No Evidence for Axonal Atrophy in Human Diabetic Polyneuropathy

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Abstract. In rats with streptozotin-induced diabetes mellitus, the caliber of distal myelinated fiber (MF) axons in relation to the number of myelin lamellae is smaller than in controls. This finding usually has been attributed to axonal atrophy, but shrinkage or maldevelopment has also been considered. For human diabetic polyneuropathy (DP), axonal atrophy has been assumed by some investigators, but convincing evidence has not been demonstrated. We morphometrically evaluated transverse sections of 33 sural nerves from carefully evaluated diabetic patients ≥ 30 years old without (8 patients) or with (25 patients) DP and compared them with 24 nerves from healthy subjects ≥ 30 years old. Nerves from diabetic patients and controls were obtained under identical conditions and processed and evaluated in the same way, using an observer blind to the disease condition. Using computer digitization of electron micrographs, we evaluated the axonal area, perimeter, index of circularity, number of myelin lamellae, and frequency of axonal sequestration of 50.4 (mean) ± 5.8 (SD) MF per sural nerve for healthy subjects and diabetic patients ≥ 30 years old. The regression lines of the natural log (ln) of axonal area on number of myelin lamellae of diabetic patients (with or without DP) were not significantly different from the regression lines of nerves of healthy subjects for large MFs—the most reliable group in which to recognize atrophy. Likewise, the regression lines of index of circularity (IC) (an index that is decreased with atrophy or shrinkage) on number of myelin lamellae for large fibers was not significantly different between the disease and control groups. The rate of axonal sequestration was not significantly higher in DP than in healthy subjects. These results do not support the hypothesis that axonal atrophy occurs in human DP. For small MF, or all MF, some significant differences in regression lines of IC or IC on number of lamellae were found, but these changes are probably explained by events of remyelination and axonal regeneration, which can affect these relationships and are known to occur in DP.

Key Words: Axonal sequestration; Axonal atrophy; Axonal shrinkage; Diabetic polyneuropathy; Index of circularity; Myelin; Nerve fiber.

INTRODUCTION

Human diabetic polyneuropathy (DP) is a major health problem. Of the people in Rochester, MN (a northern city in the United States with a predominantly white population) with diabetes mellitus, approximately 50% have DP and about 13% have symptoms due to DP (1). The mechanisms underlying DP are incompletely understood, but the duration of diabetes mellitus (DM) and the degree of chronic hyperglycemia are known risk factors (2–5).

Pathologic and morphometric studies of the sural nerve may be used to study the morphologic correlates of symptoms such as sensory loss and autonomic deficit (6). Also, pathologic studies may address (a) the class of neurons (fibers) affected, (b) whether axons or Schwann cells (or myelin) are primarily affected, (c) whether axons atrophy before they degenerate (the focus of the present study), and (d) the interstitial events or reactions that occur. Axonal degeneration, nerve fiber loss, and segmental demyelination and remyelination are characteristic features of DP (7–16). Because axonal atrophy may precede demyelination and remyelination and axonal degeneration, it might explain all these pathologic events if it were characteristic of DP (17). In experimental diabetes, axons with a small caliper relative to the amount of myelin have been reported (18–21), but the explanation for it (for example, cellular atrophy, shrinkage, or maldevelopment) is not agreed on. Some authors have inferred that axonal atrophy is characteristic of human axonal DP but have not measured whether it occurs (22). In earlier studies, atrophy was not found (23, 24).

We used sural nerve specimens from healthy subjects and patients with diabetes mellitus who had or did not have DP. This study included a larger number of nerves than previous studies and used improved morphometric endpoints to assess fiber size (the number of myelin lamellae rather than myelin spiral length).

