Degenerative Myelopathy in Three Strains of Aging Rats

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Abstract. Spontaneous paresis and paralysis associated with degenerative spinal cord and spinal nerve root lesions occurred in three strains of rats used in studies of aging. Focal or segmental spinal cord lesions had mild to severe demyelination, loss of nerve axons, and lipid-filled gitter cells. The lesions were limited to the white matter and were most severe in the lateral and ventral funiculi. The nerve roots had cholesterol clefts, focal hemorrhage, and demyelination. Atrophy of the skeletal muscle probably was secondary to the cord lesions. Vertebral lesions that involved the spinal canal and vascular blood flow were found, which may explain pathogenesis.

Spontaneous posterior paralysis with associated spinal cord and spinal nerve root lesions occurs in several rat strains [3, 5, 13]. The syndrome is usually associated with aging since the paralysis has been seen only in rats more than 2 years old. Along with paralysis, severe atrophy of skeletal muscle in the posterior part of the body also has been seen. The condition is progressive and eventually results in death. The cause and pathogenesis are unknown. Some rats had tumors in the spinal cord or canal that could have caused paralysis whilst others had large pituitary tumors. However, in most rats, no specific cause could be found.

Three different strains of rats were kept for aging studies at the Institute for Experimental Gerontology. Sporadic cases of paralysis occurred in two strains, but the incidence was very low. We have noticed that the third strain (F₁ hybrid of the other two strains) has a much higher incidence of posterior paresis and paralysis. Preliminary studies on these rats are presented and the pathologic features are compared to those in other reports [3, 5, 9, 13].
Table 1. Number of paralyzed rats versus number examined

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Number with paralysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
</tr>
<tr>
<td>BN/Bi</td>
<td>48 male, 106 female</td>
</tr>
<tr>
<td>WAG/Rij</td>
<td>51 male, 95 female</td>
</tr>
<tr>
<td>F₁</td>
<td>58 male, 68 female</td>
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</tbody>
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Materials and Methods

The Brown Norway rat strain, BN/Bi, was started by Silvers and Billingham in 1958. The original source of the rats was the Microbiological Associates Inc., Bethesda, Md., in 1963. We have inbred these rats since then, and they are now in their 20th generation. The WAG/Rij rat is a small Wistar rat obtained from Glaxo Laboratories, Greenford, Middlesex, England, in 1953 and inbred since then. It is now in its 39th generation. The third strain is the F₁ hybrid of the BN/Bi and WAG/Rij rats.

All three strains were maintained under similar conditions as described [4, 7]. The rats were allowed to complete their lifespan so that various neoplastic and non-neoplastic lesions associated with aging could be studied. All rats found dead or killed when moribund were necropsied using routine techniques [4]. Although all organs were examined, only findings in the spinal cord, spinal nerve roots, vertebral column, and skeletal muscle will be discussed.

Sections of cervical, thoracic, and lumbosacral cord were taken from all paralyzed rats. In addition, 12 F₁ rats (table II) were examined by blocking the spinal cord and taking 25–30 semi-serial sections from the length of the cord and nerve roots. Six of these rats were fixed by whole-body perfusion with formalin as described [8], and the other six were formalin-fixed without perfusion. After fixation, the spinal column with cord was decalcified, paraffin-embedded, sectioned at 6 μm, and stained with hematoxylin-phloxine-saffron (HPS). In addition, selected sections were stained with Luxol fast blue – periodic acid-Schiff (LFB-PAS).

Results

The incidence of clinical paralysis in the three strains is summarized in table I. The rats were from 15 to 42 months old. All but three were older than 24 months. The age when clinical disease began could not be determined. These rats had obvious paralysis at time of death. Rats with mild or questionable clinical disease were not considered paralyzed. For the BN/Bi and WAG/Rij the incidence was about 2%. For the F₁, however, the incidence was 21%. All but one F₁ rat with paralysis were males so the incidence in males only was 45%.
Spinal Cord and Nerve Roots

The microscopic lesions in the spinal cord consisted of severe demyelination, distended axon sheaths, swollen or absent axons, variable numbers of lipid-filled macrophages (gitter cells), and numerous swollen astrocytes (fig. 1, 2).

The lesions were limited to the white matter and were most extensive in the ventral and lateral funiculi. The lesions in the dorsal funiculi were mild compared to the lateral and ventral changes. In most cases the malacia was bilaterally symmetrical.

