The organization of the retinal nerve fiber layer of the cynomolgus monkey was studied by electron microscopy. Nerve fiber size spectra were obtained from measurements of every fiber in enlarged montages of selected bundles. Variation in spectra among nasal, arcuate, and papillomacular bundles was examined, and variation of spectra of a given bundle as it crossed the retina was determined. Among the three types of bundles, the papillomacular contained relatively more small fibers, nasal bundles relatively more large fibers. Systematic variation of fiber spectra was observed as the disc was approached by arcuate and papillomacular but not nasal bundles. Arcuate bundles sampled in the peripapillary area contained relatively more small fibers than when sampled at a greater distance from the disc. In contrast, papillomacular bundles sampled near the disc contained relatively more large fibers than near the fovea. Invest Ophthalmol Vis Sci 25:19-29, 1984

The nerve fiber layer of the primate retina contains the axons of ganglion cells and the processes of astrocytes and Muller cells, aggregated into bundles by radial fibers of Muller. The nerve fibers within a bundle are of different lengths since they originate at varying distances from the optic disc margin. Recent studies in the cynomolgus monkey have shown that long fibers, whose cells of origin are outlying, pass to the optic disc on the vitreal surface of the bundles, overlying shorter fibers that originate from cells located closer to the disc.

Although long-standing interest in nerve fiber layer retinotopy has led to a number of qualitative studies of this important retinal structure, no quantitative data concerning the variation of nerve fiber layer morphometry across the retina have been published. In particular, the proportions of nerve fibers of various sizes (nerve fiber spectra) and their organization within the nerve bundles have not been reported. Previous studies include those of Cohen who examined three adjacent bundles of fibers in a macaque monkey and found, repectively, 310, 317, and 330 fibers ranging in size from 0.2 to 3.0 microns. Ogden, in a study of primate nerve fiber layer astrocytes, described one fiber bundle that contained 508 nerve fibers but was not concerned with fiber dimensions.

Variation in fiber spectra across the retina could result from variation in the proportions of large and small ganglion cells at different retinal locations. This could be of significance because ganglion cells of different size probably have different functions. Variation in ganglion cell size with retinal position has been reported and may contribute to the peculiar susceptibility of ganglion cells of the central retina to the effects of toxic chemicals and nutritional deficiency or to the selective loss of axons of Bjerrum area ganglion cells in glaucoma and peripheral ganglion cells in papilledema. Thus the availability of more detailed data concerning nerve fiber layer structure could facilitate understanding of a number of important retinal problems.

The purpose of this study is to provide quantitative data concerning the variation in size, number, and distribution of ganglion cell axons in the nerve fiber layer of the cynomolgus monkey at various positions across the retina. These data complement the results of recent studies that revealed variations in the thickness and glial content of the nerve fiber layer, traced the paths of individual nerve fibers and bundles, and showed their retinotopic organization in the macaque and owl monkeys.

Materials and Methods

The tissues studied were obtained from ten adult cynomolgus macaque monkeys of both sexes, anesthetized with ketamine (10 mg/kg) and pentobarbital (40 mg/kg). The posterior hemisphere of the enucleated eye was rapidly incised, pinned flat in the form of a Maltese cross and immersed in cold fixative (2% glutaraldehyde, 2% paraformaldehyde, 1% osmium in 0.1 M phosphate buffer, pH 7.2). After 1 hour, the lightly osmicated tissues were post-fixed in 2% osmium,
washed in cold buffer, dehydrated in alcohol, and sectioned into blocks whose position in the retina was carefully noted. Each block contained a complete nerve fiber bundle projection. Thus the superior and inferior temporal blocks were arcuate in form to accommodate the arcuate fibers. The temporal and nasal blocks were wedge shaped. The tissues were embedded in eponaraldite and thin sectioned on an LKB V ultratome. Sections were stained with uranyl acetate and lead citrate, and examined with a Zeiss 10B electron microscope. The data presented below are not corrected for shrinkage; however, previous studies of retinas embedded in this manner showed linear shrinkage of 12–15%.

