ABNORMALITIES OF THE SCIATIC NERVES OF DYSTROPHIC MICE, WITH REFERENCE TO NERVE COUNTS AND MEAN AREA OF AXONS

BY

Eiko Okada, Vinci Mizuhira,*1 and Haruomi Nakamura*2

ABSTRACT

Ultrastructural changes of the sciatic nerves of C57BL/6J-dydy mice were examined. Nerve counts were calculated and the area of axons was measured in these nerve tissues. Many large bundles of unmyelinated nerves were observed at the proximal portion of the sciatic nerve, without cytoplasm or basement membrane of the Schwann cell. These bundles were composed of axons of various sizes and shapes, including some very large axons. The large unmyelinated axons decreased in number and size as nerves descended to the distal portion. The myelinated nerves showed irregular shapes. Some myelinated nerves were enveloped in very thin myelin sheaths for the mouse age. The destructed myelin was often observed from the radix to the distal portion of sciatic nerves. They lost their myelin sheaths at the Ranvier node, and descended for long distances with naked axons. The neighboring myelin segments of naked axons showed unusually bizarre shapes. They seemed to be the abnormal development of the demyelinated axons. Such findings were very similar to those of the embryonal developing peripheral nerves. It was concluded that the muscular dystrophy of the C57BL/6J-dydy mice might be related to dysmyelination and to the decrease in the number of myelinated nerves.

INTRODUCTION

Murine muscular dystrophy has long been considered to be a primary myopathy2,3) ever since the report of Michel-son1) (1955). McComas et al.5-7) suggested from their electrophysiological studies that neurological changes might cause this disease. Since their research5-9) supported the fact that many kinds of muscular dystrophy were related to neurogenic disease, these factors have been studied for their role in causing dystrophic changes in the muscle. Ragab10) and others11) observed ultrastructural changes in the motor end-plate of dystrophic mice. Harris and Wilson12,13) reported that myelinated nerves (M-nerve) were decreased in the intramuscular spaces. Research on homozygote-muscle transplanation14) and muscle culture of the murine muscle15,16) seemed to support the suggestion of McComas, but some workers are opposed to his theory.

Joseph17) did not recognize pathological changes in the spinal cord and peripheral nerves of dystrophic mice. Panayiotopoulos et al.18) reported that the Duchenne type of muscular dystrophy was not different from controls in electrophysiological experiments.

*1 阿田永子, 水本敏: Department of Cell Biology (Chief: Prof. V. Mizuhira), Medical Research Institute, Tokyo Medical and Dental University (Tokyo Ika Shika Daigaku).
*2 中村晴平: Division of Neuropathology, Institute of Neurological Sciences, Tottori University School of Medicine, Yonago.
Received for publication, November 18, 1974.
Recently, Bradley and Jenkison published data showing ultrastructural morphological changes in the peripheral nerves of Bar Harbor 129 Re dydy dystrophic mice. They concluded that these changes were simply co-existent abnormalities in muscular dystrophy. We have attempted to compare these neurological changes in the Bar Harbor 129 strain to those in C57BL/6J-dydy dystrophic mice (dystrophic mice), using reconstructed electron micrographs of their sciatic nerves.

**Materials and Methods**

Three control mice of the C57BL/6J-++ or C57BL/6J-dy+ (control mice) and four dystrophic mice varying in age from 3 to 16 weeks were used. The dystrophic mice weighed from 5 to 9 g and the control mice weighed from 6 to 12 g. The animals were anesthetized with ether. They were perfused through the left ventricle with a small catheter, at the perfusion rate of 4 ml/min, for 10 min with 2.5% glutaraldehyde in 0.1M phosphate buffer of pH 7.2 and at a room temperature of about 20°C. The sciatic nerves were dissected from the radix to the anterior tibial nerve without severing them. At the same time, the lumbar spinal cord with the anterior and posterior radix was extracted. After extraction, the nerves were fixed at 4°C for 2 hr with the same glutaraldehyde fixative as above, followed by washing for 10 min in 0.1M phosphate buffer containing 8% sucrose. They were then fixed in 2% osmium tetroxide for 2 hr in 0.1M phosphate buffer of pH 7.2 with 4% sucrose. They were dehydrated by immersion in increasing concentrations of ethanol and then in propylene oxide. The tissue was then embedded in Epon according to the method of Luft.

