Spinal Nerve Root Degeneration in Aging Laboratory Rats: A Light Microscopic Study

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ABSTRACT
Degeneration of unknown etiology was noted in the spinal nerve roots of aging rats. These animals (Charles River CD® rats) were included in a study of long-term effects of ionizing radiation. The initial lesion was a demyelination of an individual axon or a few, isolated, scattered axons in the ventral roots of rats between 18 and 20 months of age. As the lesion progressed, the dorsal roots showed involvement, and by 24 months the usual finding was marked degeneration in ventral roots with degenerative changes of a lesser degree in the dorsal roots. When degeneration was complete, connective tissue filled the regions normally occupied by neural tissue. The lesion was unrelated to the radiation procedure, occurring with equal frequency in both irradiated and non-irradiated animals. Furthermore, lesions occurred with equal frequency in both males and females. The one factor to which the lesion appeared to be related was age since it was not observed in animals less than 18 months old but was seen in 66.7% of those between 18 and 20 months and in 96.0% of those older than 20 months. These data indicate that this lesion needs to be considered when using aging laboratory rats.

Knowledge of the normal or usual situation in an experimental system is basic to biological research. Without this information, experimentally induced alterations cannot be determined or evaluated properly. One of the factors often complicating the interpretation of data is the lack of information regarding what might be expected in an intact, "normal" control animal of an age not commonly used in laboratory investigations. Such a situation arose recently during the terminal phases of a two-year study of the long-term effects of ionizing radiation administered to rat spinal cords during the early postnatal period. In this study, spinal nerve root degeneration was observed in the older animals; this observation, which could have been interpreted as being induced by radiation, was found to occur with equal frequency in non-irradiated control rats. Since this information is not well documented and since it can be of value to other investigators using aging laboratory rats, observations of this type need to be reported.

MATERIALS AND METHODS
The spinal cords used in this study were obtained from 52 Charles River CD® rats ranging in age from 18 months to 28 months. These animals were included in an experiment designed to study histopathologic changes in spinal cords of rats exposed to ionizing radiation when three days of age and autopsied at intervals from 11 days to 24 months following irradiation. A few animals were maintained on the study for 28 months. Details of this study will be published elsewhere. All of these animals were from litters raised in this laboratory, and the irradiated rats and their non-irradiated littermate controls were maintained under uniform conditions after weaning. The manner in which the radiation was administered to a restricted portion of the spinal cord has been published previously (Gilmore, '63), as have the details of the type of radiation used in the experiment (Gilmore, '71).

In order to carry out perfusion-fixation, the animals were injected with Nembutal® sodium (0.1 cc per 100 gm body weight). A midline incision was made in the abdomen, and the abdominal aorta was isolated so that a cannula could be placed in

Received April 24, '72. Accepted July 3, '72.
it for perfusion of 10% phosphate-buffered formalin. As soon as the perfusion was begun, the thorax was opened and the right atrium cut. The fixative was allowed to flow for at least 20 minutes, after which a portion of the vertebral column containing lower thoracic and lumbosacral segments of the spinal cord was removed and immersed in fixative for an additional 24 hours. The material was then decalcified and embedded in paraffin. After sectioning (8 μ) and mounting of interrupted serial sections, the tissues were stained by the following methods: hematoxylin and eosin, hematoxylin and van Gieson, luxol fast blue-periodic acid Schiff (PAS), Holmes’ stain for axons and Wilder’s stain for reticular fibers.

RESULTS

The initial changes were seen best in sections stained with luxol fast blue-PAS for myelin. In these instances, scattered, degenerating, individual fibers or small groups of several fibers were observed, particularly in the ventral root (fig. 1). The endoneurial connective tissue of the degenerating fibers remained intact and outlined the area formerly occupied by the degenerated myelin sheath (fig. 2). Reactive cells, probably macrophages, were sometimes present in these areas of early degeneration, as seen in figure 2. No marked proliferation of cells, however, was associated with the areas showing axonal loss and demyelination. Spinal nerve roots showing degenerative changes to this degree were noted usually in animals 18 to 20 months of age.