Nerve Fiber Layer Morphometry

Measures of nerve fibers were obtained from photographic montages of the nerve fiber layer. These were constructed from electron micrographs with a total enlargement of 16,000X. The montages varied from 25 cm square to 100 × 300 cm. Each montage was first divided into square sectors, 21 cm on a side. A microcomputer-based graphics tablet was used to perform the measurements, one sector at a time. The diameter of each fiber was traced on the tablet. The minimum diameter that could be registered reliably at this magnification was about 0.1 micron. These data, converted to microns, were stored by the computer to prevent overlap of measurements. A major goal of this study was to determine to what degree the proportions of large and small fibers in bundles change as they cross the retina. Thus it was necessary to compare a number of fiber diameter histograms. Quantitative comparison of fiber distributions was facilitated by fitting a mathematically defined function, the log-Weibull probability density function (PDF), to the data. The log-Weibull PDF is well suited to modelling of skew distributions, such as those found in this study.

The procedure for fitting a log-Weibull PDF to a frequency distribution used in this study is described in detail by Oyster et al. The Weibull function is:

\[ P(x) = \beta \alpha^{-1} [(x - \gamma)/\alpha]^{\beta-1} \exp[-((x - \gamma)/\alpha)^\beta] \]

where \( \alpha \), \( \beta \), and \( \gamma \) are parameters of the distribution. The parameters \( \alpha \) and \( \beta \) determine the position of the curve maximum and the broadness of its base. The parameter \( \gamma \) is a shift parameter held constant at the smallest histogram bin width, 0.1 \( \mu \)m. These parameters were estimated by a graphical technique, which also yielded a correlation coefficient \( (r) \) for each curve. Proper choice of the parameters consistently resulted in \( r \)-values greater than 0.9 (Table 1), indicating an acceptable fit of the curves to the data.

Terminology

Nerve fibers are called nasal if they enter the optic disc along its nasal half. Fibers that originate within 0.5 mm of the fovea are called papillomacular. Note that nasal papillomacular fibers, so-called because they originate in the nasal half of the macula, pass directly to the optic disc. The temporal papillomacular fibers arch above and below the macula then join the nasal fibers to enter the disc temporally. These curvilinear fibers are not considered arcuate. Fibers called arcuate are partly those that originate temporal to but outside the macula, within about 2 mm of the horizontal meridian. They enter the optic disc between 12 and 1 or 5 and 6 o'clock. The remaining arcuate fibers are those that originate in retina underlying the arcuate bundles. Fibers that originate within 1 mm of the disc are called peripapillary or short; those that originate more than 5 mm from the disc are called long. The terms central and peripheral are used with reference to the fovea. Obviously, temporal papillomacular fibers are much longer than nasal papillomacular fibers, although both may be equally central.

Results

The axons of the nerve fiber layer were partitioned into discreet bundles by sheets of Müller cell fibers. At progressively greater distances from the optic disc, the nerve fiber layer became thinner, and the amount of glial tissue between the bundles increased. About 5 mm from the disc, ganglion cells were observed in the fiber layer, and the identity of individual bundles became somewhat ambiguous.

A typical nerve fiber bundle is illustrated in Figure 1. This section was obtained 3.8 mm from the disc margin, along the inferior arcuate ridge, where the nerve fiber layer was 15–20 \( \mu \)m thick. Nerve fibers were easily distinguished from astrocyte and Müller cell processes within the nerve fiber layer. Most axons

### Table 1. Weibull curve shape parameters for figure 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alpha</th>
<th>Beta</th>
<th>Gamma</th>
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<td>Total bundle</td>
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<td>4.853</td>
<td>0.1</td>
<td>0.993</td>
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<tr>
<td>Inner third</td>
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<td>4.171</td>
<td>0.1</td>
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<td>Central core</td>
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<tr>
<td>Outer third</td>
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<td>5.362</td>
<td>0.1</td>
<td>0.994</td>
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</tbody>
</table>

* Parameters used to calculate the smooth curves of Figure 2.
Fig. 1. Low-power electron micrograph of an arcuate nerve fiber bundle sampled 3.8 mm from the disc margin. Total fiber count, 1,029 (Magnification, 8,500×).

contained mitochondrial profiles, and all contained neurotubules. The glial profiles contained varying amounts of glial filaments and, unlike the axons, were seldom oval or round in form. Only an occasional profile smaller than 0.1 μm in diameter could not be identified. Such processes were rarely encountered and
AXON DIAMETER (μm)

Fig. 2. Fiber size histograms and calculated log-Weibull probability density functions (PDF) for the fiber bundle shown in Figure 1. A, total population, 0.73 μm sample mean; B, outer third subpopulation, 0.79 μm sample mean; C, inner third subpopulation, 0.68 μm sample mean; D, core subpopulation, 0.79 μm sample mean; E, histograms from A, B, and C are superimposed; F, PDFs from A, B, C, and D are superimposed. In each graph, the relative frequency of fibers of different diameter is indicated on the abscissa. Diagrams indicate sampled area and numbers of fibers in the sample.