Tissue blocks were prepared from each portion shown in Fig. 1. The blocks were trimmed and then cross-sections of the nerve trunks were cut on a Porter-Blum microtome MT-1 or MT-2 using glass knives. Sections of 0.5 μ in thickness were studied with a light microscope after staining with toluidine blue (Figs. 2 and 3).

Ultra-thin cross sections of the nerve trunks were cut with the same instrument and were mounted on one-hole-grid meshes of 0.8 mm in diameter. Sections were doubly stained with uranyl acetate and lead citrate, and examined with Hitachi HU-11D-5 and HS-7 electron microscopes. Electron micrographs with an original magnification of 900 times were prepared to reconstruct cross sections of the sciatic nerve trunks, paying special attention to make sure that the neurofilaments and neurotubules were being viewed in a perfect cross section. The images of each
trunk were reconstructed with about 100 pictures (Figs. 12, 13, and 14). The number of myelinated (M-) and unmyelinated (U-) fibers in the nerve trunks was counted in the reconstructed pictures. Then area of all the axons was measured with a planimeter on a center part of one-ninth in the same reconstructed pictures. About 300 axons were measured. U-axons smaller than 1 μ in diameter were measured by taking the total area of a group of these axons and dividing by the number of U-axons present.

**Results**

1) Light Microscopy: There were striking pathological changes in dystrophic mice. Pale areas in Toluidine Blue stains were observed in the anterior and posterior roots of the lumbar spinal cord (Fig. 2). They were observed also from the radix to the distal portion of the sciatic nerve (Fig. 3). These areas proved to be groups of U-axons under the electron microscope. In the cross section, these pale areas were larger in the proximal portion than in the distal area (Fig. 3, B, D, and F). M-axons were more irregular than round in shape.

2) Electron Microscopy: U-fibers, which were seen in the pale areas of the light micrographs, were in contact with each other, separated only by the axon membrane. There was no intervening Schwann cell cytoplasm nor basement membrane. It appeared that Schwann cells localized independently, and their cytoplasm did not surround the U-axons (Fig. 5). These findings were in contrast to the control mice (Fig. 4). In addition to these findings, there tended to be numerous neurofilaments in the U-axons of dystrophic mice. Sometimes we could see a large cytoplasm with many lysosomes and dilated smooth endoplasmic reticulum and degenerated mitochondria (Fig. 6). Schwann cell cytoplasm in dystrophic mice had axons with thin myelin sheaths and demyelinated nerves (Fig. 7). Sometimes a fibrocyte...
Table 1. Nerve counts of each dissected portion

<table>
<thead>
<tr>
<th></th>
<th>Number of M</th>
<th></th>
<th>Total Number (M+U)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>D</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>C-16</td>
<td>4,856</td>
<td>4,383</td>
<td>2,688</td>
<td>9,579</td>
<td>10,156</td>
</tr>
<tr>
<td>C-4</td>
<td>—</td>
<td>3,921</td>
<td>2,693</td>
<td>—</td>
<td>8,758</td>
</tr>
<tr>
<td>C-3</td>
<td>5,626</td>
<td>5,029</td>
<td>3,289</td>
<td>6,683</td>
<td>10,094</td>
</tr>
<tr>
<td>Mean</td>
<td>5,241</td>
<td>4,444</td>
<td>2,890</td>
<td>8,281</td>
<td>9,669</td>
</tr>
<tr>
<td>± SD</td>
<td>±585</td>
<td>±556</td>
<td>±345</td>
<td>±1,298</td>
<td>±789</td>
</tr>
<tr>
<td>Dy-16</td>
<td>326</td>
<td>3,242</td>
<td>900</td>
<td>5,168</td>
<td>10,943</td>
</tr>
<tr>
<td>Dy-8</td>
<td>—</td>
<td>2,387</td>
<td>1,537</td>
<td>—</td>
<td>13,449</td>
</tr>
<tr>
<td>Dy-4.1</td>
<td>2,195</td>
<td>1,115</td>
<td>—</td>
<td>12,900</td>
<td>4,576</td>
</tr>
<tr>
<td>Dy-4.2</td>
<td>234</td>
<td>2,827</td>
<td>1,495</td>
<td>7,540</td>
<td>10,027</td>
</tr>
<tr>
<td>Mean</td>
<td>280</td>
<td>2,662**</td>
<td>1,261**</td>
<td>6,354</td>
<td>11,829</td>
</tr>
<tr>
<td>± SD</td>
<td>±46</td>
<td>±468</td>
<td>±306</td>
<td>±1,186</td>
<td>±1,612</td>
</tr>
</tbody>
</table>

