An Ultrastructural Study of Spinal Nerve Roots and Dorsal Root Ganglia in Aging Rats with Spontaneous Radiculoneuropathy

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Abstract. The spinal nerve roots and dorsal ganglia of 104- to 135-week-old rats with spontaneous radiculoneuropathy were examined by light and electron microscopy.

Demyelination was common in myelinated fibers of various diameters of both ventral and dorsal roots. The most striking alteration was wide distention of myelin sheaths, which extended throughout the entire internode. The spaces formed between separated lamellae frequently were invaded by macrophages. Subsequent vesicular degeneration of myelin seemed to be mediated by invading macrophages. These processes caused complete myelin destruction, but most axons showed no degenerative changes except for obvious reduction in diameter. Occasionally, there were clumping and partial degradation of neurofilaments and ruptured axolemma in the severely demyelinated axons. A few fibers also were undergoing wallerian-type degeneration, perhaps secondary to the severe demyelinating changes. Remyelinating fibers in various phases of repair were coexistent with markedly demyelinated ones. Demyelinating changes described above also developed within some of these remyelinated internodes.

There were no remarkable changes in neurons of the dorsal root ganglia, though accumulation of lipofuscin was common.

Our findings suggest that the changes in the nerve roots are essentially a primary segmental demyelination in aging rats with radiculoneuropathy.

It is well known that posterior paralysis occurs spontaneously in senile rats more than 2 years old. Degenerative changes of the peripheral nervous system are common in these rats [3, 6, 7, 14, 36]. These changes have been reported as “radiculoneuropathy” in Sprague-Dawley rats [3, 4], and have been found in SPF Wistar strain [36], Charles River CD\textsuperscript{®} strain [8, 14] and BN/Bi, WAG/Rij and (WAG/BN)F\textsubscript{1} rats [6, 7]. It has been shown that the lesions are prominent in the spinal nerve roots, especially from the lumbar roots to the cauda equina, but are not found in the nerve cells of the dorsal root ganglia and ventral horn of the spinal cord, the origin of these nerve fibers [3, 36]. There is still no consensus on the primary site of the lesion, however, because nerve lesions in the peripheral nervous system usually include segmental demyelination with or without wallerian degeneration. Some investigators suggested that the segmental demyelination might be a primary change [3, 14]. In contrast, another investigator stated that the lesion combines wallerian degeneration and demyelination, with the former possibly being primary [36].
We examined the spinal nerve roots and dorsal root ganglia of aging rats ultrastructurally, to investigate the degenerative changes of the nerve fibers.

**Materials and Methods**

Twelve male and 14 female SPF Sprague-Dawley rats more than 104 weeks old (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Japan), untreated controls in a 30-month chronic toxicity test, were used in this study. They were housed four per wire mesh cage in a barrier system animal room with a controlled temperature of 24 ± 1°C and a relative humidity of 55 ± 5%. They were supplied with a basic diet (Oriental MF, Oriental Yeast Ltd., Japan) and tap water *ad libitum*.

Eleven males and eight females became moribund because of spontaneous diseases or tumors at various times during the chronic toxicity test (table I). Of these 19 rats, four males and three females showed posterior paresis. The three females died of the neurological impairment. At the end of the chronic test, one male and six females survived. Of these, one female showed posterior paresis.

Among 19 rats that became moribund, three males and four females, including one male and two females with clinical signs, were anesthetized with chloral hydrate injected intraperitoneally. They were perfused through the left cardiac ventricle with physiologic saline and then with 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). The dorsal and ventral nerve roots and dorsal root ganglia of the fourth to sixth lumbar segments of the spinal cord were sampled and fixed for an additional three hours in glutaraldehyde. The remaining rats (nine males and ten females), including three males and two females with clinical signs, were killed by an overdose of ether. The same nerve tissues were exposed and fixed in situ for three to five minutes with phosphate-buffered 3% glutaraldehyde. These tissues then were removed immediately, and immersed in the same fixative for 6 to 12 hours. Both perfused and nonperfused nerve tissues were post-fixed with 1% osmium tetroxide for two hours, dehydrated in graded alcohols, cleared in n-butyl glycidyl ether and embedded in epon. Semi-thin sections, 1 µm thick, were cut both longitudinally and across, and stained with toluidine blue-basic fuchsin in borax for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined an electron microscope.

