

Ultrastructural Study on Myelination in Rat Spinal Cord during the Early Postnatal Stage

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The progress of myelination and the appearance of myelinated fibers in the anterior funiculus of the lumbar spinal cord of newborn rats were examined by electron microscopy. Myelin was seen only in the relatively larger axons on the first postnatal day, and the number of myelinated axons increased in number with age, but no tract-specific development in myelination could be observed in the anterior funiculus.

During the early development of the white matter in the spinal cord, active immature oligodendrocytes, whose cytological characteristics differed from those of mature oligodendrocytes, were seen. The cytoplasmic processes of these immature oligodendrocytes possessed electron dense material, which might be contributive to oligodendrocytic phagocytosis. This element might play a significant role in the myelination mechanism.

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Studies on the electron microscopic identification of neuroglial cells and certain aspects of gliogenesis in the CNS already have been reported by many researchers [1-4]. The morphological relationships between neuroglial cells and myelination have been reviewed by Bunge [5], Caley and Butler [6]; and recently, the serial section analysis of Knobler et al [7, 8] permitted reconstruction of the three-dimensional structure of the developing myelin sheath.

However, except for the observed morphological phenomena of myelination, the factors inducing the initiation of myelination in the CNS, as well as in the PNS, have not yet been explored, and axonal growth and the regional appearance of the myelinated fibers have not yet been satisfactorily examined.

For this reason, to obtain a better understanding of the myelination mechanism in the CNS, the present study was made by investigating the appearance and pattern of the myelinated fibers in the anterior funiculus of the lumbar spinal cord of newborn rats.

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Materials and Methods.

Sixteen rats of the Wistar strain, aged 1, 5 and 10 days after birth, were used in this study. The animals were anesthetized intraperitoneally with sodium pentobarbital and perfused through the left ventricle with 0.1 M phosphate

buffered 4% glutaraldehyde and 5% paraformaldehyde mixture containing 0.045 mM CaCl₂ (modified Karnovsky's method [9]) at room temperature for 30 min. The lumbar segments (L1, L2) were removed and immersed in the same fixative for about 24 hours at room temperature. These segments were cut transversely in 300 µm slices with Vibratome and rinsed with 0.1 M phosphate buffer solution containing 8% sucrose and 0.045 mM CaCl₂ at 0–4°C. Following postfixation with cold 1% osmium tetroxide in Caufield's buffer for 2 hours, the tissues were dehydrated in a graded ethanol series and embedded in Spurr's medium. To select the sites for electron microscopic examination, semithin sections stained with toluidine blue were examined. Ultrathin sections of the anterior funiculus of the lumbar spinal cord were then obtained using a Porter-Blum MT-2 ultramicrotome and stained with uranyl acetate and lead nitrate. After staining, the sections were examined and photographed with a Hitachi HS-9 electron microscope.

Results

The Manner of Myelination

Almost all the features of the phases of myelination in the CNS could be observed in the anterior funiculus of the lumbar spinal cord of the 1-day-old rats. Many thin oligodendrocytic processes could be seen branching and stretching into the spaces among the bare axons. At this stage, the first wrapping seemed to begin around the relatively larger axons which were embraced by the cytoplasmic arms of the oligodendrocytes. When these axons were wrapped with a few spiral folds, the cytoplasm between the spirals began to disappear in the central region of the wrapping loops and compact myelin was formed by the fusion of the major dense line. The connection between the myelin sheaths and the processes emanating from an oligodendrocyte itself was only rarely observed (Figs 2 and 3). In many cases, the myelin sheaths had both inner and outer tongues around the axons (Fig 1), but whenever the oligodendrocytic process was connected to the myelin sheath, only an inner tongue could be observed (Fig 2).

At all three postnatal stages, the number of oligodendrocytes was much smaller than the number of axons, and it could be observed that

a process of one oligodendrocyte would form the myelin sheaths for two or more axons (Fig 3). This feature was difficult to observe on a single section only.

