A Quantitative Assessment of Myelin Sheaths in the Peripheral Nerves of Dystrophic, Quaking, and Trembler Mutants*

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Summary. If myelin sheaths are relatively thin for axon caliber, this is generally taken as a sign of insufficient myelin formation. However, recent studies have shown that sheath thickness relates not only to axon caliber; the relative length of the internode is also important. Foreshortened internodes have slightly thinner sheaths than long internodes of the same fiber caliber (Friede and Bischhausen 1982).

In the present study we compared sheath thickness with internode geometry in the sciatic fibers of three murine mutants, the Dystrophic, Quaking and Trembler mice, using a new computer-assisted method. A quantitative correspondence was found between abnormally thin sheaths and internode foreshortening. The magnitude of the changes was the same as that found previously in normal and regenerated fiber populations. The data show that the geometric proportions of internodes cannot be ignored when assessing sheath thickness, and they also shed some new light on the mechanisms which produce abnormally thin sheaths.

Key words: Myelin sheath thickness — Internode length — Peripheral nerves — Trembler, Quaking, Dystrophic mice

Introduction

Peripheral nerve fibers are known to possess regular proportions between the calibers of their axons and the thickness of their sheaths. Fibers with markedly thinner sheaths are often taken as prima facie evidence of hypomyelination with the implication of an insufficiency in the Schwann cell's capacity to produce myelin. Such straightforward interpretations of sheath thickness may be misleading. A more complete analysis of internode geometry has shown that variance in axon caliber is not the only factor affecting sheath thickness. Internodes differ in their geometric proportions, some being relatively short and others relatively long for their caliber (Friede 1983). These proportions are expressed by the l/d quotient between the internode's length and its diameter. Foreshortening of an internode (lower l/d quotient) was found to be accompanied by a slight reduction in sheath thickness and vice versa (Friede and Bischhausen 1982). This interrelation helps to explain the many variations of sheath thickness in normal and pathologic fiber populations.

Considering these premises, the interpretation of hypoplastic myelin sheaths in murine mutants is open to reappraisal. One may question whether such sheaths are truly hypoplastic from a defect in myelin formation, or else whether sheath thickness is merely an adaptation to changed geometric proportions of the internode population. We report data for three murine mutants, the Dystrophic, the Quaking, and the Trembler mice (for review see Aguayo et al. 1979; Bray et al. 1981). A new computer-assisted method was used (Friede and Beuche 1985) which allows the collection of great numbers of precise measurements at the electron-microscopic level.

Material and Methods

Dystrophic mice (C57BL dy2/dy2) were obtained from the Muscular Dystrophy Laboratories in Newcastle upon Tyne, England; Quaking (B6 qk/qk) and Trembler mutants (B 6D2F1/T+) from the Laboratoire de Neurochimie, Hôpital de Salpêtrière, Paris, France. Normal controls were of the C57BL strain. Three animals of each strain, aged 16—18 weeks, were selected from a larger number of specimens for optimal tissue preservation. The animals were killed under deep anesthesia with Rompun-Ketanest; tissues were fixed by immersion with 3%
glutaraldehyde. Tissues were osmicated and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope.

A detailed account of the morphometric method was given in a previous report (Friede and Beuche 1985). Briefly, thin sections were cut as strictly perpendicular to fibers as possible, and electron micrographs were taken of every grid opening showing nerve tissue at a final magnification of $\times 3,400$. This provided randomized sampling of the nerves. Prints were made, and the inner and outer surfaces of the myelin sheaths were traced manually with a cursor and measured with a Kontron Videoplan. Care was taken to measure only compact sheaths, avoiding clefts of Schmidt-Lanterman, paranodal bulbs, or any suboptimally preserved sheaths. A special computer software, developed for these measurements, determined four primary parameters (circumference of axon, circumference of sheath, area of axon, area of sheath). From these, six secondary parameters were calculated, including the noncircularity of the axons (area of axon measured/area of a full circle having the same circumference) and the recalculation for circular profiles of axon and fiber diameters, of the g-ratio (diameter axon/diameter fiber), and of axon area. This program allows plotting of scatter diagrams of two parameters in scatter diagrams with appropriate statistics and linear regression analysis. An important part of this measuring program is its ability to automatically correct for fiber shrinkage, expressing all data for circular profiles along with documentation of the degree of shrinkage for each fiber. The data shown here are for circular profiles based on measured sheath circumference. Noncircularities are given in the Results section. There was a tendency toward lower values for thin fibers, but previous studies had shown the noncircularity trend to be quite variable among specimens.