**Results**

**Light microscopy**

Degenerative changes of various degrees of severity were seen in the large myelinated fibers of both dorsal and ventral roots of all the rats examined. The

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<th>Table I. Fate of untreated Sprague-Dawley rats</th>
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<tr>
<td><strong>No. of males</strong></td>
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<tr>
<td>Moribund; killed by 112 weeks old</td>
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<sup>1</sup> Figures in parentheses represent the number of rats subjected to perfusion fixation.
ventral root was more severely affected than the dorsal root. Degeneration of nerve fibers was more extensive in the rats showing neurologic signs than in clinically normal rats. The most conspicuous change was blebbing of myelin sheaths involving nearly the entire internodal segment in longitudinal sections (fig. 1), frequently accompanied by macrophage invasion into the intramyelinic spaces. Inflammatory cells were rare in the interstitium. Longitudinal sections showed that the demyelinating processes resulted in complete destruction of the sheath in the demyelinating internode (fig. 2). In the nerve fibers with these demyelinating changes, the axon generally looked normal, except for a reduction in diameter. Some fibers had swollen, fragmented axons and separation into a discontinuous series of myelin ovoids, which indicate wallerian-type degeneration. In spite of the evident demyelinating changes, thinly myelinated fibers also were common in these lesions, indicating that active remyelination was coexistent with demyelination (fig. 3). Several consecutive remyelinated internodes often were found in longitudinal sections (fig. 4). Intercalated nodes were seen occasionally (fig. 5). Within these remyelinated nodes, blebbing of the myelin sheath and invasion of macrophages sometimes had developed (fig. 6). There were occasional patches of collagen fibers in the severely affected nerve roots, especially prominent in the ventral root.

In the dorsal root ganglia, lipofuscin-like inclusions were seen in the nerve cells.

**Electron microscopy**

The ultrastructural changes of degenerating fibers seemed to be qualitatively similar in the ventral and dorsal roots, but were more extensive in the former.

*Demyelination.* Blebbing of myelin sheaths, the most conspicuous light microscopic characteristic in this disease, was identified ultrastructurally as wide distention of myelin sheaths or periaxonal spaces mainly due to splitting of the intraperiod lines (fig. 7, 8). These lesions were seen in myelinated fibers of various sizes, but were more prominent in those of large diameter. In some parts showing further active disintegration of myelin, major dense lines had been opened focally. While the spaces formed between separated lamellae usually were clear, they sometimes contained macrophages laden with myelin debris and clumps of damaged myelin. Stripping of the myelin lamellae by invading macrophage processes was seen occasionally in some parts of these fibers (fig. 9), though the myelin splitting was not always associated with macrophage invasion. Distended sheaths invaded by macrophages sometimes showed vesicular dissolution of myelin, resulting in reduced thickness of the myelin sheath (fig. 10). This vesiculation usually was associated with the presence of macrophages. In severely affected fibers, there was complete destruction of the myelin sheath due to advanced vesiculation and phagocytosis of degraded myelin (fig. 11). The basement membrane surrounding Schwann’s cells remained intact in most of these severely affected fibers, though there were occasional defects in these membranes through which degradative products of myelin passed into the interstitium. Occasionally, similar demyelinating changes consisting of wide distention of myelin sheaths were seen in an already remyelinated internode.
Fig. 1: Longitudinal section of ventral root from rat with clinical signs. Blebbing of myelin sheaths involves internodal segment in myelinated fiber, with invasion of macrophages in intramyelinic spaces. Node of Ranvier (arrow). 1-μm section, toluidine blue-basic fuchsin. Bar = 50 μm.