MATERIALS AND METHODS

Patients and Healthy Subjects

Diabetic patients, many from the Rochester Diabetic Neuropathy Study (RDNS) (25), provided sural nerve specimens for research purposes. The RDNS is a cross-sectional and longitudinal epidemiologic study in Rochester, MN of the prevalence, course, and risk factors for complications of diabetes mellitus (DM). The ascertainment of patients with DM within the geographical boundaries of Rochester, MN is virtually complete, and approximately 40% of the diabetic patients participate in the RDNS. The patients in the study reported herein are representative of the diabetic patients in Rochester MN, except for those 70 years or older. Participating diabetic patients were assessed for some risk factors as often as 4 times per year, and for nerve, eye, and kidney complications once per year. Severity

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of DP was staged as follows: N0, no neuropathy; N1, asymptomatic neuropathy; N2, symptomatic neuropathy; and N3, disabling neuropathy (26). All patients had evaluation of neuropathic symptoms, neurologic deficits, attributes of nerve conduction, quantitative sensory tests and quantitative autonomic tests. After giving informed consent, patients 20 to 70 years old, with or without DP, provided fascicular or whole sural nerve biopsy specimens from the level of the ankle.

Control sural nerves were obtained from the consenting healthy subject cohort of the RDSN (27). These subjects were selected at random from age and sex strata of the general population of Rochester, MN. Everyone who was drawn and who agreed to participate was evaluated for polyneuropathy by the tests listed for diabetic patients. Persons from this group who were found to have neuropathy, neurologic disease, substance abuse, or diseases known to predispose to neuropathy were excluded as healthy subjects.

Nerve Biopsy and Histologic Processing

Fascicular (or whole) sural nerve biopsy specimens from the ankle level were obtained in a hospital operating room under local anesthesia. The site, biopsy procedure, histologic processing, and morphometric assessment have been described elsewhere (17) and were the same for control and diabetic nerves. Fixation of the specimens in the operating room was by immersion in 2.5% glutaraldehyde in 0.025 M cacodylate buffer, pH 7.38, temperature 10°C. After the specimen was washed in buffer, it was additionally fixed in 1% osmium tetroxide and, after dehydration, was embedded into epoxy resin. Semithin sections of nerve specimens were inspected and re-sectioned as necessary to obtain good transverse orientation. Thin sections were stained with uranyl acetate and lead citrate, and electron micrographs of transversely sectioned myelinated fiber (MF) were photographically enlarged to a magnification of ~× 14,000. A systematic sampling technique (17) was used to get an approximately equal proportion of large and small MF. Tissue blocks that were not adequately fixed or had crush artifact or other preparatory artifact were not used to make electron micrographs.

Measurement of Ultrastructural Features

The number of major dense lines of MF was counted on electron micrographs under a dissecting microscope, with care taken not to include infolded loops of myelin (Fig. 1). Also, counts were not made in paranodal regions.

The axolemma of fibers was traced to determine perimeter length and axonal area using computerized digitization (Fig. 1) (IBM personal computer, Digi-pad, GTCO Inc., Columbia, MD, and our own software).

Statistical Assessment

Electron micrographs of MF of sural nerves from 34 diabetic patients either with (26 patients) or without (8 patients) DP and from 44 healthy subjects were prepared. To obtain comparability with respect to age (only one diabetic patient, but numerous healthy subjects were younger than 30 years), we restricted these analyses to subjects ≥ 30 years old: 8 diabetic patients without DP, 25 patients with DP, and 24 healthy subjects. The ages in these 3 groups were not significantly different (Table 1). From visual inspection of individual electron micrographs of myelinated fibers, it was not possible to recognize whether they were from healthy subjects or diabetic patients (Fig. 1). Therefore, masked evaluation was possible and performed.

For the sampled MF of representative sural nerve specimens from healthy subjects and diabetic patients, we plotted axonal area, natural log (ln) of axonal area, diameter (derived from area), and ln axonal diameter (from area) on the number of myelin lamellae or on ln number of myelin lamellae. By least squares regression, we fitted linear regression lines to the data. From visual inspection of the data, in axonal area on the number of myelin lamellae provided an approximately gaussian distribution of values around linear regression lines for small and large fibers. Regression lines were computed separately for each nerve for small, large, and all fibers. An estimate of the common line for each group was obtained using the average of the intercepts and the slopes (Fig. 2).

