FORMATION OF MYELIN IN THE CENTRAL NERVOUS SYSTEM OF MICE AND RATS, AS STUDIED WITH THE ELECTRON MICROSCOPE*

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Myelin formation and its relation to the neuroglia has been a problem of continuing interest to neurocytologists since the early work of Jastrowitz (1), Boll (2), and Wlassak (3). It was not until 1928, however, that del Rio-Hortega (4) ascribed to the oligodendroglia a definite role in the process of myelinization. Since then other workers (5, 6) have supported del Rio-Hortega’s thesis, although Alpers and Haymaker (6) proposed that not only the oligodgia but the astroglia are in some way implicated in the process of myelinization.

Schmitt, Bear, and Palmer (7) by x-ray diffraction obtained a pattern suggesting that the myelin of peripheral nerves is arranged as a laminated structure. This pattern has been corroborated by the electron microscopic observations of Fernández-Morán (8), Sjöstrand (9), Geren and Raskind (10), and Robertson (11). With the resolution afforded by the electron microscope and the now available methods of fixation and thin sectioning, the mechanism of myelinization is in need of reinvestigation. Geren (12) has shown that the laminae of the myelinated sheath of a peripheral nerve are helically disposed continuations of the plasma membrane of the contiguous Schwann cell.

The myelinated sheath in the central nervous system also is comprised of closely arranged lamellae (Fernández-Morán (13) and Luse (14)). The source of these membranes in the central nervous system is not obvious, for not one but many cells may abut upon a single axon. In an attempt to determine the origin of the lamellated membranes within the myelinated sheaths of axons in the central nervous system, the brains and spinal cords of young mice and rats were examined during the period of myelinization.

Materials and Methods

Mice and rats were killed at 1, 3, 5, and 17 days after birth. Small pieces of spinal cord, medulla, and occipital cortex were rapidly removed from decapitated animals and immedi-

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ately placed in a 1 per cent osmium tetroxide solution in potassium dichromate adjusted to a pH of 7.2 (15). After fixation at room temperature for 1 to 2 hours, the tissues were rapidly dehydrated in ethanol solutions of graded concentrations (10 to 100 per cent) and infiltrated with an 8 to 1 mixture of butyl and methyl methacrylates. The tissues were embedded in the butyl and methyl methacrylate mixture to which benzoyl peroxide was added as a catalyst. Polymerization occurred at 60°C. Thin sections were cut with a Servall Porter-Blum microtome (16) and examined in an RCA EMU-2E electron microscope without removing the plastic. Initial magnifications were at 3,000 to 9,000 diameters and the micrographs were enlarged photographically as desired.

OBSERVATIONS

Myelinization of axons proceeds in a similar manner in the brain, spinal cord, and medulla, although the times at which the fibers obtain their myelinated sheaths may vary. It is not feasible to discuss the appearance of the myelin at the various times after birth, since some axons within the same field may show the beginning of a sheath while others are in a relatively advanced stage of myelinization.

Unmyelinated axons in the newborn mouse or rat are closely approximated structures which in cross-section appear irregularly round or hexagonal (Fig. 1). The axoplasm usually contains numerous small circular profiles similar to those in the synaptic endings from adult animals (17–20). One or two mitochondria are frequently observed in cross-sections of the axons. Surrounding the axoplasm is a distinct plasma membrane, the axolemma. The plasma membranes of adjacent axons are separated by a space which is approximately 100 to 200 Å wide. Occasionally there is a focal increase in density of the axolemma as is seen in Figs. 1 and 2. Where this occurs the increased density is present in both of the adjacent membranes, and is similar to the focal thickening of the plasma membranes at a terminal bar between neighboring epithelial cells (21, 22). Similar specialized contacts between adjacent unmyelinated fibers have recently been described by Schultz, Berkowitz, and Pease (23) in the spinal cord of the lamprey. Interspersed among the unmyelinated axons are occasional larger pale appearing processes which have a scant granular intracytoplasmic component. These pale processes can be traced as extensions of the oligodendroglial cells.

