AGE CHANGES IN INTERNODAL LENGTH IN THE HUMAN SPINAL ROOTS*
—NERVE TEASING STUDY—

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INTRODUCTION

Various investigators have made quantitative histologic measurements for demonstrating pathologic alteration of peripheral neuropathies. There are two popular methods that have been used for a long time—counting nerve fibers in a unit intrafascicular area and determining distribution of fiber diameters. They are subsequently referred as fiber population and fiber spectrum.6)7)8)9)

The measurement of distances between the nodes of Ranvier of teased nerve fibers has recently been recognized as another useful procedure for investigating pathogenesis of peripheral nerve disorders.6)7)8)9) However, there has hitherto been no report on teasing study of the spinal roots.

The anterior and posterior roots have different functions: the former is concerned with motor function and the latter with sensory. Specific pathologic alterations might be found in either root or both in certain disorders of the peripheral nerves, because clinically some peripheral neuropathies only show motor dysfunction, whereas others show sensory, or both.

For demonstrating the degrees of abnormality in these cases, it becomes necessary to determine the normal variations in subjects of all age ranges.

The purpose of this study is to establish the age changes, as a number, in internodal length of the anterior and posterior roots in normal subjects.

The anterior and posterior roots of the fifth lumbar segment were chosen for this study. This segment innervates the peripheral parts of the legs. Moreover, clinical variations with growth and aging from the new born to the aged person on the motor, sensory and reflex functions of these parts have been satisfactorily established, so that this segment was considered to be the best portion for studying clinicopathological correlations.
The specimens of the anterior and posterior roots were obtained at autopsy from patients who died at the Nagoya University Hospital and related hospitals, usually within twelve hours after death. Twenty-five cases ranging in age from 1 month to 79 years were chosen. The age, sex and cause of death are given in Table 1. Patients suffering from acute diseases were selected, dying after a short illness. No patients were believed to show clinical evidences of peripheral nerve disorder.

The materials were taken at the level of the fifth lumbar segment, fixed in 10% formalin solution and stored. For examination of internodal length, after washing in distilled water, the spinal roots were stained for 3 hours in 2% osmium tetroxide, washed again, and then placed in a 2:1 glycerin-water mixture for at least one day to reduce their brittleness. Single fibers were teased out in pure glycerin with a dissecting microscope using fine needles and transferred to a slide on which a drop of clove oil had been placed. After approximately 20 myelinated fibers had been accumulated, covering a whole range of fiber diameters, the fibers were arranged in parallel array, clove oil drained off and the slide blotted with a filter paper. The fibers were then mounted in Canada balsam.

With an ocular micrometer measurements of internodal length were made

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mo.</td>
<td>F</td>
<td>Asphyxia</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>F</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>3</td>
<td>1 yr.</td>
<td>M</td>
<td>Ventricular septal defect</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>M</td>
<td>Dehydration</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>F</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>M</td>
<td>Nephritis</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>Cerebral glioma</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>F</td>
<td>Drug intoxication</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>M</td>
<td>Head trauma</td>
</tr>
<tr>
<td>10</td>
<td>27</td>
<td>M</td>
<td>Cerebral glioma</td>
</tr>
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<td>11</td>
<td>29</td>
<td>F</td>
<td>Postpartum hemorrhage</td>
</tr>
<tr>
<td>12</td>
<td>32</td>
<td>M</td>
<td>Cerebral aneurysm</td>
</tr>
<tr>
<td>13</td>
<td>37</td>
<td>M</td>
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</tr>
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<td>14</td>
<td>37</td>
<td>M</td>
<td>Pituitary tumor</td>
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<tr>
<td>15</td>
<td>43</td>
<td>F</td>
<td>Cerebral hemorrhage</td>
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<td>16</td>
<td>46</td>
<td>F</td>
<td>Head trauma</td>
</tr>
<tr>
<td>17</td>
<td>50</td>
<td>M</td>
<td>Myocardial infarction</td>
</tr>
<tr>
<td>18</td>
<td>56</td>
<td>F</td>
<td>Cerebral hemorrhage</td>
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<td>19</td>
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<td>M</td>
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<td>62</td>
<td>F</td>
<td>Pneumonia</td>
</tr>
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<td>21</td>
<td>66</td>
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<td>76</td>
<td>M</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>25</td>
<td>79</td>
<td>F</td>
<td>Bronchopneumonia</td>
</tr>
</tbody>
</table>
at a magnification of 40 and of fiber diameter at 100.