In addition, the presence of electron dense material was frequently found in the cytoplasmic processes of the myelinating oligodendrocytes (Figs 4 and 5). These were not uniform in shape; some were taken to be lysosomes having membranous elements while others were vacuoles which included some electron dense material.

The Appearance of the Myelinated Fibers

The pattern of the myelinated fibers as seen under light microscopy on toluidine blue-stained sections is illustrated in Fig 6. On the first postnatal day, a small number of myelinated fibers could already be observed, especially in the superficial region. Various features of the early stage of myelination could also be seen, and in the superficial region, neuroglial cells, including astrocytes and microglia, were frequently found.

At this stage, the myelinated axons were approximately 0.6–1.0 µm in diameter, relatively larger than the unmyelinated axons.

At the same time, the presence of three types of neuroglial cells, i.e. astrocytes, oligodendrocytes, and microglia had already become observable. The oligodendrocytes could be fairly easily distinguished from the astrocytes, because an oligodendrocyte is conspicuous in having: a) cytoplasm more electron dense than that of an astrocyte, due to the presence of many ribosomes and rosettes of ribosomes, b) a nucleus more densely packed with heterochromatins than that of an astrocyte, and c) cytoplasm containing many microtubules, whereas that of an astrocyte contains bundles of microfilaments and glycogen granules. Though it was more difficult to distinguish the oligodendrocytes from the microglia than from the astrocyte, especially during the early developmental stage, the microglia could be identified by their poorly developed organelles, their roundish processes, and the presence of large dense bodies and lipofuscin.

We noticed oligodendrocytic types similar to those described by Mori and Leblond [3]. One type had a pale profile whose nuclei were large and roundish, and whose endoplasmic reticulum and Golgi apparatus were not apprecia-