The 12 F1 rats had similar lesions with a similar distribution (table II). The changes in the cervical cord from C1 to C3 were slight or absent. There were severe lesions in the lateral and ventral columns, developing at approximately C5, 6 and 7 and becoming most severe between T1 and 4. From T4
posteriorly the lesions gradually became less severe, and by the lumbar segments the lesions were slight or absent.

The lesions in the nerve roots consisted of mild to severe demyelination, distended myelin sheaths, swollen or absent axons, and variable numbers of lipid-filled gitter cells (fig. 3, 4). Also, there were rhomboid clefts resembling cholesterol clefts, hemosiderin, and occasional hemorrhages (fig. 5).

The nerve root lesions occurred caudal to T3. Ventral roots were always affected. Dorsal roots in the thoracic region were either normal or slightly affected. Lesions in the ventral roots in this area were more severe than those in the dorsal roots.

In the lumbosacral region, the dorsal and ventral roots were both affected to about the same degree, but the severity varied between individual roots.

**Skeletal Muscle**

All rats had grossly obvious atrophy of the loin and hind legs. The muscle changes were usually advanced at necropsy. The muscle changes were atrophic muscle fibers; muscle nuclei either clumped or in rows; and swollen, hyaline, muscle fibers, with loss of cross striations (fig. 6). Fibers

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**Table II.** Distribution and severity* of lesions in the spinal cord and nerve roots of F₁ male rats

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Cervical 1 to 3 D L V</th>
<th>Cervical 4 to 7 D L V</th>
<th>Thoracic 1 to 4 D L V</th>
<th>Thoracic 5 to lumbosacral D L V</th>
<th>Spinal nerve roots in lumbosacral region</th>
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</thead>
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<tr>
<td>1</td>
<td>− − ++</td>
<td>± +</td>
<td>++</td>
<td>+++</td>
<td>− − +</td>
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<tr>
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<td>− − +</td>
<td>− − −</td>
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</tr>
<tr>
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</tr>
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<td>− − +</td>
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</tbody>
</table>

*Range of severity: − (normal), ± (questionable), +(mild), ++(moderate), +++(severe).

D = dorsal funiculi; L = lateral funiculi; V = ventral funiculi.
Fig. 2. Cross section of severely affected lateral column with swollen astrocytes, distended axon sheaths, absence of nerve axons, and occasional gitter cells. LFB-PAS.

Fig. 3. Caudal portion of lumbar spinal cord with nerve roots. Necrosis of bone (↑↑), amorphous mineral deposits (↑), and severe degeneration of nerve roots. LFB-PAS.
occasionally were fragmented. Fatty tissue often separated the remaining muscle bundles. Lesions varied between muscle bundles, and various degrees of severity could be found even in one section. A systematic study of the peripheral nerves was not done.

Vertebral Lesions

Degeneration of intervertebral disks was seen in nearly all rats with paralysis. In these animals, there was some degree of degeneration in the majority of disks available for study. Degeneration was especially noticeable in disks of the F1 rats. There was occasional protrusion or rupture of the disks resulting in compression of the spinal cord (fig. 7). Many vertebrae contained large areas of aseptic necrosis of bone (fig. 8). The areas of bone necrosis
Fig. 6. Skeletal muscle with variation in size of muscle fibers, focal loss of cross-striations, and prominence of sarcolemmal nuclei. HPS.

Fig. 7. Two protruding intervertebral disks resulting in slight compression of spinal cord. Amorphous disk material (arrow). HPS.
Fig. 8. Multifocal aseptic necrosis of bone in the vertebral body (arrows). HPS.

Fig. 9. Localized compression of the ventral spinal artery in the cervical region by amorphous disk material. HPS.

varied in size and location between individual vertebrae and, as with the disks, nearly all vertebrae were affected in some rats, whereas in others only one or two vertebrae had changes. In some sections it appeared that pieces of bone had broken away and were in the intervertebral foramen or subdural space. At times arterial or venous obstruction was seen (fig. 9).
Finally, the dura and the epidural space (cavum epidurale) were often replaced by fibrous tissue. Cartilage, bone, and even marrow elements occasionally were seen in this region. There also was fibrosis in many intervertebral foramina. Proliferative exostoses and marginal osteophytes were seen infrequently on both the dorsal and ventral surface of the vertebral canal.