In obtaining the fibers for measurement, no attempt was made to select either normal or abnormal fibers, which meant sampling was performed even-handedly.

For demonstrating the relationship between internodal length and diameter of the root fibers, the former was plotted on Y-axis against the latter on X-axis in each case, and the regression line was calculated as $Y = aX + b$, by the method of least squares, where (Y) represented internodal length and (X) fiber diameter in micra.

The variance of the (Y) values around the regression line of $(Y_p)$ was computed with the formula of $S^2 = \frac{\sum(Y - Y_p)^2}{N-2}$, for showing variation or degree of irregularities of internodal length in each case. $(N)$ demonstrated number of measurements.

**RESULTS**

1) *Age changes in internodal length and fiber diameter* (Fig. 1)

The length of individual internodes has been plotted against their diameter for the cases of 1 month- and 2-, 19-, 29-, 60-, and 79-year-old in Fig. 2.

In the case of 1-month-old the diameter of nerve fibers was smaller than 13 $\mu$ and internodal length was generally short (80–550 $\mu$) regardless of diameter both in the anterior and posterior roots.

With growth the diameter became larger and internodal length increased definitely commensurate with the increased fiber diameter. There was a same tendency both in the anterior and posterior roots, although the internodal length of the larger fibers in the anterior root was longer than that of the posterior root (Fig. 2).

In the cases of 19- and 22-year-old the fiber diameter varied up to 20 $\mu$ in both roots. Internodal length was up to 1,500 $\mu$ in the anterior root and 1,400 $\mu$ in the posterior root. These indicated maximum values respectively in this study.

With further advance in age, the fiber diameter and internodal length gradually decreased in number.

In the cases of aged persons, the scatter of internodal length for a given diameter was considerably greater than that of adult cases. The diagram for the oldest subject, aged 79, is shown in Fig. 2. Localized demyelination and irregularities of internodal length were prominent (Fig. 1, 2). The diameter ranged up to 18 $\mu$ in both roots and internodal length up to 1,150 $\mu$ in the anterior root and 1,100 $\mu$ in the posterior root; They indicated much decrease in number compared with those of the adult group.
FIG. 1. Teased root fibers: Arrows indicate nodes of Ranvier.

(A) A fiber with a small diameter has short internodes (1-year-old male; The anterior root; \( \times 120 \)).

(B) A fiber with a large diameter has a long internode. The myelin of an internodal segment is uniformly stained by osmium tetroxide (19-year-old female; The anterior root; \( 120 \times \)).

(C) Localized demyelination in internodal segments is observed (76-year-old male; The posterior root; \( 120 \times \)).

(D) Another localized demyelination is seen in a part of the internodal segment (76-year-old male; The posterior root; \( 120 \times \)).

(E) A segmental demyelination is observed in an internodal segment (79-year-old female; The anterior root; \( 60 \times \)).

2) Regression line

The relationship between internodal length and diameter was approximately linear in the groups of infants, children and adults (Fig. 2), the regression lines having been counted as \( Y = aX + b \), by the method of least squares. Although variation of internodal length was observed in the aged group (Fig. 1, 2), the linear regression lines have also been calculated by adopting the same method as for younger groups.

The age change of regression coefficient \( (a) \) in the formula of \( Y = aX + b \), which means steepness of the regression line in each case, is diagrammed in Fig. 3.

The regression coefficient \( (a) \) in the case of 1-month-old was 28.40 in the anterior root and 16.36 in the posterior root. It increased in number rapidly with growth and attained the highest value, of 97.68 in the anterior root in the case of 19 years and 68.17 in the posterior root in the case of 22 years.
AGE CHANGES IN INTERNODAL LENGTH

Fig. 2. Relationship between internodal length (Y-axis) and fiber diameter (X-axis) for the subjects of different age. The upper series are for the anterior roots and the lower ones for the posterior roots. The regression line is drawn in each root.

Fig. 3. Age changes of the regression coefficient (a). The left figure is a series for the anterior roots and the right one for the posterior roots.

The average regression coefficient (a) in 7 cases between 15 and 34 years old was $86.49 \pm 7.90$ in the anterior root and $61.62 \pm 4.03$ in the posterior root. These were the standard values for the normal adult group and would be important for estimating degrees of alteration in the spinal roots under various conditions.
On having compared the mean value of the adult group with that of the 1-month-old, the former was 3.0 times in the anterior root and 3.8 times in the posterior root larger than the latter.