To test the hypothesis that the axon caliber relative to the number of myelin lamellae is less in diabetic patients with or without DP than in controls, we assessed for differences in slopes and intercepts of regression lines for the relationship of ln axonal area on the number of lamellae using 2 sample t tests. Because diabetic nerves undergo pathologic events other than the putative axonal atrophy studied here that affect the relationship of axonal caliber to myelin thickness (e.g. nerve sprouting and remyelination after demyelination), this relationship was assessed separately for large MF (≥ 80 lamellae) and small MF (< 80 lamellae). The main test of the hypothesis was based on results from large MF.

The index of circularity is less in small fibers than in large ones and in axonal atrophy (and shrinkage). To test the hypothesis that axonal atrophy (or shrinkage) occurs in DP, we assessed for significant differences in slopes and intercepts of regression lines for the relationship of index of circularity on the number of myelin lamellae using 2 sample t tests. Additional analyses were carried out to evaluate the need to include age and sex in the analyses. We regressed the intercepts against age, sex, and group, using multiple regression analyses. For group, 2 variables were created to compare diabetic patients with (X1) or without (X2) neuropathy to controls:

\[
X1 = 1 \text{ if diabetic with polyneuropathy} \\
= 0 \text{ otherwise}
\]

\[
X2 = 1 \text{ if diabetic without polyneuropathy} \\
= 0 \text{ otherwise}
\]

We then deleted age or sex, depending on whether these effects were significant (P < 0.05). The same analysis was used for the slopes.

RESULTS

Clinical Characteristics of Healthy Subjects and Diabetic Patients

The number, sex and age of healthy subjects and diabetic patients with or without DP are given in Table 1. Of the patients, 10 had stage 1, 13 had stage 2a, and 2 had stage 2b DP.

Body mass index and laboratory values for the 3 patient groups are compared in Table 2. The fasting plasma
Fig. 1. Transverse section of large myelinated fiber from control sural nerve (×10,000). From visual inspection of such micrographs, it was not possible to judge whether the fiber was from a healthy subject or a patient with or without diabetic polyneuropathy. In this example, the number of myelin lamellae (as seen in the inset [×25,000]) is 122, the axonal perimeter is 16.9 μm, the axonal area is 21.6 μm², the natural log of axonal area is 3.07 ln μm², the diameter is 5.24 μm, the ln of diameter is 1.66 ln μm, and the index of circularity is 0.98.

<table>
<thead>
<tr>
<th>Table 1: The Age and Sex of Healthy Subjects and Diabetic Patients in the Study*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Controls (≥30 years old)</td>
</tr>
<tr>
<td>Diabetics without polyneuropathy (≥30 years old)</td>
</tr>
<tr>
<td>Diabetics with polyneuropathy (≥30 years old)</td>
</tr>
</tbody>
</table>

* No significant differences were found in age and sex.

Ln Axonal Area and Number of Myelin Lamellae in Healthy Subjects

Values of ln axonal area (mm²) on the number of lamellae of MF of a representative sural nerve of a healthy subject are plotted in Figure 2 (Top). Overlaid on the graph is a linear regression line for all fibers, small fibers, and large fibers.

In sural nerves from healthy subjects, no significant effect on intercept or slope of ln axonal area on the number of lamellae was found for sex or age for all MF, small MF, or large MF.

Axonal Area and Number of Myelin Lamellae in Diabetic Patients

Considering the large MF of sural nerves of patients with or without polyneuropathy, no statistically significant difference was found between diabetic and control nerves for intercepts or slopes of common regression...
lines of ln axonal area on the number of lamellae (Table 3). When all MFs were included, some statistically significant differences for slope and intercept were found (Table 3). Age and sex were not significant in any of these models.

Common regression lines for nerves of healthy subjects and for diabetic patients with or without DP are plotted in Figure 3. For large MF, common regression lines for the 2 diabetic patient groups were generally below those of healthy subjects, but the differences were not statistically significant. The order of the decrease did not fit the hypothesis that the greatest decrease occurred with the most severe stage of DP. For small MF, the ratio of axonal area to the number of myelin lamellae tended to be highest for diabetic patients with DP. The lines for diabetic patients without DP were very close to that of healthy subjects.