Nerve fiber bundle structure was examined with stepped serial sections at three retinal regions, including three papillomacular, three arcuate, and three nasal projections, each containing 3–5 bundles. These nine studies involved the retinas of eight monkeys. Less detailed studies involved an additional 32 papillomacular, 30 arcuate, and 21 nasal bundles. The arcuate bundle shown in Figure 1 will be used to demonstrate the details of the analysis applied to each of the bundles. At this location, the bundle contained 1,029 fibers. The histogram of the fiber population was unimodal, as it was in every bundle examined, and had a peak frequency at 0.6 μm (Fig. 2A). The bundle was unusual in that the fiber spectra of different regions showed a variation of dominant fiber size with position of the fibers. The outer third of the bundle (Fig. 2B) contained relatively more large fibers than the inner third (Fig. 2C). The central core of the bundle also contained relatively more large fibers than the inner third (Fig. 2D). The core region included in Figure 2D is shown in Figure 3B.

Histograms of raw data are inherently noisy due to inevitable biologic variation among the samples.
attempt to compare histograms directly is confounded by this variability (Fig. 2E). The smooth curves superimposed on the histograms of Figures 2A–D represent the PDF for each population. Comparison of populations is greatly facilitated by superimposition of these curves, which effectively smooth the noise inherent in the histograms (compare Figs. 2E and 2F).

The differences in the PDFs shown in Figure 2 can also be characterized by the differences in the parameters α and β used for their calculation (Table 1). The shift from small to large fibers and broadening of the base of the curves was associated with an increase in these parameters. Thus the parameter α was 4.171 for the inner third of the bundle and 5.362 for the outer third. This increase was associated with a shift of the population peak to larger fibers and a broadening of the base of the probability density function. Table 1 also shows the correlation coefficient associated with each parameter determination. The high r-values reflect the closeness of fit of the curves to the data.

The computer-based measurement system used in this study stored the position and size of every fiber within a given bundle. These data could be displayed graphically with an X-Y plotter, and it was convenient to designate fibers of a given size in such a plot to reveal subtle aspects of bundle organization. Figure 3 shows plots of the bundle shown in Figures 1 and 2. The position of every fiber in the bundle is accurately shown, and the position of every fiber smaller than 0.3 μm also is indicated in Figure 3A. These 36 very small fibers were predominantly located at the bundle surface. Figure 3B shows the location of every fiber larger than 1.4 μm. These 60 large fibers were clearly not localized in any particular part of the bundle.

The analysis shown in Figures 1–3 was applied to a total of 58 nerve fiber layer montages. Segregation of fibers by size was found in three samples and only one bundle. Thus the results shown in these figures are exceptional. In the remaining samples, no significant differences in fiber population was found within a given bundle at one retinal location, and fibers of all sizes were equally represented in all parts of the bundles.

Variation among Nasal, Arcuate, and Papillomacular Bundles

There was a significant difference among fiber spectra of nasal, arcuate and papillomacular bundles. This difference is illustrated in Figure 4A, which shows samples obtained 2–3 mm from the disc. The papillomacular bundle (p) contained proportionately many more small fibers than the arcuate bundle (a), which in turn contained proportionately more small fibers than the nasal bundle (n). These differences were consistently found among bundles sampled in each of the 11 retinas, at all distances from the optic disc. Mean fiber diameter in 44 papillomacular bundles sampled within 1 mm
of the macula was 0.4 μm (SD ± 0.1 μm). In 38 arcuate bundles sampled at the disc margin, mean diameter was 0.6 μm (SD ± 0.2 μm). Mean diameter of 30 nasal bundles was 0.8 μm (SD ± 0.2 μm) at all locations. The full range of fiber sizes, 0.1–3.9 μm, was found in all bundles except the papillomacular bundles, which lacked fibers larger than about 2.5 μm.