Teased fibers were measured with the Kontron Videoplan using an Abbe drawing apparatus. Internode length was measured between nodal gaps; fiber caliber was determined from four measurements per internode, two on either side of the nucleus. Further details were given previously (Friede 1983). Fibers were particularly difficult to tease and measure in Trembling mice. Light-microscopic photographs were taken of each teased fiber. The fiber was then traced with an oil immersion lens, and positively identified nodes were marked on the photographs. The latter were used as a guide when measuring internode length and fiber caliber. Corresponding sets of data were then fed into the computer.

Results

General Remarks

The manual morphometry used in this study involves considerable effort in time and photographic material. Therefore, data collection was restricted to the sciatic nerves of three animals of each mutant plus three controls, all animals having similar ages. A separate set of curves was obtained for each animal including basic statistics. These sets were always nearly identical, and the scatter diagrams shown here are the superimposed data for all three animals. There was a total of 1,902 fibers for the Dystrophic mutants; 2,314 for the Quaking mutants; 1,753 for the Trembler mutants; and 2,303 for the controls. The computer program permits comparison of various parameter combinations, e.g., the thickness of the sheath, or the area of the sheath to be compared with the diameter of the fiber, the diameter of the axon, the area of the axoplasm, etc. All of these curves were printed. We include here only the scatter diagrams for the g-ratio, which defines sheath thickness by the quotient axon diameter/fiber diameter. These diagrams were found to be the most informative set of curves. The g-ratio was always calculated for circular fiber profiles based on measurements of circumference; this eliminates noncircularity from fiber shrinkage in situ or during tissue processing. Mean noncircularities were 0.73 for the Dystrophic mutants; 0.84 for the Quaking mutants; 0.94 for the Tremblers; and 0.82 for the controls. There were no consistent trends in noncircularity in these fiber populations other than that of introducing irregular scatter.

Scatter Diagrams of Sheath Thickness (Fig. 1)

Scatter diagrams of myelin sheath thickness (g-ratio) have a characteristic cornucopia shape in normal mice. This shape is known to result from the overlap of two fiber populations. A group of very thin myelinated fibers with very thin sheaths (high g-ratios) occupies the left upper quadrant of the scatter diagram. The rest of the fibers shows a linear regression which indicates a slight decrease in sheath thickness with increasing fiber caliber. The two fiber populations are separate in some nerves, e.g., in the sciatic nerve of the frog or cat (Friede and Beuche 1985), in the sciatic nerve of the rabbit (unpublished data), or in the human sural nerve (unpublished data). Their overlap in mice and rats causes the characteristic cornucopia shape of the scatter diagram.

Because of the irregular shape of the scatter diagram one must take caution against calculation of unqualified means for the entire fiber population. Means are affected by the number of fibers in each caliber class. There is also no true Gaussian distribution of fiber histograms. Furthermore, fibers with relatively thin sheaths, for instance, are composed of a large population of very thin fibers and a small population of very thick fibers representing the two ends of the cornucopia profile. For all these reasons, we made judicious, sparse use of statistics in this study, placing greatest emphasis on the comparison of the characteristic shapes of the scatter diagrams (Fig. 2).

The scatter diagram of dystrophic mice resembled that of normal mice, but the cornucopia profile was "filled in" from the top, and values tended to be slightly higher. The mean was 0.76 ± 0.06 for Dystrophic mice, as compared with 0.70 ± 0.06 for the controls. Fibers were also slightly thicker in dystrophic nerves: 4.3 ± 1.7 vs. 3.1 ± 0.7. This was seen also for the diameters of the teased fibers in Fig. 3.