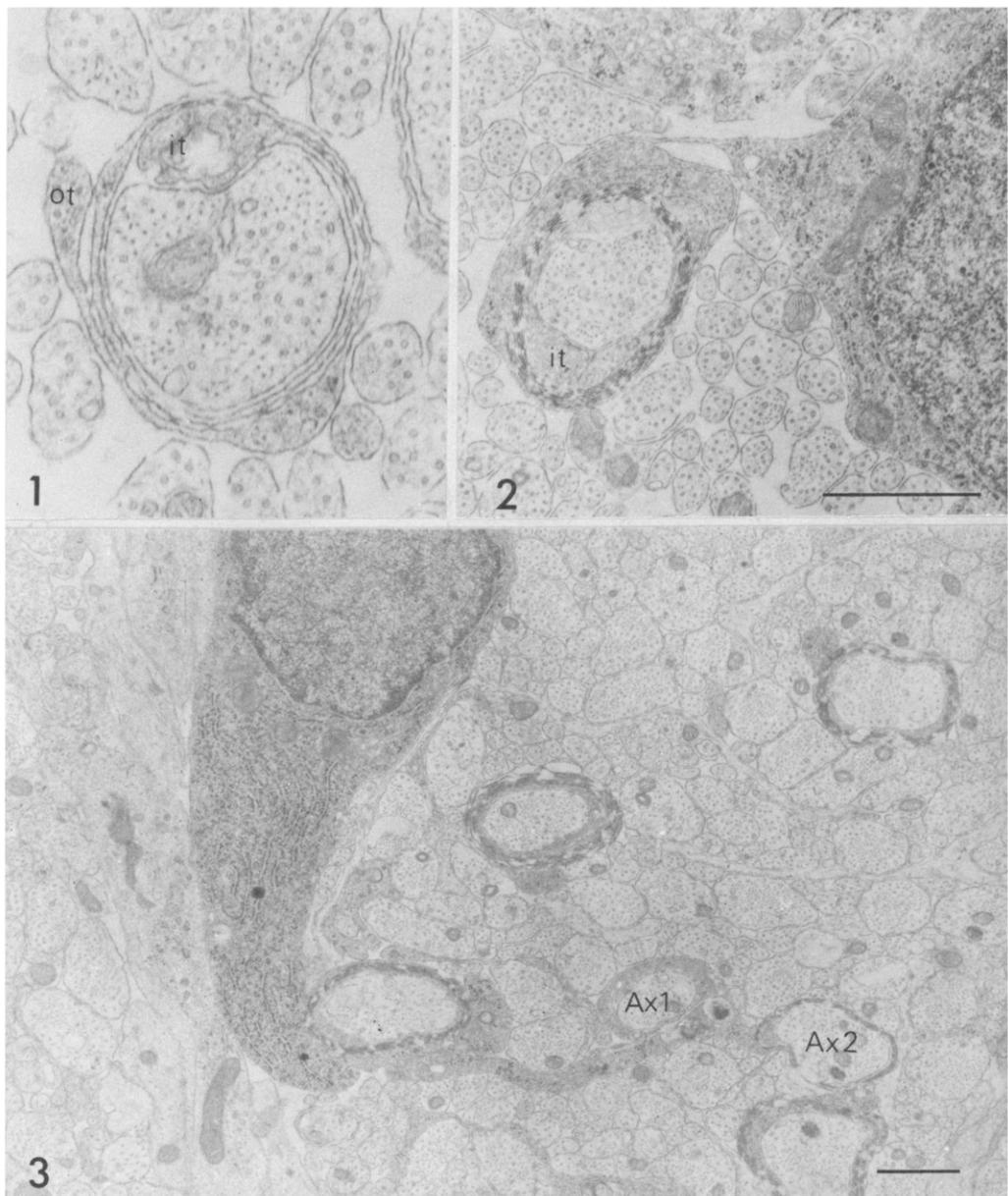


Fig 1 Early stage of myelination: First postnatal day. Myelin sheath surrounding the axon has inner (it) and outer (ot) tongues. $\times 45,500$.

Fig 2 A myelinating oligodendrocyte: First postnatal day. The profile is pale and the myelin loop has only an inner tongue (it). The scale on the photograph is 1 μm .

Fig 3 An oligodendrocytic process forming the myelin sheaths for two axons (Ax1, Ax2): First postnatal day. This oligodendrocyte has a somewhat dense profile and a well-developed endoplasmic reticulum. The scale on the photograph is 1 μm .

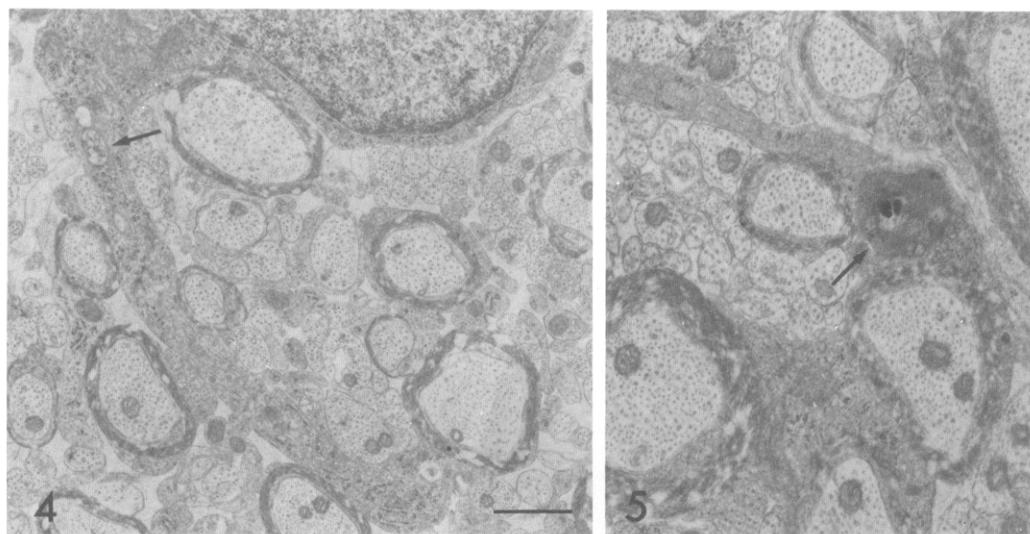


Fig 4 An active immature oligodendrocyte: Tenth postnatal day. In its process, some vesicles, including electron dense materials (arrow), can be seen. The scale on the photograph is 1 μ m.