As the degenerative process progressed, the differential degree to which the dorsal and ventral roots are affected became more obvious (fig. 3). The earliest evidence of degeneration, as described above, was seen in the ventral roots, with alterations in the dorsal roots occurring later. In no case was degeneration noted in the dorsal roots and not in the ventral.

The number of degenerating fibers appeared to increase with age of the animal; by 24 months extensive degeneration was the usual observation, with the ventral roots continuing to show a greater degree of degeneration than the dorsal roots (fig. 4). By this time, multiple, cystic areas of various sizes (fig. 5), outlined by connective tissue of the endoneurium, contained debris and/or reactive cells. In some of these, myelin had degenerated completely with a darkly stained, shrunken axon remaining (fig. 6); in others, both myelin and axon had degenerated. In other regions it appeared that myelin and axonal loss was complete and that these structures had been replaced by connective tissue. Areas such as these were PAS positive (figs. 6, 7), and connective tissue was evident also in sections stained with hematoxylin and van Gieson or Wilder’s method for reticular fibers. Foci of cell proliferation were present in areas having marked degeneration (fig. 8), but extensive or generalized proliferation was not observed.

The same types and patterns of histopathological changes were seen in the few animals autopsied at 28 months of age.

With respect to incidence, degenerative changes were seen in 42 of the 52 animals (80.7%) examined in this study. There was no difference in incidence in animals that were irradiated (80.0%) and those not irradiated (81.0%). The occurrence of the lesion was essentially the same in both males and females, with 16 of the 20 males (80.0%) and 26 of the 32 females (81.3%) showing involvement. The only difference in the occurrence of the lesion was seen with respect to age. Of the animals autopsied when 18 to 20 months of age, 66.7% (18 of 27) exhibited degenerative changes, whereas 96.0% (24 of 25) of those older than 20 months showed some degree of root degeneration. In spite of what appeared to be a relatively extensive spinal nerve root involvement, the neurologic status of the animal appeared to be normal with no loss of motor function having been observed.

DISCUSSION

The radiculoneuropathy described above was completely unanticipated and was considered at first to be a delayed response to ionizing radiation. When the initial microscopic examinations were made, the data were recorded according to the code number on the slide, with no information being available on the source of the tissue. However, when the results of the microscopic
studies were sorted and separated as to irradiated and non-irradiated groups, it became obvious that these histopathologic changes occurred with essentially equal frequency in both irradiated and non-irradiated rats. It therefore seemed improbable that this lesion was a result of radiation damage. Furthermore, the appearance of the histopathologic changes was the same, whether or not the animal had been irradiated, and this, too, supported the view that the lesion was not radiation induced. Since degeneration was not found in rats less than 18 months of age, the question was raised regarding a possible relationship between the lesion and the age of the animals.

A search of the literature revealed only one study (Berg et al., '62) describing radiculoneuropathy in laboratory rats. In that study, degeneration was observed in spinal nerve roots, and to a lesser degree in peripheral nerves, of aging Sprague-Dawley rats. Myelin degeneration was noted in the posterior columns in a few instances. The changes observed in spinal nerve root of these animals consisted of degeneration of myelin with preservation of axons, some macrophage infiltration, and usually little proliferation of sheath cells. The latter was variable but did not correlate with the alteration in myelin. These changes increased in frequency with age, being a small percentage in animals less than 700 days and increasing to 80% for males and 75% for females from 700 to 1300 days. In most (90%) of these cases, the neural changes were regarded as being slight, as based on a subjective, qualitative evaluation of the degree of myelin changes and the number of myelin sheaths involved.