The first evidences of beginning myelinization are an increase in the density of the plasma membranes surrounding the axon, and a doubling of these membranes as is shown in Fig. 2. Completely surrounding the axon and external to the axolemma in Fig. 2, is a zone of pale cytoplasm containing occasional small vesicles and a scant number of dense ergastoplasmic granules. This process is enclosed by a membrane. At the upper left of the axon can be seen the prolongations of the plasma membrane enclosing the pale zone. The axolemma and the two membranes surrounding the pale zone account for the three inner membranes about the axon, while the plasma membranes of adjacent cellular proc-
esses form the outer or fourth membrane as is shown in the lower portion of Fig. 2.

Two other examples of early myelinization are illustrated in Fig. 3. In the fiber on the right (A₁) the number of lamellae surrounding the axon is inconstant, there being more membranes visible on one side of the axon than on the other. The external lamella of the membranes is comprised of plasma membranes of contiguous glial processes and thus is not continuous around the entire axon. The middle two membranes are those of an attenuated glial process, an expansion of which is visible at the lower left. The lamellar coat surrounding the other myelinating axon (A₂) is more complicated in its arrangement. The distinctly increased density of part of the axolemma and the adjacent external membranes is evident at the lower right margin of this axon. Its left margin is noteworthy, with the refolded attenuated glial cytoplasmic processes and their surrounding plasma membranes which form the laminated covering of the axon.

One of the outstanding differences between the mechanisms of central and of peripheral myelinization is seen in the axon described above. Myelinization in the central nervous system is not accomplished by the wrapping of the plasma membrane of a single cell about the axon with the same number of layers on all sides of the axon as seems to be true in the peripheral nerve (12), and with a single mesaxon as has been shown by Geren (12), Robertson (11), and Gasser (24). Rather, it is the result of a plicating of glial membranes with the possibility of a variable number of lamellae in different parts of the sheath, and with numerous attachments of glial plasma membranes to the sheath rather than with a single mesaxon as in the peripheral nerve. Whether these glial processes are from one or several cells is not yet clearly shown.

Astrocytes are relatively few in comparison with oligodendroglial cells in the early postnatal period. However, astrocytes do occur, as can be seen in Fig. 4, and are more often seen where myelinization is partially completed. The astrocyte illustrated in Fig. 4 is typical of those seen at this period with a scant perinuclear cytoplasm which extends out as tenuous delicate processes some of which surround medullated and unmedullated axons.

An oligocyte with its voluminous cytoplasm and close association with unmyelinated processes is illustrated in Fig. 5. During the period of myelinization the oligodendroglia seem to be more prominent than later. Whether this is due to an actual increase in the number of cells or to their more abundant cytoplasm is difficult to answer with certainty. Their cytoplasm is, however, quite different from that in adult animals. It is not only more abundant, but the processes are more numerous and plump. Both the Golgi apparatus and the ergastoplasmic material are prominent. The isolated granular cytoplasmic particles are more distinct and composed of larger particles in these young oligodendroglial cells than in those of older animals. The mitochondria in some
of the young oligocytes are larger and more numerous than in similar cells in adult animals but this is not a consistent observation. Occasional, dense, moderately large structures are present within the cytoplasm of oligodendroglial cells during the early postnatal period. They have no distinct inner structure and their significance is unknown.