Fig 5 A lysosome-like feature (arrow) in the process of a myelinating oligodendrocyte: Tenth postnatal day. $\times 19,000$.

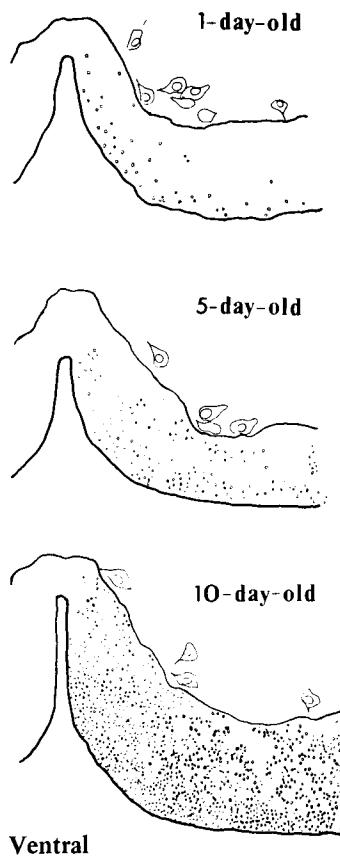
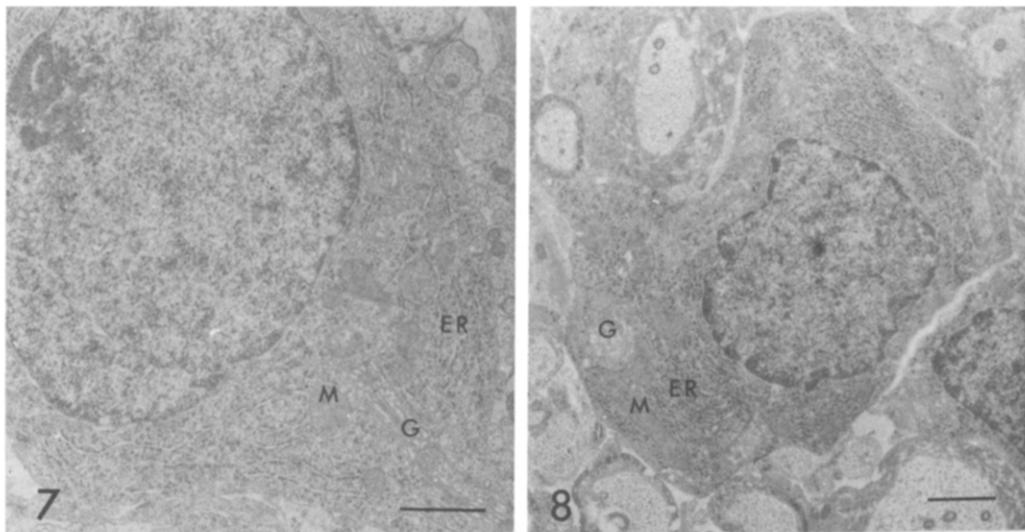


Fig 6 Distribution of myelinated axons in the anterior funiculus of the lumbar spinal cord (L1) of 1-, 5- and 10-day-old rats. These were schematically figured by dotting each myelinated fiber observed on light micrographs of 1 μ m Spurr's medium transverse sections, stained with toluidine blue. Magnification: $\times 150$.

bly developed (Fig 2). Another type possessed a well-developed endoplasmic reticulum and Golgi complex and had a more dense profile (Figs 7 and 8).

