Segmental Variability of the Trabecular Meshwork in Normal and Glaucomatous Eyes

Cheryl Buller and Douglas Johnson

Purpose. Although numerous studies have examined morphologic changes in the trabecular meshwork of glaucomatous eyes, few have systematically looked for segmental variations around the circumference of the meshwork. If segmental variations occur, studies based on random sections or on single trabeculectomy specimens may not give a complete picture of the histologic changes in glaucoma. The purpose of this study was to determine the extent of segmental variability in human trabecular meshwork.

Methods. Eyes from five normal donors and eyes from five donors with primary open-angle glaucoma were obtained at autopsy. Histologic sections of each quadrant from nine of the normal eyes and eight of the glaucomatous eyes were analyzed. Light microscopy was used to measure the length of Schlemm’s canal, the thickness of the trabecular lamellae, and the area of empty space in the juxtacanalicular (JCT) region. Transmission electron microscopy was used to examine the thickness of the JCT and the ultrastructural components of the JCT, which were quantitated with a computerized image analysis system. Ultrastructural analysis included JCT area, area of empty space, area of empty space touching Schlemm’s canal, area of solid tissue, and area of sheath and tendon material.

Results. Differences among quadrants were qualitative rather than quantitative, because significant variations in structure were not found within single eyes. The variability among quadrants was similar for normal and glaucomatous eyes, with a coefficient of variation of approximately 20% for most tissue components. A greater variability was found for the thickness of the JCT. The largest variability was found for the area of empty space touching Schlemm’s canal: 81.4% for normal eyes and 86.5% for glaucomatous eyes. No quadrant had consistently higher or lower values of any parameter.

The length of Schlemm’s canal did not differ significantly between normal and glaucomatous eyes (normal: 256.6 ± 66.0 μm, glaucomatous: 298.8 ± 57.7 μm), nor did the mean thickness of the trabecular lamellae (normal: 4.7 ± 0.8 μm, glaucomatous: 4.8 ± 0.5 μm). Light microscopy underestimated the total amount of empty space in the JCT by 20% compared with electron microscopy.

Conclusions. Significant segmental differences were not found within single eyes in either normal or glaucomatous eyes. The magnitude of the variability of most tissue components was similar for normal and glaucomatous eyes. The most variable component was the area of empty space touching Schlemm’s canal, which may represent the effective pathway for aqueous flow to enter Schlemm’s canal. Future quantitative studies of the JCT should include samples from at least three quadrants per eye. Quantitative analysis of the JCT from a single quadrant, as occurs in the study of trabeculectomy specimens, may overestimate or underestimate the amount of solid tissue or empty space by 200%. Invest Ophthalmol Vis Sci. 1994;35:3841-3851.

A fundamental question in the study of primary open-angle glaucoma is whether histologic sections from only one area of the trabecular meshwork are representative of the pathologic changes throughout the meshwork. Although numerous studies have examined the morphologic changes in primary open-angle glaucoma,1-11 few have systematically examined segmental variations around the circumference of the
meshwork. If segmental variations occur, studies based on single trabeculectomy specimens may not give a complete picture of the histologic changes in glaucoma. Calculations of outflow resistance or conclusions based on the study of only one sample of tissue may not be representative of the meshwork at other locations or of the overall outflow status of the eye.

Pathologic changes described in primary open-angle glaucoma include thickening and fusion of the trabecular lamellae, shortening and loss of area of Schlemm’s canal, decreased numbers of trabecular cells, and increased density of extracellular material in the juxtacanalicular region. Of these findings, changes in the JCT may represent the pathophysiologic basis for the elevated intraocular pressure found in glaucoma. The juxtacanalicular tissue is composed of electron-lucent spaces and several types of extracellular materials: amorphous basement membrane, elastic-like tendons, and collagenous tendon sheaths (earlier described as “plaques” by Rohen: types 1, 2, and 3, respectively). Trabecular cells are present in stellate or free-form shape. With advancing age, the extracellular materials may increase in amount. In eyes with primary open-angle glaucoma, larger amounts of these extracellular materials are often found, in accord with an early study that concluded that glaucoma may be caused by a “pathologic excess of a physiologic process.”