Fig. 2: Longitudinal section of ventral root from rat with no clinical signs. Area where disintegrating myelin sheath with blebbing passes into myelin destruction (arrows). Axon (A) looks normal. 1-μm section, toluidine blue-basic fuchsin. Bar = 25 μm.

Fig. 3: Cross section of ventral root from rat with clinical signs. Numerous remyelinated axons coexistent with intact fibers, and demyelinated ones with blebbing of myelin. Toluidine blue-basic fuchsin. Bar = 40 μm.

Fig. 4: Consecutive remyelinated internode. Node of Ranvier (arrows). Toluidine blue-basic fuchsin. Bar = 25 μm.

Fig. 5: Intercalated internode. Node of Ranvier (arrow). Toluidine blue-basic fuchsin. Bar = 20 μm.

Fig. 6: Demyelination (D) in remyelinated internode. Toluidine blue-basic fuchsin. Bar = 30 μm.

Fig. 7: Distended myelin sheath. Axon reduced in diameter. Bar = 5 μm. Inset: Detail of axon. Increase in density of neurofilaments and microtubules. Bar = 2 μm.
Axonal changes. Most of the fibers with marked distention of the myelin sheath had axons of reduced diameters (fig. 7, 8, 11). The density of neurofilaments and microtubules was increased strikingly within these axis cylinders. In severely affected fibers showing complete destruction of the myelin sheath, the axolemma occasionally ruptured and its axoplasm was exposed to the surrounding degraded myelin (fig. 12). Some of these axons contained an increased number of neurofilaments. Partial degradation of these neurofilaments with a finely granular dark profile was seen in several parts.

In some parts of the fibers where no demyelinating process was evident, there was occasionally axonal degeneration consisting of accumulations of mitochondria of various sizes, multilamellated bodies, vesicular elements and neurofilaments (fig. 13). The myelin sheath surrounding these axons usually looked normal. Some shrunken axons were surrounded by collapsed segments of the myelin sheaths. Several Schwann's cells contained large masses of myelin fragments (fig. 14).

The axonal changes were less common than the demyelinating changes. In severely damaged roots, there were scattered areas in which nerve fibers were replaced by endoneurial collagen.

Remyelination. Thinly myelinated axons of various diameter usually were concur-
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Fig. 9: Invading macrophage extends cytoplasmic process between myelin lamellae (arrow). Bar = 2 μm.

Fig. 10: Vesicular dissolution of myelin sheath. Macrophage laden with myelin debris contacts altered myelin sheath. Thickness of sheath (arrows) remarkably reduced. Bar = 2.5 μm.

Fig. 11: Completely demyelinated axon (A). Cytoplasmic process of macrophage stretches through disconnected basement membrane (arrow). Macrophage, M; Schwann's cell, S. Bar = 2 μm.

rent with those subjected to obvious demyelination, as seen by light microscopy, in severely affected nerve roots. There were several proliferative Schwann's cells and macrophages containing myelin debris surrounding the denuded axon. In addition, an axon enclosed by proliferated Schwann's cells also was found within a single basement membrane (fig. 15). These axons showed increased density of neurofilaments and microtubules, possibly attributable to a reduction of axon diameter found in demyelinated axons. There were also some axons invested by new myelin sheaths consisting of several major dense lines, which indicate an early phase of remyelination (fig. 16). Occasionally, outside of the basement membrane enclosing remyelinated axons, there was a profusely folded basement membrane that presumably represented the basement membrane of the original fiber before demyelination had taken place. On the other hand, the bands of Büngner with multiple axonal sprouts, which indicate the early phase of regeneration, rarely were seen. Bundles of three to five regenerating myelinated and unmyelinated fibers surrounded by a common basement membrane were seen infrequently. No onion bulb formation was seen.
Fig. 12: Cross section of nerve fiber: complete destruction of myelin sheath. Degradative myelin products spread to interstitium. Disruption of axolemma and increased number of neurofilaments (NF) in axon. Partial degradation of neurofilaments with finely granular dark profile (arrows). Bar = 2 μm.