In rare sections it is possible to see relatively longitudinal sections of myelinating neural processes isolated from other similar processes so that one can delineate the structure of the surrounding membranes. In Fig. 6 such a longitudinal section is illustrated. Here, in particular, the variation in composition of the myelinated sheath is striking. On the right, it is a dense structure resembling that seen in the adult except that it is narrower. The axolemma is distinct throughout. Elsewhere, the sheath is formed by overlapping glial membranes which enclose a moderate amount of cytoplasm. The presence of focal condensations of dense material on the plasma membranes is well demonstrated. In Fig. 7, another longitudinal section of a myelinating axon, the discontinuity of the membranes forming the myelin is distinct. The folding back on themselves of some of the membranes indicates the interruptions in the sheath which is formed of membranes from more than one process, and possibly more than one cell. In a later stage of myelinization multiple connections of the myelin lamellae with the plasma membranes of adjacent glial processes are prominent as well as the folded and reduplicated character of the glial processes (Figs. 8 and 9). By the 17th day the myelinated sheaths about many of the axons in the spinal cord have assumed their adult multilayered form with closely placed lamellae (Fig. 10).

The intimate proximity of some myelinating axons to definite oligodendrocyte processes is illustrated in Figs. 8 and 9. Frequently pale processes of indisputably oligodendroglial origin surround axons undergoing myelinization. Sometimes, as in Fig. 8, the continuation of the osmiophilic lamellae of the myelin with that of the plasma membrane of the oligodendroglial cell is apparent and inclusions of the pale oligocyte cytoplasm can actually be identified within the myelinated sheath.

The composition of the cytoplasm trapped within membranes forming the myelin sheath may differ from that of any of the cytoplasmic processes ordinarily seen in the central nervous system (Fig. 9). In these places the cytoplasm is enclosed by the membranes forming the myelinated sheath which are not yet in close apposition to each other. The enclosed cytoplasm is dense and is filled with finely granular material. Not only are fine granules present in the cytoplasm of the membrane-surrounded spaces about the axon, but occasionally there is a distinctly vesicular component (Figs. 7 and 9).

A peculiar configuration is seen repeatedly in myelinating fibers, in particular in those with a distinctly lamellar coat resembling that of the mature myelinated axon. In these axons (Fig. 11), the myelin sheath frequently appears too large for the axon it surrounds, thus forming a handle-like projection not yet
filled by the axoplasm. Since this type of configuration is rarely seen in adult material it suggests that the myelinated sheath is built larger than the axon and that the axon later grows to fill its sheath. That this might occur is not surprising, since in peripheral nerve it is known that the regenerating fibers are first small and only later attain their usual size (25). Also, in myelinating peripheral nerves (sciatic nerves of 3 day old rats) an occasional overlarge sheath has been observed (26).

Rarely complete interruptions of the myelin sheaths are observed (Fig. 12). Whether or not these represent the types of interruptions of myelin considered to be nodes of Ranvier within the central nervous system by Hess and Young (27), Bodian (28), and Pease (29) is not clear. However, distinct interruptions in the continuity of the myelinated sheath are definite. In one fiber an abrupt cessation of its myelinated sheath has been observed near the region where the axon emerges from its neuron (Fig. 13).

**DISCUSSION**

The observations presented above concerning the myelinated sheath in the central nervous system help to elucidate its structure and its mechanism of formation. Myelinization in the central nervous system differs in some ways from that in the peripheral nervous system, and in other ways resembles it.

In both the peripheral and the central nervous system the myelinated sheath is comprised of flattened laminated membranes which are derived from the plasma membranes of adjacent cells. In the peripheral nerve, in each node, the laminae of the myelinated sheath are apparently formed by the plasma membrane of the contiguous Schwann cell. In the central nervous system there is no single cell adjacent to any segment of the axon. Rather, many glial and possibly some neural elements may abut upon the axon, so that centrally many glial cell processes and their plasma membranes may be involved rather than a single cell and its processes. Thus, the sheath about a central axon may be of heterogeneous origin and need not necessarily have the same number of lamellae in all regions. Furthermore, in the peripheral nerve there is a single mesaxon; while centrally there is not a mesaxon, but rather multiple points at which glial membranes are continuous with the laminae of the myelinated sheath.