We examined the variability of the trabecular meshwork around the circumference of the eye, looking for evidence of significant regional differences. Examination included measurement of the length of Schlemm’s canal and trabecular beam thickness with light microscopy and quantitation of the extracellular matrix of the JCT with both light and transmission electron microscopy using an image analysis system. Differences among quadrants appeared to be more qualitative than quantitative, because significant variations in structure among quadrants was not found. The coefficient of variation of the amounts of most tissue components, approximately 20%, indicates that quantitative studies of the meshwork from a single quadrant may overestimate or underestimate the amount of solid tissue or empty space by as much as 200%.

**MATERIALS AND METHODS**

Eyes obtained at autopsy from five donors with normal eyes and five donors with primary open-angle glaucoma were studied. Only one eye from each donor was used for the statistical analysis. Fellow eyes were studied when available and used in subsequent analyses; a total of nine normal and eight glaucomatous eyes were studied (Tables 1, 2). Each eye was compared with itself to determine circumferential variability, and eyes fixed by both immersion and perfusion were included in each group.

Normal eyes were obtained at autopsy and processed within 18 hours of death. The mean age of the patients from which the normal eyes were obtained was 76 ± 9 years (mean ± SD; range, 69 to 89 years; Table 1). No eyes had had prior surgery or a history of eye disease. Six eyes were fixed by perfusion (17 mm Hg), and three eyes were fixed by immersion after opening the eye at the equator.

Glaucomas were obtained at autopsy from the Mayo Clinic eyebank (7 eyes) and from the Foundation for Glaucoma Research. All eyes had a history of primary open-angle glaucoma, and clinical histories were obtained from the patients’ ophthalmologists in most cases. Several stages of severity of glaucoma were included, from pressure elevation only (no disc or field damage) to cupping with field loss (Table 2). All patients had undergone topical therapy; none had required filtration surgery. Eyes were processed within 18 hours of death. Mean age of the patients was 78 ± 7 years (range, 68 to 85 years; Table 2). Three eyes had posterior chamber intraocular lenses (IOLs); no other ocular surgery had been performed. Two eyes were fixed by perfusion (17 mm Hg), and six were fixed by immersion.

**Tissue Processing**

Fixative was 1% paraformaldehyde–2% glutaraldehyde in 0.1-M cacodylate buffer, pH 7.4. Wedges of the limbal region including the trabecular meshwork were taken from each quadrant, dehydrated in ascending alcohols, and embedded in Araldite 502 (Ted Pella, Reading, CA). Thick sections (1-μm) were stained with 1% toluidine blue. Adjacent thin sections were cut for transmission electron microscopy and mounted on either copper slot grids (coated with 3% nitrocellulose) or 135 hexagonal mesh grids. Grids were stained with uranyl acetate and lead citrate and examined in a Phillips CM-12 electron microscope (Phillips Electronic Instruments, Mahwah, NJ).

**Morphologic Analysis**

Schlemm’s Canal. Light microscopy was used to measure the anterior-to-posterior length of Schlemm’s canal. This measurement was also expressed as a ratio proportionate to the length of the meshwork, which keeps the canal size relative to the size of the meshwork and also lessens the effect of oblique or nonradial histologic sections. Meshwork length was measured from the end of Descemet’s membrane to the posterior end of Schlemm’s canal. Measurements were made at 400× magnification with a graticule in the
TABLE 1. Characteristics of Normal Eyes