Dorsal root ganglia. There was no significant damage, such as neuronal necrosis with subsequent proliferation of satellite cells to form nodules of Nageotte. Many ganglion cells contained lipofuscin granules in various amounts (fig. 17). There was some mild degeneration of the nerve cells such as a reduction of the rough endoplasmic reticulum, indicating central chromatolysis; slight distention of the endoplasmic reticulum (fig. 17); and hyperchromatic nuclei. Some satellite cells showed an increase in the size of the nucleus and nucleolus.

Discussion

It has been reported that focal blebbing of the myelin sheath is a significant histological feature in the radiculoneuropathy of aging rats [3, 14, 17]. This change is seen frequently in the spinal nerve roots, and to a lesser extent in the distal nerve trunk. In our study, this blebbing was identified as segmental demyelination, which was characterized by wide distention of the myelin sheath due to interlamellar splitting, accompanied by reduction of axon diameter. This separation of myelin has been seen in immune-mediated diseases such as experimental allergic encephalomyelitis [18, 27], experimental allergic neuritis [28, 29], and Landry-Guillain-Barré
Fig. 13: Axonal degeneration: accumulation of mitochondria and multilamellar bodies. Axon covered by intact myelin sheath. Bar = 1 μm.

Fig. 14: Schwann’s cell contains large mass of collapsed myelin ovoid in cytoplasm. Bar = 2 μm.

Fig. 15: Axon enclosed by Schwann cell. Axon shows increasing density of neurofilaments and microtubules. Bar = 2 μm.

syndrome in man [25], as well as in toxic neuropathies of lead [21], alkyl-tin [1], and tellurium [20]. Especially in experimental allergic neuritis [28], it was shown that this demyelination resulted in a reduction of axon diameter. On the other hand, similar splitting has been seen in uremic neuropathy in man, in which the nature of the fiber degeneration is that of distal axonal degeneration, and has been considered to be secondary to axon shrinkage [12]. This type of demyelination also may occur in the peripheral nerves of patients with Friedreich’s ataxia showing fiber degeneration of
axonal atrophy [13]. The question thus arises as to whether the demyelinating changes found in our study are secondary to axon shrinkage. In uremic neuropathy, this demyelinating change is seen in the distal portion of the nerve where striking loss of nerve fibers is found [2, 34], and no abnormality is seen in the spinal nerve roots [2]. It has been thought that this axonal degeneration may result from a metabolic failure of the peripheral neuron [12]. In Friedreich's ataxia, there are atrophy and disappearance of many cells of the spinal ganglia [16]. Thus, axon shrinkage due to primary neuronal disorders may cause the demyelinating changes in the distal nerve fibers. We did not do single teased-fiber studies nor morphometric studies on nerve fibers. Our ultrastructural observations, however, showed that even in severe cases, obvious demyelination and remyelination were common in many nerve fibers, whereas loss of nerve fibers and regeneration were less common. There was no evidence that axon shrinkage in the fibers with obvious demyelination passed into complete axon death. Furthermore, no conspicuous change was found in the dorsal root ganglia. Therefore, the present findings in the spinal nerve root may be inconsistent with those in diseases that cause segmental demyelination secondary to axon degeneration or atrophy.
The other type of myelin destruction, an invasion of macrophages into the distended sheaths, was common in our study. Stripping of myelin lamellae by invading macrophages was seen rarely. Although this stripping by macrophages is seen most commonly in experimental allergic neuropathies [18, 19, 27, 29] and virus-induced demyelinating diseases [9, 22], it also has been reported in other types of demyelinating diseases unrelated to immunologic or infectious mechanisms [20, 21, 33]. In addition to this demyelination, vesicular dissolution of myelin was seen in the distended sheaths, usually in the presence of macrophages. This vesiculation, a well-known feature in myelin breakdown, has been found in many diseases associated with degeneration of myelin sheaths [10, 18, 19, 27, 29, 30, 38]. Thus, these two types of demyelination, macrophage invasion and vesiculation, are not specific to this radiculoneuropathy, but are common patterns of myelin breakdown in general. These demyelinating changes are considered to be caused by macrophages invading to eliminate the degraded myelin.