**Variation among Adjacent Bundles is Small**

The fiber spectra of adjacent bundles at one retinal location were determined for the three projections in each of the retinas. The spectra of adjacent bundles were very similar. The PDFs superimposed in Figure 4B, obtained from 3 adjacent arcuate bundles sampled 4 mm from the disc margin, are seen to be nearly identical. The superimposed PDFs of 3 papillomacular and 4 nasal bundles (Figs. 4C and D) also show relatively little variation among adjacent bundles sampled at the same retinal location. This is reflected in the similarity of the shape parameters for these curves, shown in Table 2.

**Bundle Variation across the Retina**

The fiber spectrum of individual papillomacular and arcuate, but not nasal, bundles varied as the bundles crossed the retina en route to the disc. The degree of variation was dependent on the position of the bundle with respect to the area centralis. Bundles that picked up papillomacular fibers as they crossed the retina varied more with position than bundles whose fibers all originated at about the same retinal eccentricity. Fiber histograms and probability density functions from a single arcuate bundle sampled at five locations are shown in Figures 5A–E. The location of each sample is indicated in the diagram of Figure 5A. As this bundle approached the disc, it increased in size from 542 to 18,351 fibers and showed a striking increase in the proportion of smaller fibers. Superimposition of the probability density functions demonstrates the trend clearly (Fig. 5F). Curve A, adjacent to the disc, has a narrow base and tall peak indicating a large proportion of fibers were 0.2 to 0.6 μm in diameter. Curve E, from well out in the periphery, shows proportionately many more fibers larger than 0.6 μm in diameter. These changes in curve shape were associated with changes in the α and β parameters of the log-Weibull functions. The graphs of Figure 6A show the means of the shape parameters, calculated from adjacent arcuate bundles at each location. Both parameters decreased as the disc was approached.

Figure 7 shows a similar study of a temporal papillomacular bundle sampled at three locations between the disc and macula. In contrast to the arcuate bundle,
this bundle acquired relatively more large fibers as it approached the optic disc and increased from 1,317 to 13,088 fibers. This is reflected by the relatively broader probability density curve at position A as compared to the curve of position C (Fig. 7D). The trend of Weibull curve parameters from small to large with approach to the disc is shown in Figure 6B, and clearly differs from the graph of arcuate parameters shown in Figure 6A. Nasal bundles, followed up to 4 mm from the optic disc, showed no consistent variation of fiber spectra with distance from the disc.

Segregation of Fibers by Size within Bundles

There is recent evidence that fibers of more peripheral origin are segregated from peripapillary fibers within arcuate bundles. The above results show that the arcuate bundles near the disc contain proportion-
Fig. 8. X/Y plotter write-out of an arcuate bundle to show the position of every fiber (dots) and every fiber larger than 1.4 μm (large circles) at various distances from the disc: A, 5.5 and 5 mm; B, 4 mm; C, 3 mm; D, 2 mm; E, 1.5 mm; F, 1 mm. The large fibers were randomly distributed throughout the bundle (Calibration: 20 μm).

It was of interest to determine where the large fibers, which are proportionately more common in the periphery, come to lie within the bundles as they approach the disc. It was predicted that the large fibers should be segregated either at the vitreal border of the bundles or at the scleral border of the bundles. In fact, the large fibers were randomly scattered throughout the bundles all along their course to the disc. This is illustrated in Figure 8, which shows plots of an arcuate bundle sampled at seven locations across the retina. The positions of all fibers greater than 1.4 μm in diameter are specifically indicated, and it is apparent that they are not segregated in one particular part of the bundle. An analysis of the position of fibers smaller than 0.3 μm gave similar results. Also, similar studies of 11 other arcuate bundles, eight papillomacular bundles, and nine nasal bundles did not show evidence of segregation of either large or small fibers within the bundles. The arcuate bundle shown in Figures 1–3 was the only bundle encountered in this study that showed such an effect.
Comparison of Cynomolgus and Rhesus Monkey Fiber Spectra

The range of fiber diameters observed in this study was 0.1 to 3.9 μm. This includes the range (0.2–3.1 μm) found in the rhesus monkey by Cohen. The histogram of Figure 9A shows his data, obtained by summing the spectra of three adjacent bundles (see ref. 3, Table I). A probability density curve was calculated for these data and is shown superimposed on the histogram in Figure 9A. The same family of curves used in the above study also describes the data of Cohen. This is shown in Figure 9B, where the log-Weibull curves from Cohen’s data and an arcuate bundle of the cynomolgus monkey, sampled 4 mm from the optic disc, are superimposed. The similarity of the curves shows that the data obtained from these different species and laboratories is highly comparable.