<table>
<thead>
<tr>
<th>Specimen (Age, Sex)</th>
<th>Fix</th>
<th>SC Length (μm)</th>
<th>SC length:TM Length</th>
<th>Beam Thickness (μm)</th>
<th>JCT Thickness (μm)</th>
<th>JCT: % Solid Tissue</th>
<th>JCT: % Empty Space</th>
<th>Tendon and Sheath: % of JCT</th>
<th>JCT: % Empty Space</th>
<th>Empty Space Touch SC: % of JCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>345 (69 M)</td>
<td>PF</td>
<td>310 ± 87</td>
<td>0.39 ± 0.11</td>
<td>4.8 ± 1.0</td>
<td>7.4 ± 3.2</td>
<td>65.7 ± 5.1</td>
<td>19.1 ± 10.5</td>
<td>34.2 ± 5.1</td>
<td>6.0 ± 4.7</td>
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<tr>
<td>L*</td>
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<tr>
<td>633 (72 M)</td>
<td>PF</td>
<td>184 ± 35</td>
<td>0.28 ± 0.05</td>
<td>4.7 ± 0.8</td>
<td>7.8 ± 3.8</td>
<td>61.6 ± 10.4</td>
<td>24.2 ± 13.4</td>
<td>38.2 ± 10.4</td>
<td>3.6 ± 3.7</td>
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<tr>
<td>R</td>
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<td></td>
<td></td>
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<tr>
<td>L</td>
<td>PF</td>
<td>216 ± 42</td>
<td>0.37 ± 0.06</td>
<td>4.5 ± 0.4</td>
<td>5.7 ± 3.4</td>
<td>52.7 ± 1.7</td>
<td>34.4 ± 6.2</td>
<td>47.1 ± 1.7</td>
<td>9.7 ± 8.6</td>
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<tr>
<td>1265 (81 F)</td>
<td>PF</td>
<td>236 ± 95</td>
<td>0.31 ± 0.05</td>
<td>5.2 ± 0.8</td>
<td>15.5 ± 4.4</td>
<td>64.0 ± 10.3</td>
<td>23.6 ± 10.1</td>
<td>35.9 ± 10.5</td>
<td>3.4 ± 3.9</td>
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<tr>
<td>1352 (81 F)</td>
<td>IM</td>
<td>291 ± 45</td>
<td>0.47 ± 0.08</td>
<td>5.6 ± 0.8</td>
<td>7.4 ± 2.4</td>
<td>75.5 ± 10.1</td>
<td>21.1 ± 4.9</td>
<td>24.3 ± 10.1</td>
<td>0.8 ± 0.4</td>
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<tr>
<td>R</td>
<td>PF</td>
<td>298 ± 83</td>
<td>0.46 ± 0.09</td>
<td>5.2 ± 0.9</td>
<td>4.6 ± 1.8</td>
<td>70.4 ± 2.1</td>
<td>33.7 ± 13.3</td>
<td>29.4 ± 2.1</td>
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<tr>
<td>1355 (89 F)</td>
<td>IM</td>
<td>218 ± 63</td>
<td>0.41 ± 0.14</td>
<td>3.6 ± 0.7</td>
<td>9.5 ± 6.3</td>
<td>60.5 ± 9.3</td>
<td>22.6 ± 3.9</td>
<td>39.3 ± 9.3</td>
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</tr>
<tr>
<td>R</td>
<td>PF</td>
<td>224 ± 34</td>
<td>0.41 ± 0.04</td>
<td>4.4 ± 0.7</td>
<td>5.2 ± 1.7</td>
<td>60.0 ± 11.5</td>
<td>17.5 ± 4.1</td>
<td>39.8 ± 11.5</td>
<td>3.0 ± 2.4</td>
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</tbody>
</table>

Mean variability

- 5 donors (n = 5 eyes)
  - 19.1% ± 0.06% 20.6% ± 7.4% 15.6% ± 2.8% 40.4% ± 17.7% 16.0% ± 1.8% 32.1% ± 16.3% 31.2% ± 7.0% 81.4% ± 33.3%

- All eyes (n = 9)
  - 20.7% ± 6.3% 19.8% ± 7.0% 15.6% ± 3.5% 41.7% ± 14.3% 12.4% ± 6.3% 33.8% ± 14.8% 23.3% ± 12.5% 79.1% ± 24.8%

* Eye used in analysis of variability.

Im = Immersion fixation; PF = perfusion fixation; SC = Schlemm's canal; TM = trabecular meshwork; JCT = juxtacanalicular tissue.
### TABLE 2. Characteristics of Glaucomatous Eyes