C: Control mice; Dy: Dystrophic mice; (Nos.: Age in weeks)
R: Sciatic radix; P: Proximal portion near the sciatic notch;
D: Distal portion near the sciatic notch;
M: Myelinated fibers; U: Un-myelinated fibers.

* P<0.05    ** P<0.01

contained myelin ovoids like destructed nerves and cytolyosomes among nerve bundles (Fig. 8). The myelin sheaths in dystrophic mice had varying thickness, and irregular and bizarre shape (Figs. 13 and 14). These findings are similar to embryonal myelination. These differences between dystrophic mice and control mice will be discussed later.

In longitudinal section, naked axons without myelin sheath could be seen descending for a long way. They were partly covered with the cytoplasm of Schwann cells. Sometimes they lost their myelin sheaths at the Ranvier node, which irregular in shape in contrast to the control mice (Figs. 10 and 11). They were many lysosomes, cored vesicles, dilated smooth endoplasmic reticulum, and irregular dilated neurotubules in U-axons (Fig. 9). The mitochondria were not so degenerated. Morphological figures taken from montage pictures were not very different from the light micrographs of M-fibers. Since U-fiber membranes could be seen more clearly than in light micrographs, many small U-axons could be observed in detail for calculation. Some vessels were dilated severely at the radix in dystrophic mice (Fig. 14). This could not be seen in the P and D areas.

3) Evaluation of nerve counts and the area of axons: Measured values were examined by Student’s t-test and by analysis of variance. The analysis of percentage index was given official approval after angular transformation.

A) Nerve counts (Table 1): Nerve counts of the sciatic radix (R) could not be compared because the nerve trunks were not yet formed. Therefore, the P and D portions were used for comparison. Dystrophic mice and control mice have a similar total number of M- and U-axons in the proximal area. The mean total number of axons was 14,492±1,284 in Dystrophic mice, and 14,113±1,276 in control mice. The total axons in the distal area
Table 2. Proportion of U-nerves and the rate of nerve diminution

<table>
<thead>
<tr>
<th></th>
<th>U</th>
<th>M+U</th>
<th>M_p-M_D</th>
<th>U_p-U_D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>C-16</td>
<td>66.3 (54.51)</td>
<td>69.9 (56.73)</td>
<td>65.4 (53.97)</td>
<td>38.7 (38.47)</td>
</tr>
<tr>
<td>C-4</td>
<td>55.4 (48.10)</td>
<td>66.7 (54.76)</td>
<td>63.7 (52.95)</td>
<td>34.6 (36.03)</td>
</tr>
<tr>
<td>C-3</td>
<td>55.4 (48.10)</td>
<td>66.7 (54.76)</td>
<td>63.7 (52.95)</td>
<td>34.6 (36.03)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>60.9±5.5</td>
<td>68.6±1.4</td>
<td>63.9±1.2</td>
<td>34.9±3.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>U</th>
<th>M+U</th>
<th>M_p-M_D</th>
<th>U_p-U_D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Dy-16</td>
<td>94.1 (75.94)</td>
<td>77.1 (61.41)</td>
<td>61.8 (51.83)</td>
<td>72.2 (58.18)</td>
</tr>
<tr>
<td>Dy-8</td>
<td>—</td>
<td>84.9 (67.15)</td>
<td>76.5 (61.00)</td>
<td>35.6 (36.63)</td>
</tr>
<tr>
<td>Dy-4.1</td>
<td>—</td>
<td>85.5 (67.62)</td>
<td>80.4 (63.72)</td>
<td>49.2 (44.54)</td>
</tr>
<tr>
<td>Dy-4.2</td>
<td>97.0 (80.02)</td>
<td>78.0 (62.03)</td>
<td>76.9 (61.27)</td>
<td>47.1 (43.34)</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>95.5±1.5**</td>
<td>81.4±3.8**</td>
<td>78.9±7.1</td>
<td>51.0±13.3</td>
</tr>
</tbody>
</table>