With advance of age after the third decade the regression coefficient \((a)\) decreased again in number. In the case of 79-year-old it was 43.26 in the anterior root and 36.60 in the posterior root, which meant milder slope of the regression line than that of the adult group.

For 7 subjects over the age of 60, the mean value of the regression coefficient \((a)\) was \(52.07 \pm 9.96\) in the anterior root and \(50.70 \pm 9.70\) in the posterior root, which was 60.2\% in the anterior root and 82.2\% in the posterior root of that of the adult group, aged between 15 and 34. The difference between these two groups was statistically significant \((p < 0.0001\) in the anterior root and \(p < 0.02\) in the posterior root).

3) Variance of Internodal length

In the cases of younger and adult groups the irregularity or variance of internodal lengths in each fiber was less marked. However, it was marked in cases of aged person (Fig. 2).

For demonstrating the variance of internodal length in each case as a number, the variance around the regression line has been computed and presented in Fig. 4 as \((S^2)\).

The variance \((S^2)\) remained under 10,000 until about the age of 50. Then it increased in number to more than 10,000 after 50 years of age. The highest value of \((S^2)\) in this study was 55,845 in the case of 76-year-old in the anterior root, and 51,848 in that of 62-year-old in the posterior root.

The average of variance \((S^2)\) in the aged group, age ranging between 60 and 79, was \(30,975 \pm 14,431\) in the anterior root and \(29,785 \pm 15,096\) in the posterior root.
root. As the average variance ($S^2$) in the adult group, aged between 15 and 34, was $8,519 \pm 2,969$ in the anterior root and $5,463 \pm 1,772$ in the posterior root, the ratio of ($S^2$) in the aged group to that in the adult group was $3.63 : 1$ in the anterior root and $5.45 : 1$ in the posterior root. The difference between these two groups was statistically significant ($p<0.002$ in the anterior root and $p<0.0005$ in the posterior root).

4) Comparison between sexes
There was no significant difference statistically between males and females in all age groups, either in the regression coefficient ($a$) or the variance around the regression line ($S^2$), or in the anterior or posterior roots.

DISCUSSION

The present study showed that internodal length was generally short and the fiber diameter was small in the case of the new-born. They increased in number during growth. Slope of the regression line increased correspondingly from 1-month-old to about 20-year-old. The highest values were obtained both in the anterior and posterior roots in cases around 20 years of age. With further advance in age, internodal length and fiber diameter gradually decreased in number.

Variance or irregularity of internodal length was not prominent below age of 50. However, it became prominent after this age.

There has hitherto been no report about teasing study on the spinal roots, so the results of this study should be compared with similar works performed on other peripheral nerves.

Mukoyama and Matsuoka examined 15 autopsy materials of the sural nerves in cases without known peripheral neuropathies, age ranging between 20 and 70, and found that internodal length of the larger fibers was longest and the slope of the regression line steepest in the third decade. Internodal length was becoming shorter, the slope of the regression line milder and irregularities of internodal length more prominent with increase in age.

Vizoso compared subjects of different ages in the study on the ulnar and anterior tibial nerves and found that increase of internodal length with fiber diameter was steepest in the case of 18-year-old and that some fibers had internodes unduly short relative to their diameters in subjects of 67- and 80-year-old.

Lascelles and Thomas examined the sural nerve and Arnold and Harriman studied several kinds of peripheral nerves in normal subjects and their results could be closely comparable to the present study, investigated on the spinal roots.

The only difference between this study and theirs was the size of fiber diameter. Their descriptions on the peripheral nerves showed slightly smaller
diameter than any seen in the present study. This difference might be related
to the sites of the nerve sectioned. The site examined in this study was the
spinal root, which was proximal in comparison with those in their studies.
Lavarack et al.\(^b\) made a special comment that fiber branching tended to reduce
maximum diameter when sections at different levels of the same nerve were
examined.

The mode of development of internodal length and fiber diameter in the
spinal roots with growth from new-born to adult, presented in this study,
would be adequately explained by the fundamental studies on experimental
animals\(^{16,17}\). The internodal length was uniformly short at the beginning of
myelination during development; All fibers did not become myelinated at the
same time and the fibers medullated earlier possessed larger diameters; The
fibers of larger diameter had greater distances in between nodes of Ranvier\(^{19,17}\).

As shown in Figs. 1 and 2, the larger fibers which had longer internodes
would be considered to have had myelin earlier in the developmental stage
than the smaller fibers which had shorter internodes.

