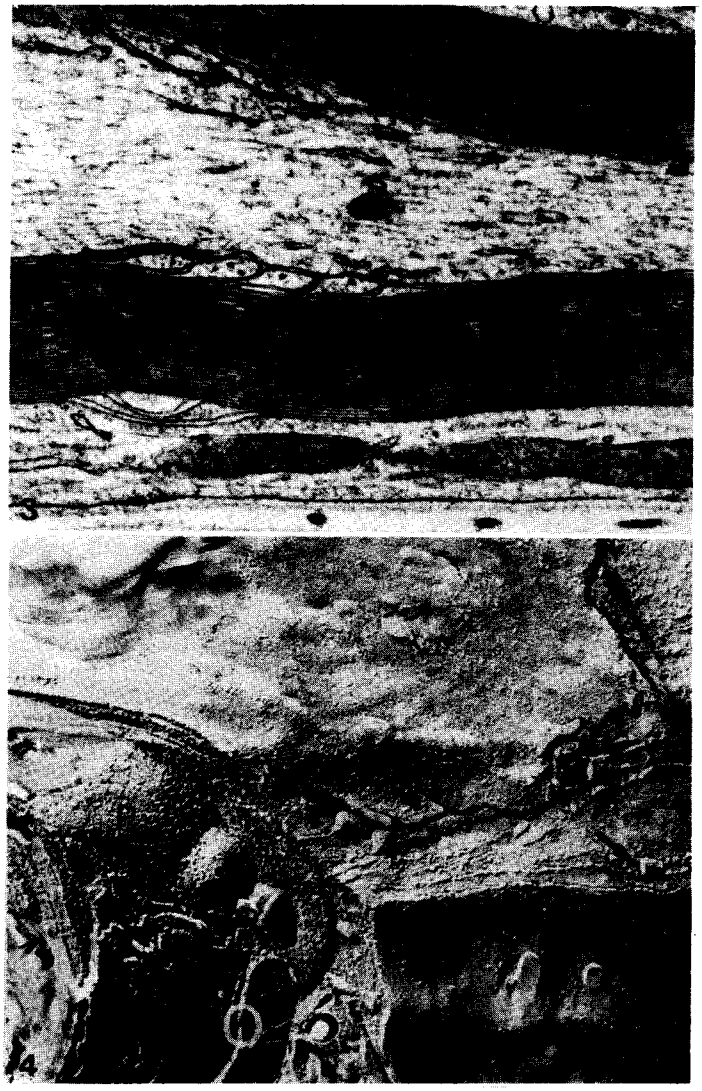

Fig. 3. 38-day-old *Shi/Shi*, ventral root of spinal cord. Peripheral myelin is compact with major and minor dense lines. × 57,000

Fig. 4. 38-day-old *Shi/Shi*, spinal cord. Freeze-fracture replica showing usual abundance of particles on myelin P faces (P) and tight junctions. The thin sheath of cytoplasm enclosed between two planes of fracture is encircled. × 70,000

was very dense and commonly filled with electron-lucent vacuoles, lined by a unit membrane, whose internal leaflet appeared thickened. These vacuoles were frequently found in continuity with the Golgi apparatus, and some of them contained dense lamellar inclusions. The cytoplasm contained also an abnormally large quantity of microtubules, swirling in small bundles. Large processes, rich in microtubules and other organelles, were found abutting onto myelinating axons.

Astrocytes appeared slightly reactive, with enlarged processes and large bundles of gliofilaments. Microglia did not show evidence of reactivity.

In 38-day animals (as compared to the previous stage) ODs were reduced in size: their cytoplasm was less abundant and of variable density. Vacuoles were rare, but some Golgi cistern had still a dense content, and tubules persisted in large numbers. Large processes were no longer seen, and the perikarya were frequently covered by two or three turns of abnormal myelin. At 90 days, the appearance of most ODs was normal: they had a dark nucleus with large chromatin clumps and a scanty cytoplasm, as dense as the nucleus. Some light and large ODs were still present.

Jimpy [16,20,23] and Quaking [3,13], the dysmyelinic mutants so far studied, do not exhibit a specific defect in the formation of the myelin sheath. Jimpy has a defect in the maturation of the OD line, but the extremely few myelinated fibers appear normal; Quaking has also a reduction of the myelin, less pronounced than in Shiverer, but again without ultrastructural abnormality. The new dysmyelinic *Shi/Shi* mutant is different from both because, in addition to the reduction of the amount of myelin, it has a specific ultrastructural malformation of myelin. The MDL, which is formed by the apposition of the cytoplasmic faces of the inner leaflet of the OD membrane, is lacking, whereas the apposition of outer leaflets, resulting in the intraperiod line, is present; tight junctions are also present, as well as the usual content of intramembranous particles, as seen on freeze-fracture replicas. It has been suggested [1,4,10,11] that the myelin basic protein (MBP) is located in the MDL, though others located it in the intraperiod line [7]. Mendell and Whitaker [12] using immunocytochemical methods found MBP in the intraperiod line of the peripheral nervous system, but were unable, for technical reasons, to see it in the CNS.

In a companion paper [8] evidence is given with biochemical and immunocytochemical techniques that MBP is lacking in this mutant. Thus, our results, taken together, strongly support the notion of localization of MBP in the MDL. There are two possible explanations for the lack of the MDL in Shiverer myelin: failure in synthesizing the basic protein or failure in the mechanism of transporting it to the myelin sheath. The involvement of MBP in experimental allergic encephalomyelitis and, possibly, in multiple sclerosis underlines the importance of the Shiverer mutation as a model for the analysis of immune and genetic mechanisms in demyelinating diseases.

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