( ) : Angular transformation  * P<0.05  ** P<0.01

were less in dystrophic mice than in control mice. The sum of axons in the distal portion decreased to 5,269±1,982 in dystrophic mice and to 8,004±986 in control mice. In the M-fibers, control mice had about 1,000 more axons than dystrophic mice in analogous portions. That is, they decreased from 4,444±556 to 2,890±345 in control mice as the trunk progressed down to the periphery, and from 2,662±468 to 1,261±306 in dystrophic mice. Although plots of each dissected portion of the sciatic nerve showed a decreasing slope in both control mice and dystrophic mice, comparative counts of M-fibers between control mice and dystrophic mice was significantly different in each P portion (P<0.05) or D portion (P<0.01) (Fig. 15). U-fibers of dystrophic mice had about 1,000 more axons than control mice in the proximal area, but that of dystrophic mice had about 1,000 fewer axons than control mice in the distal area. The mean number of control U-axons in the proximal area was calculated as 9,669±789 in control mice and 11,829±1,612 in dystrophic mice. In the distal area the U-axons decreased to 5,114±648 in control mice and 4,007±1,714 in dystrophic mice. These counts were not significantly different in U-axons (Fig. 15).

B) The ratio of U-nerves (Table 2): The ratio of U-axons to total nerve counts was about 60% in each portion of dissected R, P, and D areas in the control mice. This ratio was constant. In dystrophic mice, however, the ratio of U-axons was about 95% at the R portion, 81% at the P portion, and 74% at the D portion. There was a significant difference (P<0.01) between R and P portions. These ratios in dystrophic mice decreased as the nerve descended. It seemed to approach the normal rate. There was no significant difference at the D portion. The ratio of M-axons increased normally in dystrophic mice.

C) The rate of nerve diminution (Table 2): Let the number of M-axons at the proximal portion be M_p, and the number at the distal portion be M_d. Also, let the number of U-axons at the P area be U_p, and the number at the D area be U_d. The ratio of nerve diminution is expressed as \( \frac{M_p - M_d}{M_p} \).
Table 3. Mean area of M- and U-axons

<table>
<thead>
<tr>
<th></th>
<th>Square measure of M ($\mu^2$)</th>
<th></th>
<th>Square measure of U ($\mu^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
<td>D</td>
</tr>
<tr>
<td>C-16</td>
<td>11.861±0.727</td>
<td>12.088±0.595</td>
<td>12.325±0.606</td>
</tr>
<tr>
<td>C-4</td>
<td>----</td>
<td>12.244±0.628</td>
<td>12.653±0.542</td>
</tr>
<tr>
<td>C-3</td>
<td>3.958±0.152</td>
<td>4.679±0.190</td>
<td>5.905±0.226</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.909±0.514</td>
<td>9.670±0.511</td>
<td>10.294±0.487</td>
</tr>
<tr>
<td>Dy-16</td>
<td>10.934±0.677</td>
<td>7.234±0.265</td>
<td>10.801±0.183</td>
</tr>
<tr>
<td>Dy-8</td>
<td>8.185±0.393</td>
<td>7.656±0.566</td>
<td>7.277±0.384</td>
</tr>
<tr>
<td>Dy-4.1</td>
<td>----</td>
<td>7.655±0.310</td>
<td>6.540±0.253</td>
</tr>
<tr>
<td>Dy-4.2</td>
<td>8.929±0.481</td>
<td>9.395±0.444</td>
<td>8.129±0.318</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>9.349±0.505**</td>
<td>7.985±0.413**</td>
<td>6.687±0.292**</td>
</tr>
</tbody>
</table>

* P<0.05  ** P<0.01

for M-fibers, and \( \frac{U_P - U_D}{U_Y} \) in U-fibers, where this is indicated in Table 2. The rate of U-fibers diminution was larger than that of M-fibers (P<0.05) in both control and dystrophic mice. There was no significant difference between dystrophic and control mice, although the diminution rate of M-axons in dystrophic mice was larger than that in C mice. It was thought that the facts outlined in above (A) to (C) were applicable to all ages of mice, since they were not related to the time course of the dystrophic stages.