Index of Circularity as a Measure of Decreased Axonal Caliber

The slope and intercept of common regression lines of the index of circularity (IC) on the number of myelin lamellae for large MF was not significantly different among the 3 patient groups (Table 4). No statistically significant differences were found in slopes or intercepts of regression lines of IC on the number of lamellae of small MF and all MF of diabetic nerves without neuropathy as compared with controls. For large MF, sex was a statistically significant variable in the relationship of IC on the number of myelin lamellae. Because there was approximately the same number of nerves from men and women in the control and diabetic groups, the lack of a difference could not be explained by differences in sex. Statistically significant differences in both intercept and slope were found for small MF and all MF for diabetic patients with polyneuropathy and controls (Table 4). The differences for small MF are shown graphically in Figure 4.

Frequency of Adaxonal Sequestration as a Measure of Axonal Atrophy

Infolding of the axolemma with or without sequestration was recognized in fibers from 2 control subjects and

![Graph of axonal area vs. number of lamellae](image)

**Fig. 2.** Each point represents the plotted value of the natural log (ln) of axonal area on number of myelin lamellae of a transverse section of a myelinated fiber of a control (top) and diabetic (bottom) sural nerve. Regression lines were fitted to the data for small (< 80 lamellae), large (≥ 80 lamellae), and all myelinated fibers. As described in the text, among the transformations of axonal area (mm²) or number of lamellae considered, ln axonal area and number of lamellae provided the best fit. For each nerve evaluated in this study, similar regression lines were generated. Comparison of these regression lines among the 3 groups (healthy subjects, diabetic patients with polyneuropathy and diabetic patients without polyneuropathy) formed the basis of the analysis to answer the question whether axonal atrophy occurs in diabetic polyneuropathy.

<table>
<thead>
<tr>
<th>TABLE 2</th>
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<tbody>
<tr>
<td>Mean Values of Physical and Laboratory Characteristics of Persons ≥30 Years Old Who Had Sural Nerve Biopsy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Glucose, mg/dL</th>
<th>HbA1c, %</th>
<th>Cholesterol, mg/dL</th>
<th>Triglyceride, mg/dL</th>
<th>Creatinine, mg/dL</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls*</td>
<td>92</td>
<td>5.3</td>
<td>202</td>
<td>126</td>
<td>0.96</td>
<td>26</td>
</tr>
<tr>
<td>Diabetics with neuropathy*</td>
<td>183†</td>
<td>11.5†</td>
<td>213</td>
<td>146</td>
<td>0.96</td>
<td>26</td>
</tr>
<tr>
<td>Diabetics without neuropathy</td>
<td>186‡</td>
<td>10.8‡</td>
<td>199</td>
<td>101</td>
<td>0.88</td>
<td>25</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index.

* Generally, the test result was available for most persons studied except for cholesterol and triglyceride (approximately one-half of patients).

† Values that were significantly different from those of controls are shown by these symbols: † = $P < 0.001; \ddagger = 0.001 < P < 0.01.$
from 2 diabetic patients. Thus, we found no evidence for increased adaxonal sequestration in specimens from diabetic patients.

**DISCUSSION**

Genetic and developmental factors, including the synthesis, assembly, and transport of neurofilament proteins, are factors that determine axonal size (28, 29). The caliber of axons may be altered by disease. Axons may become focally swollen when neurofilaments accumulate in spheroids, as in certain intoxications and inherited disorders. Dystrophic axon alterations have been reported in chronic alloxan diabetes (30). Axons may also be distended when fast axonal transport is altered, as in ischemia (31). The focal accumulation of organelles such as polyglucosan bodies may distend axons (17). Also, axonal caliber can be decreased acutely by a shift of fluid, as in hyperglycemic hyperosmolar coma (32).

Axonal atrophy occurs in various neurologic disorders and neuropathies (17). Frequently, however, the term is used loosely and measurable evidence for its occurrence is lacking. In early neuropathologic reports, the terms fiber or axonal atrophy were used imprecisely to describe the finding of smaller-than-normal MF in a tract or nerve, without distinguishing whether it was the result of mal-development, selective loss of large fibers, occurrence of regenerating fibers, cellular atrophy (the disease process by which fibers become smaller), or shrinkage. Acute shrinkage may occur in acute hyperglycemic hyperosmolar coma and with fixation of a nerve in hyperosmolar fixative (17).