Discussion

It has been shown that bundles of retinal nerve fibers at all retinal locations contain populations of fibers with a skewed unimodal distribution of sizes. The fiber spectra of adjacent bundles were relatively uniform within a local area. The proportion of small fibers in a bundle depended on the relative contribution to it of the area centralis. This is in agreement with the observations of Polyak and others that the proportion of small-sized ganglion cells is substantially higher in the area centralis than in the periphery. The great preponderance of small fibers in the papillomacular bundles is also consistent with past studies of the retro-laminar optic nerve, which showed similar fiber spectra in the temporal quadrant of the nerve.

Substantial and systematic variation in the spectra of single arcuate and papillomacular bundles sampled at serial positions across the retina was found. The extent of the variation along a bundle was dependent on its trajectory. Bundles that passed close to the area centralis varied more than those whose path remained equidistant from the fovea. Absence of positional variation of spectra in the nasal bundles was associated with their extreme peripheral position and suggests uniformity in proportions of different sizes of ganglion cells at eccentricities greater than 20°.

Nerve fiber spectra obtained from the inner thirds of most fiber bundles were very similar to those obtained from the middle and outer thirds. Thus fibers were not segregated within the bundles by size. This was an unexpected finding in view of recent evidence of retinotopic layering of fibers within fiber bundles of the cynomolgus monkey. Ogden labeled long fibers of arcuate bundles with local, outlying retinal injections of horseradish peroxidase and was able to follow the fibers to the optic disc margin in serial sections of the retina. The labeled long fibers maintained a vitreal position in the bundles as they crossed the retina. Since the bundles have proportionately more large fibers in the periphery, it was predicted that the vitreal portion of arcuate bundles should have a spectrum shifted toward large fibers, but this was not found. A possible explanation for the similarity of the spectra of inner and outer portions of bundles is that the peripheral contribution of a few hundred relatively larger fibers is masked by the many thousands of relatively smaller fibers added to the bundles as they approach the disc. Thus the expectation of large fiber segregation in the nerve bundles may have been unwarranted, and the absence of segregation does not invalidate the earlier findings of nerve fiber layer retinotopy, which showed unequivocal evidence of gross separation of long from short fibers.

Ganglion cells may be classified as of several types on the basis of functional or structural characteristics. Classifications that include both types of properties are the most helpful in emphasizing meaningful differences among cells. Thus the association of X and Y receptive field properties with the morphologic β and α ganglion cell types in the cat had led to a substantial advance in our understanding of ganglion cell function. Primate ganglion cells also have X-like and Y-like response properties. The midget ganglion cells of Polyak are clearly X-type and have been shown to project to the parvocellular layers of the lateral geniculate nucleus in the primate. These cells are analogous to the B cells of the cat and have been called B cells by Leventhal et al or Pβ cells by Perry and Cowey. A high percentage of ganglion cells of the macula are of the midget type and have X-like properties. Thus, the papillomacular bundles sampled near the fovea should contain primarily the axons of the B cells. Average fiber diameter in the papillomacular bundles sampled in this study was 0.4
also be trimodal. Examination of whole cat optic nerve bundles as they approach the disc, from a mean of about 0.4 μm to about 0.6 μm.

Functional Significance of Nerve Fiber Spectra

The fiber spectra of cat and monkey optic nerves are unimodal, however, their compound action potentials are strikingly multi-modal, suggesting the activity of clearly distinct groups of fibers of different sizes. The relationship of a compound action potential to the spectrum of fiber diameters in a population of fibers is complex. Even a cursory examination of published data provides convincing proof that the relation is nonlinear. For instance, most recordings from the optic disc or nerve show a short latency deflection of large amplitude that represents the largest fibers of the population. Yet these large fibers constitute no more than 5–10% of the total population. A second deflection of similar amplitude represents medium-sized fibers, which constitute perhaps half the total. The late deflection, representing the smallest fibers, probably almost half the total, is usually poorly defined. A number of factors contribute to this noncorrespondence of action potentials and population statistics. The use of bipolar stimulation in the brain results in unequal excitation of different-sized fibers at different distances from the cathode, so placement of the stimulating electrode is of crucial importance, and slight changes in electrode position may result in dramatic changes in the form of the compound action potential. There can never be assurance that all fibers at a given location are activated by electrical stimulation in the optic chiasm, optic tract, or lateral geniculate nucleus. The problem is compounded by electrode bias at the recording site, where large fibers generate more extracellular current flow per unit time than small fibers. This results both from the geometry of the large fibers and temporal dispersion of activity in the small fibers.