D) Mean area of axons (Table 3): In developing animals the mean area of axons varies. It also varied in our experimental animals.

In the control mice, the mean area of M-axons tended to increase as the nerve descended but there was no significant difference among the dissected areas of each portion. In the dystrophic mice, the mean area of M-axons tended to decrease as the nerve descended. There was a significant difference among the dissected areas of each portion (P<0.01).

In the control mice, the area of U-axons showed no variation among the dissected portions. The mean area was from 0.46 to 0.52 $\mu^2$. On the contrary, in the dystrophic mice, mean area of U-axons had large variety inter dissected portions (P<0.01). The area was 2.22 $\mu^2$ at the radix and 0.80 $\mu^2$ at the proximal portion. At the distal portion, the area was near the control value, 0.69 $\mu^2$, but it was significantly different between control and dystrophic mice (P<0.01).

**DISCUSSION**

Many large groups of U-axons, which were observed at the radix and proximal portion of the sciatic nerve, were similar to the peripheral nerve of the developing fetal nerve, but there were differences in this phenomenon between the dystrophic mice and the developing fetuses, that is, U-axons in the dystrophic mice were enveloped without intervening Schwann cell cytoplasm and basement membrane. Abnormally large U-axons were seen in dystrophic mice. Although the peripheral nerves of the normal fetus have several different figures depending on the stage of development, many groups of U-axons are divided into small groups which tend to be enveloped with the cytoplasm and base-
ABNORMALITIES OF THE SCIATIC NERVES OF DY MICE

![Graph](image)

--- Control mice
--- Dystrophic mice

Fig. 15. The sum of M- and U-fibers in the proximal area is nearly the same in both the control and dystrophic groups but in the distal portion, the total number of M- and U-fibers in dystrophic animals is much less than that in the control animals.

A small group of U-axons in control mice was enveloped with the cytoplasm and basement membrane of Schwann cells. It was different from the U-axons of dystrophic mice.

In the cutaneous nerve of the human fetus after 10–22 weeks, bundles of U-axons were enveloped without the cytoplasm and basement membranes of Schwann cells. These were called "very immature nerves" by Gamble. Therefore, very immature nerves were localized in the proximal portion of dystrophic mice. On the other hand, Peters and Muir reported that the diameter of U-axons varied from 0.2 to 0.4 μ in the fetus of the rat. It is thought that axons more than 1.8 μ in diameter have myelin in the human fetus. In the dystrophic mice, many U-axons of from 1.8 μ to 6 μ in diameter were abnormally enveloped. These findings could not be considered normal growth. Bradley reported that these blocked the function of Schwann cells in some stage of the fetus. Schwann cells originate from the neuronal crest and then migrate along axons down to the peripheral area. Therefore, the groups of large axons are thought to result from the defect of migration of Schwann cells. The dysfunction of Schwann cells can be shown by the varying thickness and abnormal shapes of the myelin sheaths. Many naked axons without myelin sheaths were observed for long distances. They lost their myelin sheaths at the Ranvier nodes and became naked axons. The Ranvier nodes were usually of bizarre shape (Figs. 10 and 11). This might indicate ab-
normal budding of M-axons with dysmyelination. Then these might disappear among the nerve fibers. It is certain that the U-axons decreased in number toward the periphery. Although morphological changes in dystrophic mice were similar to those in the 129 strain which Bradley used, there is a difference in the relation of the groups of axons and Schwann cells. One is that U-axons of dystrophic mice have incomplete cytoplasmic lamella of Schwann cells, while the cytoplasm of 129 strain surrounded groups of axons completely. Therefore, these findings suggest that the C57 strain of dystrophic mice is more immature than the 129 strain of dystrophic mice. Another difference from the 129 strain is that the cross section of the nerves has an irregular shape in the C57 strain. This is also a sign of immaturity.