On the fifth postnatal day, the number of myelinated fibers and myelin lamellae had increased, and the sizes of the axons with myelin sheaths had also increased (Fig 9). The distribution of the myelinated fibers and neuroglial cells was as yet limited to the superficial region of the white matter, and the number of neuroglial cells seemed to have increased since the first postnatal day.

On the tenth postnatal day, myelinated fibers had appeared in the deep region of the white matter around the gray matter and their number had rapidly increased in the period during the fifth and tenth postnatal day, but no difference in the progress of myelination in the nerve tracts, i.e. tract-specific development, could be observed. Further progress in myelin ensheathment and an increase in the diameters of axons which had begun to myelinate in the



Figs 7 and 8 *Two phases in the course of continuous oligodendrocyte differentiation: Tenth postnatal day. These oligodendrocytes come to differ in their heterochromatin distribution, electron density and endoplasmic reticular development. ER: endoplasmic reticulum, G: Golgi apparatus, M: mitochondria. The scale on the photographs is 1 μ m.*

earlier stage was noted along with new myelination of the smaller bare axons. During this stage, the neuroglial cells were dispersed into the deep region of the white matter, but their number seemed to be nearly constant.

Between the fifth and the tenth postnatal day, the anterior funiculus had developed in volume, but the population density of the neuroglial cells had decreased, and the continuity between the oligodendrocytes and the myelin sheaths had become indistinct in many cases.

Discussion

The postnatal development of the white matter in the spinal cord should be considered in terms of the interaction between axonal growth and the functional differentiation of the neuroglial cells, mainly that of the oligodendrocytes.

The electron microscopic identification of various neuroglial cells in adult animals is rather easily accomplished, but it is difficult to identify neuroglial cells in early postnatal life because of their immature cytological characteristics [1, 4]. Mori and Leblond [3] classified the oligodendrocytes into three classes: light, medium shade and dark oligodendrocyte. Ling [2] showed, in studying the changes in neuroglial population in the cervical

spinal cord of newborn rats, that these three classes of oligodendrocytes appeared in turn, light, medium shade and dark oligodendrocytes, respectively, in the process of the postnatal development.

We also encountered oligodendrocytic types similar to those described by Mori and Leblond [3], but could not distinctly classify these oligodendrocytes into three classes, because of their minor differences. Actually, each oligodendrocytic type may be a transient phase in a continuous course of differentiation. One type of oligodendrocyte, observed in the white matter of the earlier postnatal stage, had a large round nucleus containing dispersed chromatin, and a high nucleo-cytoplasmic ratio (Fig 2) suggesting the possibility that this type of oligodendrocyte has a high synthetic activity.

As illustrated in Fig 6, myelinated fibers in the anterior funiculus of the lumbar spinal cord had appeared on the first postnatal day and they increased in number with age. Myelination in this area progressed rapidly from the superficial region to the deep region during the period between the fifth and tenth postnatal day. The distribution of the neuroglial cells tended to be similar to that of the appearance of the myelinated fibers, but the population density of the neuroglial cells decreased after the fifth post-