A comparison of observations by Berg et al. ('62) with those in the present study reveals several points which deserve comment. The general characteristics of the lesion are quite similar in both studies. The lesion was noted first in both studies as a demyelination of a few axons. The number of demyelinated fibers gradually increased, but the demyelinating process became much more extensive in this study than in that reported by Berg et al. The changes classified as slight by the latter investigators and occurring in 90% of their animals appeared to be comparable to the early changes described in this study. They did not observe the areas of marked degeneration with development of connective tissue as were found in the present investigation. The fact that the demyelinating process was accompanied by little cell proliferation was common to both studies. A further difference between the two studies is in the incidence, onset and severity of the lesion noted in the dorsal and the ventral roots. No differential was reported by Berg et al. ('62), but there was no question that lesions in the ventral roots from rats in the present study were seen at an earlier time and progressed to a greater degree of severity than did those of the dorsal root. The nearly equal incidence of lesions in males and in females and the increased incidence with advancing age are patterns common to both studies.

The results of these two studies indicate that degeneration of spinal nerve roots, or also of peripheral nerves as described by Berg et al. ('62), may be the usual situation in aging rats. The etiology of these degenerative changes is unknown, but Berg et al. considered them to be pathological rather than aging changes. The data obtained in the present study provide no further insight into the cause of the degenerative alterations. It would appear, however, that radiculoneuropathy should be anticipated in aging laboratory rats and that this condition should be taken into account when working with rats 18 months of age or older. Furthermore, there is an indication in these two studies that the severity of the lesion is different in the two strains of rats studied, Sprague-Dawley and Charles River CD® rats, since changes of a greater degree were seen in the Charles River CD® rats of this study. Whether or not this difference is real, as well as questions regarding etiology of the lesion, can be answered only by further careful studies of normal, aging rats.

ACKNOWLEDGMENTS

Supported by USPHS grant NB-04761 from the National Institute of Neurological Diseases and Stroke.

The technical and photographic assist-
ance of Mr. Napoleon Phillips is acknowledged.

LITERATURE CITED


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PLATE 1
EXPLANATION OF FIGURES

1 Cross section of ventral spinal nerve root from 19-month-old rat. The large, single cystic area in the center of the photograph contains debris from the degenerated axon and its myelin sheath. The remainder of the nerve root appears to be normal. Luxol fast blue-PAS. \( \times 400 \).

2 Ventral spinal nerve root from 18-month-old rat. This Wilder stain for reticular fibers demonstrates the thin, darkly-stained connective tissue covering, the endoneurium. This structure remains intact around the cystic areas. Note the darkly-stained nuclei, probably macrophages, within the cysts. \( \times 400 \).

3 Low power view showing both dorsal and ventral nerve roots and portion of spinal cord from 20-month-old rat. The dorsal root appears to be essentially normal, whereas degeneration is obvious in the ventral root. Luxol fast blue-PAS. \( \times 35 \).

4 Spinal nerve roots and portion of spinal cord from a 24-month-old rat. Degeneration is obvious in both roots by this age but is much more advanced in the ventral roots. Luxol fast blue-PAS. \( \times 35 \).
PLATE 2

EXPLANATION OF FIGURES

5 Higher power view of figure 4 showing extensive degeneration in ventral nerve roots. Multiple cysts of varying sizes indicate some degenerating areas; elsewhere it appears that degeneration is complete with connective tissue having developed in the areas formerly occupied by the neural tissue. Luxol fast blue-PAS. × 125.

6 Ventral nerve root from 24-month-old rat showing relatively intact fibers as well as degenerated areas. Shrunken, darkly-stained axons are evident in some of the cysts. A PAS positive region, containing connective tissue, is indicated by the arrow. Luxol fast blue-PAS. × 400.

7 Extensively degenerated area in ventral nerve root from 24-month-old rat. Groups of relatively normal appearing myelin sheathes are present even though a substantial portion of the ventral root has undergone complete degeneration with replacement by connective tissue. The areas not occupied by intact axons are PAS positive. Luxol fast blue-PAS. × 400.

8 Hematoxylin and eosin stain of degenerating nerve root from 24-month-old rat. Several foci of cell proliferation are evident in the degenerating areas (compare with cell population in relatively normal portion of nerve root in lower left corner of photograph). × 400.