The sum of U- and M-fibers in the C57 strain was the same as in control mice. This suggests that the axonal budding from the nerve cells of the spinal cord and spinal ganglion was normal. It has been said that the number of anterior horn neurons in Bar Harbor 129 strain dystrophic mice is higher than normal rather than lower.\textsuperscript{17,30,31} The number of M-axons in dystrophic mice decreased significantly in the proximal area. This is similar to the nerve counts in the 129 Re dydy strain.\textsuperscript{12,19} The M-fibers decreased toward the periphery. Though U-fibers were decreased, they showed no significant disappearance. This is why U-fibers in dystrophic mice contained a large number of axons (e.g., 81% at the proximal area near the sciatic notch) in the sciatic bundle. U-fibers made up about 95% of the total fibers at the radix in dystrophic mice. The mean area of the axons was about 2.2 $\mu^2$. Their number and area decreased strikingly as the nerve descended to the distal portion. The mean area and number were near the value of control mice in these area. These phenomena indicated that the rate of diminution of U-axons was larger than that of M-axons. It is thought that larger U-axons would disappear by branching off or changing to myelinated axons or by degeneration. The most important reason for diminution of nerves is thought to be degenerating nerves such as shown in Fig. 7, 8, and 9. We have no laboratory findings about branching off of the axons. It is thought that M-fibers are derived from the large U-axons, because there were more U-axons in dystrophic mice than in control mice at the radix level of the sciatic nerve, but the diminution rate of M-axons in dystrophic mice is higher than that in control mice. Therefore, myelination of U-axons is unthinkable. One condition for myelin formation is that the axon be enveloped by the cytoplasm of the Schwann cells and the ratio of axons to Schwann cells be 1:1.\textsuperscript{21,24} These conditions did not exist at the proximal and distal area of dystrophic mice. In addition, there was the phenomenon of dysmyelination. We could see small changes in the U-axons such as cored vesicles and enlarged cytoplasmic reticulum (Fig. 9). Therefore, we cannot deny the existence of degenerated axons but, except for the abnormal demyelination, these findings exist only to a very small degree.

Dystrophic changes of a muscle is severe in the lower body, especially at the proximal area of the lower extremities. This might have some relation to the fact that large U-axons disappear as the nerve descends to the distal portion. It is suggested that muscle disease in dystrophic mice is modified by the large U-axons with dysmyelination.
CONCLUSION

A study of nerve counts and axonal area was performed in montage pictures of the sciatic nerve trunks, and details of these pictures were examined by light and electron microscope.

1) Several large groups of axons with large U-axons were observed at the proximal area of sciatic nerves in dystrophic mice. These axons contained many neurofilaments and had no intervening cytoplasm or basement membrane of Schwann cells. Although various sized U-axons were found in dystrophic mice, these axons appeared to be very immature.

2) M-axons of dystrophic mice were bizarre in shape and had varying thickness in contrast to control mice. These findings were severe especially at the proximal area. It is suggested that these M-axons are immature like the U-axons.

3) The total number of M- and U-axons at the proximal area showed no significant difference between dystrophic mice and control mice.

4) U-fibers of dystrophic mice occupied 95% of the total number at the sciatic radix. The mean area of these U-axons was 2.22 \( \mu^2 \) which was abnormally large. As they descended to the proximal portion of the sciatic notch, the U-fiber proportion became 81% and the mean area of the axons decreased to 0.80 \( \mu^2 \). The proportion of U-fibers at the D portion decreased but the rate was close to that of control mice. The mean area was 0.69 \( \mu^2 \). This value was larger than in control mice.

5) M-fibers of dystrophic mice were fewer than in control mice at the P and D area. The mean area of M-axons was smaller than that of the controls. M-fibers tended to decrease as the nerve descended to the distal portion.

6) Many naked axons were observed from the radix to the distal portion in longitudinal sections. They lost the myelin sheath at the Ranvier node. The neighboring segment of the naked axons had abnormally shaped Ranvier nodes (Figs. 10 and 11). These endings indicated that dysmyelination also occurred in this disease. In other words, dysmyelination should play an important role in the appearance of clinical syndromes.

7) The blood vessels were enlarged in the radix of the sciatic nerve (Fig. 14).