The origin of the glial processes involved in myelogenesis is of interest. Many of them, as has been illustrated here, are of oligodendroglial origin, but there is no clear indication that all of them are derived from oligocytes. And though the oligocyte is of increased prominence at the time of myelin formation, later it is the astrocytic folded membranes that closely invest all myelinated fibers centrally (14). This raises the question of functional significance of the two cell types. It is possible that the oligodendroglia supplies the membranes and the conditions necessary for myelinization, and that later, the nutrition of these fibers is subserved by the omnipresent astrocytic processes. Furthermore it is
entirely possible that one cannot with complete assurance distinguish between
the cytoplasmic processes of astrocytes and oligocytes at this period of develop-
ment. It is an accepted fact that both astrocytes and oligodendrogial cells are
being formed during the early postnatal days (30, 31) and the possibility exists
that mixed cellular forms may occur which have the ability to develop later in
either direction.

The fact that regeneration of axons is easily accomplished peripherally and
with difficulty, if at all, centrally, may be related to differences not only in form
of their myelinated sheaths, the latter being contributed to by many cells, but
also to the inability of the neural and oligocytic processes to penetrate the dense
meshwork of astrocytic processes which for the most part is formed after
myelinization is completed.

Perhaps isolated from the problem of the mechanism of myelinization and
perhaps in some way associated with it, is the presence, not only between axon
and axon, but between glial fibers and axons, and between adjacent glial fibers,
of numerous focal alterations in cell membranes. These are loci of increased
density, with a finely granular material accumulated on both plasma membranes
(Fig. 6). They are similar in appearance to the alterations present in plasma
membranes at the region of terminal bars, (21, 22), at the point of junction of
synaptic ending and neuron (17–20), and at intercalated discs in the myo-
cardium (32, 33). Their presence in immature brain and cord, and their rarity in
the adult brain and cord, other than at synapses and the terminal bars between
ependymal cells, is an observed but unexplained fact.

SUMMARY

Sections of brain and spinal cord of mice and rats at 1, 3, 5, and 17 days after
birth were examined with the electron microscope. In the early stages of myeli-

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EXPLANATION OF PLATES

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Fig. 1. Micrograph of non-myelinated axons in the spinal cord of a one day old mouse. Within the axoplasm there are numerous small circular profiles similar to those seen in synaptic endings. Occasional mitochondria are present within the axons. In a few foci the axolemma is more dense than at others as is indicated at the arrow. At the upper left is part of a pale oligodendroglial process [O]. × 42,000.

Fig. 2. Micrograph of a larger axon in the spinal cord of a day old mouse illustrating an early stage of myelinization. At the lower margin of the axon four lamellae are visible. The middle two membranes surround a zone of pale cytoplasm evident at the right, while the most external membrane is that of abutting noncontinuous glial processes. The pale region contains cytoplasm similar to that of oligodendrogial processes (See Figs. 1 and 5). At the upper left of the axon the neck of part of the invaginated glial process can be identified at the arrow. × 35,000.
(Luse: Formation of myelin in the central nervous system)
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Fig. 3. Micrograph of the spinal cord of a one day old mouse. At the lower part of the figure is a portion of an oligocyte. Its cytoplasm extends upward at the right. Several pale oligodendroglial processes (O) are present as well as unmyelinated axons and two axons (A₁, A₂) which are beginning to lay down a myelinated sheath. The myelinating fiber at the right (A₁) has four membranes at the lower right margin while only two membranes are evident at the lower left. The external of the membranes is that of contiguous glial processes, while the middle two are those surrounding an attenuated glial cytoplasmic extension which can be seen at the lower left. The lamellae surrounding the axon A₂ are more complicated, but they can be seen to continue as the limiting membranes of glial processes at the left of the axon. X 35,000.
Fig. 4. Micrograph of an astrocyte in the cerebral cortex of a rat 5 days old. The irregularly rounded nucleus occupies the central portion of the figure. The cytoplasm adjacent to the nucleus is scant and granular. It extends out as ill defined, complexly folded processes which surround processes of other cells. × 15,000.
(Luse: Formation of myelin in the central nervous system)
Fig. 5. Micrograph of part of an oligodendroglial cell in the cerebral cortex of a 3 day old rat. The cytoplasm is pale and contains a scant finely granular material. At the upper portion of the field many Golgi membranes (G) as well as some ergastoplasmic membranes (E) are present. Note that mitochondria, Golgi, and ergastoplasm are concentrated in one portion of the cytoplasm. Other pale oligocyte processes (O) are interspersed among unmyelinated axons and other cellular processes. × 12,000.
(Luse: Formation of myelin in the central nervous system)
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Fig. 6. Micrograph of a longitudinally sectioned axon in the spinal cord of a rat 5 days old. The axon is in an early stage of myelinization. At the right of the figure the sheath is similar to those in fully myelinated fibers except that it is thin. There is a dense cytoplasm (C) within the membranes forming the myelinated sheath. At the upper left the lamellae are distinctly separate with cytoplasm again being included between them. In particular, note the zones of increased density (indicated by arrows) which are similar to the terminal bars of adjacent epithelial cells. × 15,000.