<table>
<thead>
<tr>
<th>Specimen (Age, Sex)</th>
<th>Stage of Glaucoma (Maximum IOP)</th>
<th>RX</th>
<th>Duration</th>
<th>Fix</th>
<th>SC Length (μm)</th>
<th>SC Length: TM</th>
<th>Beam Thickness (μm)</th>
<th>JCT Thickness (μm)</th>
<th>JCT: % Solid Tissue</th>
<th>JCT: % Empty Space</th>
<th>Tendon and Sheath: % of JCT</th>
<th>Empty Space Touch SC: % of JCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GL 9 (76 M)</td>
<td>110P (? mm)</td>
<td>β</td>
<td>14 years</td>
<td>Im</td>
<td>318 ± 54</td>
<td>0.44 ± 0.05</td>
<td>4.8 ± 0.5</td>
<td>9.9 ± 2.2</td>
<td>58.3 ± 7.8</td>
<td>24.9 ± 6.8</td>
<td>41.6 ± 7.8</td>
<td>3.6 ± 2.2</td>
</tr>
<tr>
<td>GL 7 (82 F)</td>
<td>110P (? mm)</td>
<td>β</td>
<td>14 years</td>
<td>Im</td>
<td>288 ± 68</td>
<td>0.44 ± 0.08</td>
<td>4.6 ± 0.3</td>
<td>8.4 ± 2.5</td>
<td>71.2 ± 14.0</td>
<td>33.7 ± 11.0</td>
<td>27.4 ± 12.0</td>
<td>1.6 ± 1.5</td>
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<tr>
<td>GL 21 (80 M)</td>
<td>1IOP (25 mm)</td>
<td>β</td>
<td>1 months</td>
<td>Im</td>
<td>313 ± 75</td>
<td>0.47 ± 0.13</td>
<td>5.4 ± 0.5</td>
<td>7.9 ± 3.9</td>
<td>60.3 ± 6.2</td>
<td>31.1 ± 6.7</td>
<td>37.9 ± 7.0</td>
<td>4.9 ± 5.7</td>
</tr>
<tr>
<td>GL 5 (85 F)</td>
<td>1IOP (27 mm)</td>
<td>β</td>
<td>2 years</td>
<td>PF</td>
<td>257 ± 81</td>
<td>0.36 ± 0.07</td>
<td>4.0 ± 0.7</td>
<td>9.0 ± 2.2</td>
<td>58.0 ± 15.0</td>
<td>25.4 ± 7.4</td>
<td>41.4 ± 13.0</td>
<td>1.1 ± 1.1</td>
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<tr>
<td>GL 8 (68 F)</td>
<td>1IOP (30 mm)</td>
<td>β</td>
<td>3 years</td>
<td>FF</td>
<td>377 ± 66</td>
<td>0.47 ± 0.09</td>
<td>5.1 ± 0.7</td>
<td>8.7 ± 2.5</td>
<td>56.0 ± 3.5</td>
<td>15.7 ± 3.5</td>
<td>44.4 ± 4.2</td>
<td>11.3 ± 6.2</td>
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<tr>
<td>Mean variability</td>
<td>5 donors (n = 5 eyes)</td>
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<tr>
<td></td>
<td>All eyes (n = 8)</td>
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</tr>
</tbody>
</table>

* Ears used in analysis of variability. IM = Immersion fixation; PF = perfusion fixation; β = β blocker therapy; P = pilocarpine, E = epinephrine.
Ears GL 9R, 9L, and 7L had posterior chamber IOLs.
1cup = Definite glaucomatous enlargement of cup; VF: N = normal visual field; IV = definite abnormal scotomata present in glaucomatous pattern.
Meshwork Variability

Lamellar Thickness. The thickness of each trabecular lamella was measured in the region underlying the middle of Schlemm's canal. Measurements were made perpendicular to the length of the lamella at 1000× magnification with the same graticule used to measure Schlemm's canal. At this magnification, measurements could be made to the nearest 0.6 μm.

Juxtacanalicular Region. The Zeiss IBAS 2000 image analysis system (Zeiss, Thornwood, NY) was used. This system is an automated, computerized densitometer capable of resolving up to 256 gradations of gray (image density) with user-interactive software. The operator outlines the areas to be analyzed on the video image, selects the densities or gray levels that are of interest, and analyzes these by area. This system has been used and described previously by our laboratory and by others.

The juxtacanalicular tissue was defined as the tissue underlying Schlemm's canal, extending from Schlemm's canal endothelial cells to the empty space adjacent to the first trabecular lamella (Fig. 1). Although these boundaries were clear in many regions, some areas were less well defined and required the judgment and extrapolation of the operator to delineate uncertain areas. In these instances, the first trabecular lamella was defined either as having a central "core" area of elastic-like tissue with some surrounding cortex, or as having a horizontally elongated trabecular cell overlying such tissue (Fig. 2). Schlemm's canal endothelial cells and giant vacuoles were not included in the juxtacanalicular tissue area.

Light Microscopy. Light microscopic images were entered into image analysis system at 1000× with a video camera attached to the microscope. Images were compensated for background light; pale or darkly stained sections were not used. Three aspects of the JCT could be analyzed with light microscopy: total JCT area, total empty space, and total solid tissue. A comparison was made between light and electron microscopy, considering electron microscopy as the "gold standard" because of its better resolution of tissue details and boundaries. For this comparison, light microscopic sections (1 μm) were cut first, and the immediately adjacent thin section was used for transmission electron microscopy.