8) It is thought that the nerve changes are present at birth and have nothing to do with the time course of the stages of muscular dystrophy.

ACKNOWLEDGEMENT

We thank the Central Institute for Experimental Animals for providing dystrophic mice, Prof. Hiroshi Maeda, Department of Public Health, and Dr. Tamenobu Kubota, Department of Hygiene, both of this University, for advising us on the statistical work, and members of the Department of Cell Biology, Medical Research Institute of this University, for their technical assistance.

This work was supported in part by a research grant for specific diseases from the Ministry of Health and Welfare.

REFERENCES


3) Banker, B. Q.: A phase and electron microscopic study of dystrophic muscle. II. The pathological changes in the Newborn Bar Har-


ABNORMALITIES OF THE SCIATIC NERVES OF DY MICE

EXPLANATION OF FIGURES

Plate 1
Fig. 5. Light micrographs of transverse sections prepared from (A) the sciatic radix, (C) the proximal portion, and (E) the distal portion of a control mouse. The corresponding levels ([B] the sciatic radix, [D] the proximal portion, and [F] the distal level of a dystrophic mouse) show the grossly abnormal appearance (see text). Toluidine blue stain. ×700.

Plate 2
Fig. 4. Electron micrograph of M- and U-fibers from a control mouse, 4 weeks old. A cluster of U-fibers is wrapped with the cytoplasm and basement membrane of a Schwann cell (arrows). Double stained with uranyl acetate and lead citrate.

Fig. 5. U-fibers from a dystrophic mouse, 4 weeks old. Around the abnormally large U-axons, there is neither basement membrane nor cytoplasm of Schwann cells, and the axons appear to be naked (arrows). The scales are the same as in Fig. 4. Double staining.

Plate 3
Fig. 6. The Schwann cell cytoplasm which intervened in the group of U-axons from the P portion of a dystrophic mouse showing many lysosomes and dilated smooth endoplasmic reticulum and degenerated mitochondria. Double staining.

Fig. 7. Schwann cell cytoplasm with a younger myelinated axons from the P portion of a dystrophic mouse showing destructed axons. Double staining.

Plate 4
Fig. 8. A fibrocyte from the P portion of a dystrophic mouse showing degenerated M-axon, myelin ovoid, cytolyosomes, and phagocytosis of degenerated myelin (arrow). Double staining.

Plate 5
Fig. 9. There are many lysosomes, cored vesicles, and dilated endoplasmic reticulum in the U-axons of the dystrophic mouse. Double staining.

Plate 6
Fig. 10. A longitudinal section from the P level of a dystrophic mouse showing an abnormally shaped myelin sheath which disappears at the Ranvier node before the neighboring segment. Double staining.

Fig. 11. The Ranvier node from the R level of a dystrophic mouse showing asymmetric shape, and the myelin sheath not seen in the next segment. Double staining.

Plate 7
Fig. 12. The P portion of a control mouse, 3 weeks old, reconstructed from more than 100 pictures taken at an original magnification of 900 times. The M-fibers are generally round. Double staining.

Plate 8
Fig. 13. The same P portion as that in Fig. 12 from a dystrophic mouse, 8 weeks old. The M-fibers are irregular and bizarre in shape. There are many large groups of axons showing U-axon bundles. Double staining.

Plate 8
Fig. 14. A cross-section of the R portion of a dystrophic mouse, 4 weeks old, showing many large groups of U-axons, bizarrely shaped M-axons and dilated vessels. Double staining.
ABNORMALITIES OF THE SCIATIC NERVES OF DY MICE
E. OKADA, V. MIZUHIRA, AND H. NAKAMURA

Plate 1

A

B

C

D

E

F

×700

Fig. 3
ABNORMALITIES OF THE SCIATIC NERVES OF DY MICE
E. OKADA, V. MIZUHIRA, AND H. NAKAMURA

Plate 2

Fig. 4  ×12,000

Fig. 5.  ×12,000
ABNORMALITIES OF THE SCIATIC NERVES OF DY MICE
E. OKADA, V. MIZUHIRA, AND H. NAKAMURA

D8-P

Fig. 13

Plate 7
D4.2 R

Fig. 14