Fig. 7. Micrograph of part of an axon in the spinal cord of a mouse 5 days old. Neurofilaments can be seen in the axoplasm. The discontinuous nature of the myelinated sheath in the central nervous system is evident. At the points indicated by arrows the folding back of some of the membranes forming the sheath is clear. Note the vesicular cytoplasm adjacent to the sheath at the lower left, and the similar but denser cytoplasm included in the sheath at the upper central portion of the axon. × 40,000.
(Luse: Formation of myelin in the central nervous system)
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Fig. 8. Micrograph of a portion of the spinal cord from a mouse 5 days old. An axon surrounded by its sheath is present centrally. Within the axoplasm are four mitochondria. Surrounding the axon is pale cytoplasm similar to that of the oligocyte illustrated in Fig. 5. At the left and upper margins of the axon, between the myelin sheath and the axolemma, is similar pale cytoplasm. Three definite connections of the myelin lamellae with the plasma membranes of the glial processes are illustrated at the arrows. Another may be present at the lower margin of the axon. × 35,000.

Fig. 9. Micrograph of a portion of the spinal cord of a mouse 5 days old. Two axons are present centrally. Partially surrounding them are pale oligodendroglial cytoplasmic processes (O). At the upper margin of both axons similar pale cytoplasm is present within the sheaths. In several places the sheath and the glial membranes are continuous, thus illustrating the manner in which separate glial processes are flattened to form the lamellae of the myelin sheath. This figure also illustrates that the number of lamellae may vary in different parts of the myelinated sheath. × 30,000.
(Luse: Formation of myelin in the central nervous system)
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FIG. 10. Micrograph of a small area in the cerebral cortex of a 17 day old rat. Relatively thick myelinated sheaths surround many of the axons. Although myelination is relatively advanced, in the axon to the upper left an area of incomplete myelination is present at its left margin (at the arrow). × 28,000.

FIG. 11. Micrograph of two axons which have myelinated sheaths larger than the axoplasm. The myelin extends upward without any central axoplasm. From the cerebral cortex of a rat 17 days old. × 15,000.

FIG. 12. Micrograph of a section of cerebral cortex from a rat 17 days old. At the left is a myelinated nerve fiber (M). At the right, coursing horizontally across the field is an axon, the myelin sheath of which is interrupted forming a possible "node" in the brain. × 20,000.

FIG. 13. Micrograph of a section of spinal cord from a rat 17 days old. An axon is present centrally. At the right it is covered by a myelinated sheath which is interrupted centrally. To the left the axon enlarges. This is the beginning of a myelinated sheath as the axon emerges from its neuron. × 12,000.
(Lase: Formation of myelin in the central nervous system)