Transmission Electron Microscopy. A preliminary study was performed to compare the analysis of the entire JCT region as seen on slot grids with sample micrographs of the JCT from the anterior, middle, and posterior regions (2300× magnification). Six different tissue blocks were analyzed. No significant difference was found between slot grids and the sample areas in the relative proportions of the amount of empty space, the amount of empty space touching Schlemm's canal, and the amount of tendon and sheath material (P = 0.25, P = 0.23, P = 0.16, respectively). Although the anterior and posterior region of Schlemm's canal may be more variable in structure than the middle region, giant vacuoles can be found throughout the length of the canal. This indicates that all regions of the canal contribute to aqueous outflow, and thus, both the anterior and posterior regions were included in the analysis. Micrographs of these regions included the anterior or posterior boundary of the canal at one edge of the picture.

Micrographs were taken of the anterior, middle, and posterior regions of the JCT at 2500×. The photog.
The thickness of the JCT was measured from the micrographs of each sample region, measuring perpendicular to the long axis of the canal using the boundaries outlined above. The three measurements were combined to give the mean thickness per quadrant, and the quadrant values combined to give the mean thickness per eye.

Areas of most juxtacanalicular tissue components were determined by densitometry, including empty space, sheath and tendon material, and pigment granules. Although the elastic-like tendon and tendon sheaths (types 2 and 3 plaques) are clearly distinguishable by visual examination, their image densities as determined by the IBAS were similar enough that they could not always be differentiated, and hence were included together. Two components required hand tracing: the overall boundaries of the JCT and the small areas of empty space touching Schlemm’s canal. The total amount of solid tissue was determined by subtracting the amount of empty space from the total JCT area.

Reproducibility

The reproducibility of the densitometer measurements was determined by reanalysis of one negative from each of five eyes. The operator was masked to the previously determined values of area or gray-level settings. In addition, one negative was reanalyzed on four separate days to determine day-to-day variability of the entire process.

Statistical Analysis

One eye from each donor was included in the statistical analysis. This included three donors with immersion-fixed eyes and two donors with perfusion-fixed eyes in each group. In donors with both eyes fixed by the same technique, the right eye was arbitrarily chosen for the analysis. Data from all four quadrants were combined to determine the range of values, mean, standard deviation, and coefficient of variation ($CV = \sigma/\mu$). The coefficients of variation for each eye were added together for all eyes in each group to produce the mean variability. Comparisons were made using a paired, two-tailed $t$-test for two groups and a repeated measures analysis of variance for comparisons among three or more groups.

Fellow eyes were used for a comparison of differences between fellow eyes ($n = 4$ pair with similar fixation) and for a preliminary comparison of differ-
Meshwork Variability

ences between the immersion and perfusion fixation techniques (n = 3 pair).

Sample size calculations were performed by the method of Lachin and by a confidence interval approach. This approach estimated sample sizes using the t distribution and used the following equation for the half-width of the confidence interval:

\[ t_{n-1, 1-\alpha/2} \sigma / \sqrt{n} \leq c \]  \hspace{1cm} (I)

where c is a predetermined value within which the estimate of the true value is desired. Sample sizes were then investigated in terms of the percentage of the sample mean. This resulted in the following equation used in the calculations:

\[ (1/x)(t_{n-1})\sigma = (t_{n-1})(CV/n) \leq c/x = p \]  \hspace{1cm} (2)

where p is a percentage of the mean value.

RESULTS

Each eye served as its own control, as comparisons of the differences among quadrants within each eye were made. Although the main purpose of the study was to determine the regional variability of the meshwork, the actual values of the measurements are also given to allow preliminary comparisons among eyes.

Schlemm’s Canal

The mean length of the canal was 256.6 ± 65.9 μm for the group of normal eyes (n = 5). The ratio of the length of Schlemm’s canal to the total meshwork length in normal eyes was 0.38 ± 0.08. The mean variability of this ratio among quadrants for all eyes in the normal group, as expressed by the coefficient of variation, was 20.6% ± 7.4% (Table 1). In glaucomatous eyes, the mean length of the canal was 298.8 ± 57.7 μm (n = 5). The ratio of the length of the canal to the total meshwork length was 0.42 ± 0.06 (Table 2). The mean variability of this ratio among quadrants was 20.5% ± 5.6% (coefficient of variation). Statistically, none of these values was significantly different between the normal and glaucomatous groups.

Schlemm’s canal was multichannel in 1.3 ± 1.1 quadrants per eye in the normal eyes and in 1.5 ± 1.1 quadrants per eye in the glaucomatous group.