Information is available about how and how not to evaluate for axonal atrophy (or shrinkage) by morphometric approaches (17, 33). Smaller diameters of myelinated fibers (or of the axons) may be caused by events other than atrophy; for example, by the selective loss of large fibers. Also, a shift of histogram peaks to smaller size categories may be caused by events other than atrophy; for example, shrinkage or inclusion of regenerated sprouts or remyelinated profile. Assessment of pathologic

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**TABLE 3**

Intercepts and Slopes of Regression Lines of La Axonal Area on Number of Myelin Lamellae of Sural Nerves from Healthy Subjects and from Diabetic Patients ≥30 Years Old with or without Neuropathy

<table>
<thead>
<tr>
<th>MF</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>P*</th>
<th>Slopes</th>
<th>Mean</th>
<th>SD</th>
<th>P*</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large</td>
<td>Controls</td>
<td>1.853</td>
<td>1.359</td>
<td></td>
<td>0.011</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics w/o neurop</td>
<td>1.528</td>
<td>1.168</td>
<td>NS</td>
<td>0.012</td>
<td>0.010</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics with neurop</td>
<td>1.782</td>
<td>1.389</td>
<td>NS</td>
<td>0.010</td>
<td>0.012</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>Controls</td>
<td>0.626</td>
<td>0.532</td>
<td></td>
<td>0.025</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics w/o neurop</td>
<td>0.783</td>
<td>0.414</td>
<td>NS</td>
<td>0.024</td>
<td>0.012</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics with neurop</td>
<td>0.696</td>
<td>0.487</td>
<td>NS</td>
<td>0.032</td>
<td>0.013</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>Controls</td>
<td>0.917</td>
<td>0.399</td>
<td></td>
<td>0.018</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics w/o neurop</td>
<td>1.029</td>
<td>0.409</td>
<td>NS</td>
<td>0.017</td>
<td>0.004</td>
<td>NS</td>
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<td></td>
<td>Diabetics with neurop</td>
<td>1.219</td>
<td>0.421</td>
<td>0.013</td>
<td>0.016</td>
<td>0.005</td>
<td>0.029</td>
<td></td>
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MF = myelinated fiber; neurop = polyneuropathy; w/o = without.
* NS, P > 0.05.

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**TABLE 4**

Intercepts and Slopes of Regression Lines of Index of Circularity on Number of Myelin Lamellae of Myelinated Fibers of Sural Nerves from Healthy Subjects and Diabetic Patients ≥30 Years Old with or without Neuropathy

<table>
<thead>
<tr>
<th>MF</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>P*</th>
<th>Slopes</th>
<th>Mean</th>
<th>SD</th>
<th>P*</th>
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<tr>
<td>Large</td>
<td>Controls</td>
<td>0.848</td>
<td>0.143</td>
<td></td>
<td>&lt;0.001</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics w/o neurop</td>
<td>0.734</td>
<td>0.165</td>
<td>0.068</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td></td>
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<tr>
<td></td>
<td>Diabetics with neurop</td>
<td>0.796</td>
<td>0.216</td>
<td>NS</td>
<td>0.001</td>
<td>0.002</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>MF</td>
<td>0.722</td>
<td>0.076</td>
<td></td>
<td>0.002</td>
<td>0.001</td>
<td></td>
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<tr>
<td></td>
<td>Controls</td>
<td>0.720</td>
<td>0.088</td>
<td>NS</td>
<td>0.002</td>
<td>0.002</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetics w/o neurop</td>
<td>0.816</td>
<td>0.101</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.018</td>
<td></td>
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<tr>
<td></td>
<td>Diabetics with neurop</td>
<td>0.739</td>
<td>0.068</td>
<td></td>
<td>0.001</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>All</td>
<td>MF</td>
<td>0.732</td>
<td>0.097</td>
<td>NS</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>0.812</td>
<td>0.093</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

MF = myelinated fibers; neurop = polyneuropathy; w/o = without.
* NS, P > 0.05.
conditions of teased fibers (e.g. myelin irregularity) is not ideal for assessing axonal atrophy in DP because myelin irregularity may precede or accompany axonal degeneration and segmental demyelination, which are typical fiber changes encountered in DP (17).