The lack of correspondence of electrophysiology and morphology is more apparent in studies of cat than primate. There is sound morphologic evidence that cat ganglion cells are of at least three distinct size populations. Since axon diameter is generally proportional to soma diameter, nerve fiber spectra should also be trimodal. Examination of whole cat optic nerve reveals a unimodal population of fibers. A peripheral zone of the nerve, however, may have discreet populations of fibers of different sizes, in keeping with the measurements of ganglion cell somata. The monkey, in contrast, has an essentially unimodal population of different-sized ganglion cells. Thus morphometric studies of optic nerve fiber size and ganglion cell soma size are in agreement in the case of the primate.

Landau et al. successfully modelled the optic nerve compound action potential of the cat on the pragmatic assumption that the contribution of different-sized fibers to the extracellularly recorded potential was proportional to the third power of fiber diameter. They based their modelling exercise on the spectra published by Bishop et al. An attempt of Stone and Hollander to apply this approach to spectra of retinal nerve fibers in the cat gave poor results. However, this should not be surprising. In any particular recording situation, the relationship of fiber size to extracellular current flow through the surrounding tissue must be influenced not only by the nature of the active fiber population, but also by the particular geometry and impedance of the tissues at the recording site, which differ greatly between optic nerve and retina.

The conduction velocities of the X-type and Y-type ganglion cell axons of the monkey show considerably more overlap than is the case with the cat, so it is not clear from the single cell electrophysiology that the primate should have a polymodal distribution of fiber sizes. The conclusion that the primate has a unimodal population of optic nerve fibers, as indicated by this study, is also supported by the observations of Ogden and Miller, who recorded antidromic compound action potentials from several locations around the optic disc of the rhesus monkey. Several conduction velocity groups were observed at each location. However, multiple recordings revealed a wide range of overlapping groups consistent with a locally biased sampling of a unimodal population. Thus, unlike the cat, the morphologic and physiologic data for the primate are probably not in conflict in suggesting an essentially unimodal population of different-sized fibers.

Validity of Nerve Fiber Spectra

Studies of nerve fiber diameter are based on the tacit assumption that the data are meaningful despite fiber shrinkage and distortion caused by histological processing. This assumption has been seriously questioned by Freeman who studied with the electron microscope serial sections of seven cat optic nerve fibers. The diameters of some of the fibers varied as much as 110% over distances as small as 4 micra. He suggested that random variation of fiber diameter along the length of fixed fibers might mask the appearance of separate
fiber groups in nerve fiber spectra obtained from the optic nerve.

The irregular shrinkage observed in the cat optic nerve by Freeman\textsuperscript{30} may have been the result of his method of fixation. The optic nerve undergoes substantial distortion during fixation unless it is mechanically stabilized or fixed in situ. Freeman excised his optic nerves and fixed them by immersion without stabilization. Also, he did not measure the shrinkage of his tissue or evaluate its uniformity. Non-uniform shrinkage of tissue floating in fixative or dehydrating fluids could result in longitudinal fiber size variability. In the present study, the retina was mechanically stabilized prior to fixation and linear shrinkage was 15\% or less. These procedures should not have been associated with large fluctuations in fiber diameter. Retinal nerve fibers processed without these precautions and stained with silver or methylene blue frequently exhibit beading at regular intervals. This beading results from agonal collections of intracellular organelles and does not occur in properly prepared tissues, where long uniform diameter segments of fibers may be seen. Thus it is concluded that the unimodal fiber spectra observed in this study are not artefactual. As noted above, this conclusion is supported by studies of ganglion cell soma size.\textsuperscript{11}

It is probable, however, that some longitudinal variability in fiber size is always present in fixed tissues. It was not sufficient to mask the similarity of adjacent bundles or the variation of spectra along a bundle observed in this study. Longitudinal fiber variation may have contributed to the variability of shape parameters along single bundles, as illustrated in Figure 6. Point-to-point variation of shape parameters could also result from interchange of fibers among bundles, a common feature of nerve fiber bundle organization in the primate.\textsuperscript{2,12}

Key words: primate, retina, nerve fiber layer, morphometric analysis, electron microscopy

References