Discussion

The changes in the spinal nerve roots of the BN/Bi, WAG/Rij rats were similar. Complete semi-serial sections of spinal cords were not available from the three BN/Bi and three WAG/Rij rats, but based on the available material, spinal cord changes and vertebral bone lesions were comparable to those of the F₁ rats. The most severe lesions in the spinal cord were usually found between C5 and T4. The remaining spinal cord had less severe changes or appeared normal. The nerve roots had the most severe lesions in the lumbosacral region.

All these strains had multiple vertebral bone changes including intervertebral disk protrusions, dural fibrosis, fibrosis of the intervertebral foramina, aseptic necrosis of bone, osteophytes, and exostoses. Most rats had more than one of these conditions. The F₁ rats had more degenerative bone disease than the BN/Bi or WAG/Rij rats. It also appeared that the bone lesions were more pronounced in male F₁ rats. It has been reported that there are similar disk changes, prolapsed intervertebral disks and associated demyelination of nerve roots in the mastomys (*Praomys natalensis*) and that the disease is more severe and more frequent in males than females [12]. Older rats have some degree of aseptic necrosis of bone, and in some cases, the bone necrosis of the vertebral epiphyses leads to thoracolumbar kyphosis [11]. Thoracolumbar kyphosis has been seen in a few F₁ rats from our colony. Chondromucoid degeneration of articular cartilage of old rats is frequently seen in all three strains [10]. This condition does not appear to be related to the severe bone necrosis and other lesions described in this report.

In studies on paralysis in rats, the nerve root lesions have been discussed and parts of the spinal cord and skeletal muscle examined. Vertebral bone or disk changes have not been mentioned. The nerve root lesions are identical to those in our rats. The nerve root lesions described in the mastomys [12] are also similar to the changes in rats. Various authors have given different descriptions of the lesions in the spinal cord. In one paper [5], only the
caudal thoracic and the lumbosacral cord were studied, and in these regions the lesions are similar to those presented in our paper. No specific area of the cord was affected in rats from another study [13], and the dorsal column lesions appeared more pronounced than those in the rats reported here. The lesions in spinal cords from Sprague-Dawley rats were not confined to any particular region [3]. A demyelinating disease of two rats has been described [9]. The localization in the lateral and ventral columns is similar to that in our rats. Also, the lower sacral cord was normal in one rat. In the other and more severely affected rat, the dorsal column in the cervical cord region was also affected. However, the two rats were young, and nerve root lesions were not found.

The muscle changes we noted in the three rat strains were microscopically similar to changes in other reports [2,13]. Because the peripheral nerves and muscle changes have not been examined systematically, the cord lesions and muscle disease cannot be correlated. In spite of this, the muscle changes are thought to be secondary to the nerve root and spinal cord demyelination. The fact that muscle atrophy is seen only in animals with some degree of paresis or paralysis tends to support this. All rats with muscle atrophy had severe cord and nerve root lesions. Also, many animals with advanced disease had obvious urinary incontinence, and some had enlarged urinary bladders at time of death. This suggests damage to the innervation of the urinary bladder.

An idiopathic cervical myelopathy occurs in man, and many causes have been suggested [1,6]. Among them are narrowing of the spinal canal with cord compression and spasm of the lateral spinal arteries along with fibrosis of nerve root-sleeves. These changes may lead to ischemia, especially in the cervical region. The types of lesions in our rats suggested that similar conditions could occur. There was fibrosis of intervertebral foramina in most rats. At times it appeared there was some vascular compression. In other rats, chips of necrotic bone were found compressing blood vessels. In a few sections, intervertebral disk protrusion did cause localized compression of the ventral artery and vein in the cervical region.

Most of the lesions in the spinal cord and nerve roots could be the result of the multiple bone and disk lesions with the accompanying subdural fibrosis. These changes may result in interference or obstruction to the cord’s arterial supply, or venous return, or both. The muscular atrophy seems secondary to the spinal cord disease. To prove a relationship between the various lesions, it will be necessary to conduct more detailed systematic studies with serial killings, especially of F1 rats, which show the most
pronounced lesions in high frequency. The sequence of events may thus be studied to find the cause and pathogenesis of the posterior paralysis and accompanying lesions in aging rats.

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References