Morphometric approaches have been used to assess for axonal atrophy or shrinkage in various experimental and human neuropathies (17, 18, 33, 34). For the morphometric approach to provide valid results, test and control nerve specimens must (a) be of the same anatomical nerve and level and obtained under optimal conditions (at biopsy and not post mortem) and under the same condition; (b) be fixed with the same iso-osmolar fixative (35) and processed with the same histologic techniques; (c) be evaluated in electron micrographs so that axonal area, index of circularity, number of neurofilaments, and number of myelin lamellae can be assessed adequately; (d) be adequately orientated and photographed so that truly transverse-sectioned axons are measured and the number of lamellae be counted reliably. Also, the myelinated fibers must be selected randomly, but must include nerves with a sufficient number of large fibers. We did not assess for the number of neurofilaments because this is prohibitively time-consuming. We assessed for the frequency of adaxonal sequestration on the assumption that it might be a mechanism or a result of fiber atrophy (20).

In the present studies, a major test of the hypothesis that axonal atrophy occurs in diabetic polyneuropathy rests on accurate assessment of the cross-sectional area of axons and the number of myelin lamellae as an indicator of the former size of myelinated fibers. The assumption that the number of myelin lamellae might serve as a marker of the former size of fibers is based on our studies of the permanent axotomy model. In this model, the number of myelin lamellae remains reasonably constant (for a period of time and depending on the severity of axonal atrophy) despite the decreasing size of axons. In previous studies, we used several measures of the amount of myelin (measured thickness, number of lamellae, and myelin spiral length). It now appears that the number of lamellae is a more constant measure of a fiber’s former size than myelin spiral length (36–38).

Two important questions are, what is the size class of myelinated fibers that should be studied and what analysis should be used? Nerves without large fibers may not be studied because remaining small fibers might represent a mixture of normal fibers, remyelinated internodes, and...
regenerated sprouts. Because remyelinated internodes and regenerated sprouts generally fit in the small fiber size class, assessing the relationship of axonal area (or index of circularity) on the number of myelin lamellae of large fibers would prevent the inclusion of remyelinated internodes and sprouts.

The present studies provide a direct test of whether axons of large fibers are significantly smaller in patients with diabetes mellitus (or in diabetic polyneuropathy) than in healthy subjects. Atrophy or shrinkage was not found. Based on previous studies of experimental diabetes (18–20), acute hyperglycemic hyperosmolar coma (32), uremia (39), Friedrich's ataxia (40), hereditary motor and sensory neuropathy type 1 (41), and the permanent axotomy model (33, 34), axons smaller than those in controls can be demonstrated by the morphometric techniques used in our study. In these examples in which statistically significant shrinkage or atrophy was demonstrated using a smaller number of nerves, one can exclude atrophy or shrinkage of a comparable degree. The present results also confirm our earlier results that did not show atrophy or shrinkage (23).

For small myelinated fibers, an almost significant altered relationship of In axonal area to number of lamellae was found, but only for the group with DP. Axons of DP nerves were slightly larger (or perhaps more correctly, myelin was slightly thinner) than it should be. This is the result that might be expected when remyelinated internodes and regenerated sprouts are included with normal fibers.

The present results have important implications for understanding fiber degeneration in diabetic polyneuropathy. Axonal atrophy cannot be seen as an event that precedes myelin remodeling and axonal degeneration. Our previous results of acute axonal degeneration and proximal loss of fibers might suggest a more rapid mechanism for fiber degeneration (7, 8). The present results also suggest that experimental diabetic neuropathy and human diabetic polyneuropathy are different. Although experimental neuropathy and human neuropathy have functional similarities, axonal atrophy does not appear to be one of them.

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