The longest internodal length and the steepest slope of the regression line
of the spinal roots were found in the cases of around 20 years in the present
study. As mentioned above, same trends have been reported in teasing studies
on the peripheral nerves for normal subjects\(^{6,7,8,9}\).

Corbin and Gardner\(^1\) examined a population of myelinated fibers in the
thoracic anterior and posterior roots on 34 human cadavers, varing in age
from 1 day to 84 years and disclosed the highest number was obtained in the
second and third decades.

These facts would indicate a maturation of the spinal roots as well as
peripheral nerves completed at around these ages.

The present study on the spinal roots showed that internodal length became
shorter and the slope of the regression line milder gradually with further
advance in age after the third decade. Furthermore, from the subjects aged
over 50, an irregularity of internodal length became prominent, that is, some
abnormally short internodes were observed in the same fibers that also possessed
internodes of normal length, whereas in others, internodes were uniformly
short.

From the results of Vizoso\(^{15}\) and Fullerton \textit{et al.}\(^5\), it is known that fibers
which have regenerated after Wallerian degeneration show uniformly short
internodes, whereas those that have undergone segmental demyelination and
remyelination show internodes of various lengths.

However, it is usually difficult to disclose such simple models in human
cases. So far, it seems reasonable to consider that both Wallerian and
segmental demylinations have been mixed in the aged subjects in this study.
Wallerian type degeneration in the spinal roots will appear to be associated
with degenerative changes of neurons in the anterior horns and spinal ganglia.
In aged persons the neurons decrease in number in the spinal cord and spinal root ganglia as well as in the brain\textsuperscript{18,19,20}, the causes of which have been explained as circulatory disturbance, lowered metabolism and simple atrophy of individual nerve cells.

Segmental demyelination would appear after dysfunction of the Schwann cells\textsuperscript{9} in the spinal roots, which is produced by focally disturbed circulation and low metabolic rate, and latent anemia seen in aged person.

Anyway, a condition of blood circulation should be considered for understanding the morphologic and physiologic alterations seen in the spinal roots. Although Mannen\textsuperscript{21} and Takahashi\textsuperscript{4} have reported that blood vessels in the spinal cord and roots themselves showed scarce arteriosclerotic changes, it should still be mentioned that a circulatory disturbance at the spinal roots would be produced by sclerotic changes of the aorta and the relating incoming arteries into the roots\textsuperscript{22,23,24}.

The deformation and abnormal calcification of the spines in the elderly, especially deformation and narrowing of the intervertebral foramina, would produce a localized compression to the spinal roots, which inhibit circulation of the associating blood vessels and also damage the roots directly\textsuperscript{22,25}.

The clinical experiences\textsuperscript{10,11,12,13,14} have revealed that maturation of the nervous system is completed around 20 years old in the aspects of motor, sensory, reflex and coordinating functions, and that a depression or a loss of these functions becomes prominent with increase in age after the 3rd decade, which are thought to be well related not only to the age changes of the structures in the brain, spinal cord and peripheral nerves reported in the literatures, but also the age changes of internodal length and fiber diameter in the spinal roots as demonstrated in this study.

The present report would be the first systematic study showing the age changes of the spinal roots with reference to the correlation of internodal length and fiber diameter on teased nerve fibers.

The data obtained in this study would provide useful informations for undertaking further studies on the spinal roots in various pathologic conditions at the spinal roots themselves and at the peripheral nerves.

**SUMMARY**

Internodal length and fiber diameter were measured on the isolated root fibers after staining with osmium tetroxide, from the anterior and posterior roots at the 5th lumbar level of subjects aged between 1 month and 79 years.

The relationship between two variables were computed for the regression coefficient ($a$) and the variance around the regression line ($S^2$) in each case; the former would show the degree of steepness of the regression line and the latter irregularity of internodal length.
In the case of 1-month-old, internode was generally short regardless of fiber diameters, and the diameter was small in size. Internodal length and fiber diameter as well as the value of the regression coefficient \( (a) \) increased in number during growth. The highest values were obtained both in the anterior and posterior roots in cases aged around 20. Internodal length and fiber diameter, and subsequently, the regression coefficient \( (a) \) decreased in number with further advance in age after the 3rd decade.

Variance or irregularity of internodal length was not marked in cases below age of 50. However, it became prominent, and subsequently, the number of the variance \( (S^2) \) appeared to be high after 50 years of age.

This would be the first report on a teasing study of the spinal roots demonstrating the age changes as a number. On undertaking further study on the spinal roots in various pathologic states, the normal data at a certain age could be detected in this paper.

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