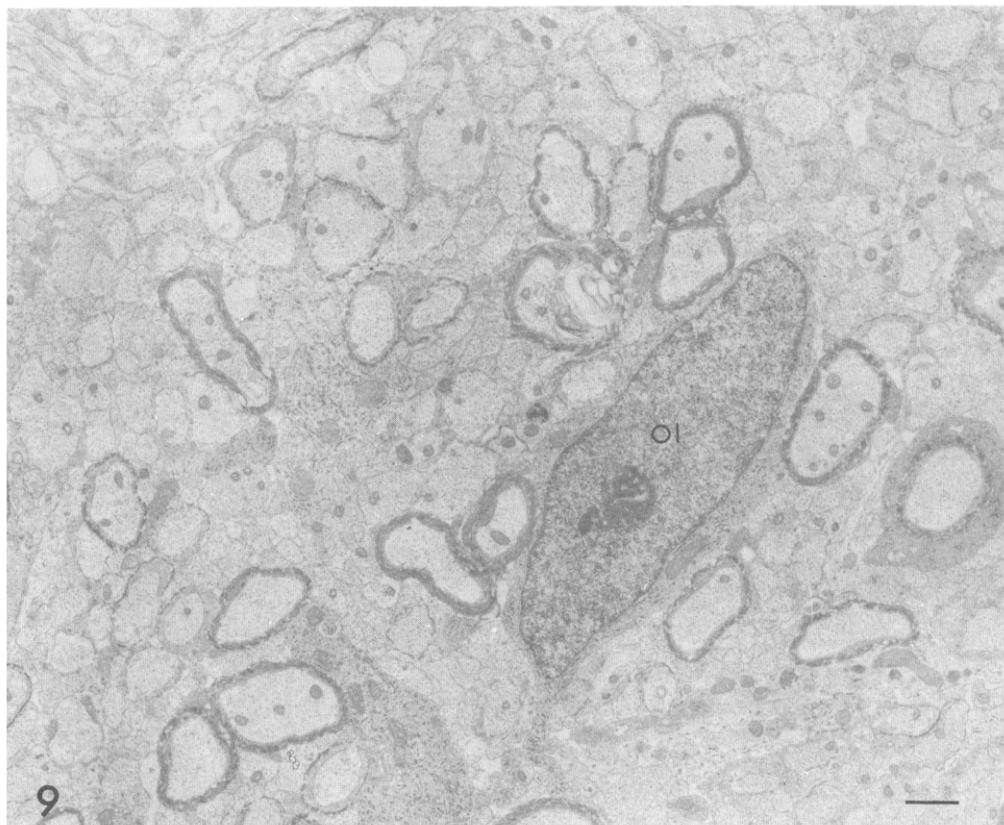


Fig 9 *Myelinated fibers in the superficial region of the white matter in lumbar spinal cord: Fifth postnatal day. Myelination can be seen beginning on the relatively larger axons.*
Ol: oligodendrocyte. The scale on the photograph is 1 μ m.

natal day. In the dorsal funiculus of the cervical spinal cord of rats, two major tracts, the cuneate and gracile fasciculi, were having distinct developmental trends and adult characteristics in terms of fiber size and myelin amount [10, 11]. In the present case, we could not show such a tract-specific development in the anterior funiculus of the lumbar spinal cord.

Myelination in the early postnatal stage is initiated on the relatively larger axons, and the number of myelin lamellae and the axonal diameters increase with age. However, on the tenth postnatal day, myelin appeared on the smaller axons, which had not formed in the earlier stages. These facts might suggest that the axonal diameter is a possible factor in the initiation of myelination in the CNS, but a strict critical diameter for myelination, such as that reported for the PNS [12], is not to

be found for the CNS [11]. And then, there is the possibility of the existence of intercellular recognition mechanisms between axons and oligodendrocytes serving as inductive factors for the initiation of myelination on the completion of axonal and oligodendrocytic maturation.

Cytologically interesting features were displayed by the myelinating oligodendrocytes. Much of the electron dense material observed in the oligodendrocytic processes exhibited lysosome-like features. This electron dense material may relate to oligodendrocytic phagocytosis. In the CNS, it is generally believed that the role of phagocytosis is played by the microglia and astrocytes. However, Inomata [13] recently reported oligodendrocytic phagocytosis in his study of the rat spinal cord following phenol-glycerin block. Moreover, in studies on the early postnatal development of the CNS, oligo-

dendrocytes were observed which contained electron dense bodies cytochemically demonstrating acid phosphatase and/or other oxidative enzyme activities [14-16]. If activity of these enzymes does in fact contribute to the normal functions of the oligodendrocyte, the dense bodies in the oligodendrocytic processes revealed in the present study might be relevant to the mechanism of myelination.

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