A few axons showed reactive changes consisting of accumulations of axoplasmic organelles, but were surrounded by normal myelin sheaths. These reactive axonal changes developed at the proximal and distal portions of severely affected axons in experimental allergic encephalomyelitis [18]. Further axonal lesions accompanied by collapsed myelin sheaths, consistent with wallerian degeneration [23], also were seen occasionally in our study. In addition, disruption of axolemma and partial degradation of neurofilaments were found occasionally in the completely demyelinated axons. It is known that any damage to the neurofilaments may lead to wallerian-type degeneration of the nerve fiber, since they probably are involved in axoplasmic transport [32]. From these observations, it is suspected that the reactive axonal change and wallerian-type degeneration in this radiculoneuropathy may extend from the area where degradation of the axolemma and neurofilaments of the axon occurred in association with complete demyelination.

An increased number of neurofilaments in some axons showing complete destruction of the myelin sheath, as in our rats, has been reported in several regenerating axons in isoniazid neuropathy [31] and in sprouting axons soon after nerve crushing [11, 24]. This accumulation of neurofilaments in our study may imply the reactive or reparative action of axons subjected to severe demyelination. This change, however, is considered to be a different sort of age-related change from that in the radiculoneuropathy, because this change resembles the ultrastructural features of eosinophilic axonal swellings or spheroids in the gracile and cuneate nuclei of aged rats and cats [26, 37].

Reparative changes of nerve fibers concomitant with obvious demyelination are suggestive of remyelination, and are similar to those reported in demyelinating diseases [25, 28]. Frequent occurrence of consecutive remyelinated internodes rather than intercalated nodes may imply that progressive and continuous demyelination took place in the radiculoneuropathy. Demyelination that develops in an already remyelinated internode is a peculiar type of demyelinating process, similar to that in
isolated nerve fibers proximal to amputation neuromas [33], though ultrastructural analysis of single teased fibers has yet to be done. This finding may suggest that repeated demyelination and remyelination occur in the radiculoneuropathy. Typical onion bulb formation, however, was not found in our rats. For this reason, it is assumed that further episodes of repeated alterations may not occur in this disease.

Except for the presence of lipofuscin granules, which are common in the dorsal root ganglion cells of aging animals [5, 15], there were no remarkable changes leading to severe damage of the distal portion of the nerve fibers in the dorsal root ganglia. In the distal peripheral nerve of aging rats with this radiculoneuropathy, wallerian-type fiber degeneration and loss of myelinated fibers have been seen in addition to demyelination [8, 36]. Furthermore, there is nerve fiber degeneration in the distal end of the long tract such as the gracile tract of the spinal cord [36]. From these findings, we suspect that the lesion in the peripheral nervous system of this radiculoneuropathy is a primary axonal degeneration of the “dying back” type. Recently, ultrastructural studies on the tibial and plantar nerves of Wistar rats from 18 to 24 months old have shown that morphological evidence of a distal axonal degeneration of “dying back” type increased with increasing age [35]. This report, however, did not document any focal ballooning of the myelin sheath. It is uncertain whether the lesion in the spinal nerve root in this radiculoneuropathy is related to this age change in the distal nerve.

The cause of this radiculoneuropathy is unknown. Our findings suggest that the changes of the nerve roots in aging rat radiculoneuropathy are essentially a primary segmental demyelination. Further investigation is required, however, to ascertain the total picture of this disease. Ultrastructural examination of the distal peripheral nerve in these rats is now in progress.

References

6 Burek, J.D.: Pathology of Aging Rats, pp. 191-197. CRC Press, Fla., 1978
9 Dal Canto, M.C.; Lipton, H.L.: Primary demyelination in Theiler’s virus infection: an ultrastructural study. Lab Invest 33:626-637, 1975
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