Lamellar Thickness

The mean thickness of the trabecular lamellae in normal eyes was 4.7 ± 0.8 μm, (n = 5; Table 1). The mean variability among quadrants, as expressed by the coefficient of variation, was 15.6% ± 2.8%. In glaucomatous eyes, the mean lamellar thickness was 4.8 ± 0.5 μm (n = 5; Table 2). The mean variability among quadrants was 12.4% ± 3.3% (coefficient of variation). These values were not significantly different from those of the normal group.

Juxtacanalicular Region

Light Microscopy. In comparison with results from adjacent sections on slot grids analyzed by transmission electron microscopy, light microscopy yielded similar absolute values for total area of the juxtacanalicular tissue (1656 ± 502 μm² and 1705 ± 650 μm², respectively). Light microscopy yielded values approximately 20% greater for solid tissue (P = 0.04) and 20% less for empty space (P = 0.05; data not shown).

Electron Microscopy. Thickness of Juxtacanalicular Tissue. In normal eyes, the mean thickness of the JCT was 10.1 ± 3.2 μm (Table 1). In glaucomatous eyes, the mean JCT thickness was 9.5 ± 1.5 μm. Note that these values include both immersion- and perfusion-fixed eyes. The mean variability among quadrants was 40.4% ± 17.7% for normal eyes and 29.2% ± 11.8% for glaucomatous eyes, as expressed by the coefficient of variation (n = 5 eyes for each group).

Empty Space. In normal eyes, the mean amount of empty space relative to the JCT area in the sampled regions ranged from 24.3% to 39.3% (n = 5; Table 1). The mean variability among quadrants, using the coefficient of variation as an indicator, was 31.2% ± 7.0%. In glaucomatous eyes, the mean amount of empty space relative to the JCT area ranged from 35.1% to 44.4% (n = 5; Table 2). The mean variability among quadrants was 20.3% ± 8.0%. Note that these values include both immersion- and perfusion-fixed eyes. No one quadrant had consistently higher or lower values than other quadrants (data not shown).

The amount of empty space touching Schlemm’s canal relative to the total JCT area was highly variable among eyes and among the individual quadrants within each eye. In normal eyes, the mean value ranged from 0.8% to 5.2%. The mean variability among quadrants of individual eyes, as expressed by the coefficient of variation, was 81.4% ± 33.3%. In glaucomatous eyes, the mean values ranged from 1.1% to 11.3%. The mean variability among quadrants was 86.5% ± 26.9% (n = 5 eyes in each group; Tables 1, 2).

Solid Tissue. In normal eyes, the mean amount of solid tissue relative to the JCT area in the sampled regions ranged from 60.5% to 75.5% (Table 1). This solid tissue value included all components of solid tissue (nonlucent spaces): cytoplasm, nuclei, basal lamina, plaques, and pigment particles. The mean variability among quadrants, using the coefficient of variation as an index, was 16.0% ± 1.8%. In glaucomatous eyes, the mean amount of solid tissue relative to the JCT area ranged from 56.0% to 64.7% (Table 2). The mean variability among quadrants was 13.0% ±
6.0%. These values include both immersion- and perfusion-fixed eyes.

Measurements of the various components of the solid tissue revealed the amount of tendon and sheath material to be 20.9% ± 4.6% of the total JCT in the normal group, with a mean variability of 32.1% ± 16.3% among quadrants (Tables 1, 4). In glaucomatous eyes, the amount of this material was 23.7% ± 5.6%, with a mean variability among quadrants of 26.6% ± 4.7% (coefficient of variation; Tables 2, 4).

Reproducibility
Reanalysis of one negative from each of five eyes revealed values within 7% for area of juxtacanalicular tissue and total empty space and within 14% for area of tendon and sheath material, the most subjective of the parameters. Reanalysis of one negative on four separate days revealed a variability of 1.3% for total juxtacanalicular tissue area, 6.2% for empty space, and 12% for tendon and sheath material.

Other Comparisons
Although the primary purpose of this study was to examine the regional differences within individual eyes, several of the eyes studied were paired and examined for a preliminary look at differences between fellow eyes. With four pairs of eyes, both eyes were fixed with the same technique. Values for most tissue components differed by approximately 15% between fellow eyes. The area of empty space adjacent to Schlemm’s canal was less correlated between fellow eyes, with values differing by 62%.

With three pairs of eyes, one eye was fixed by immersion and the fellow eye was fixed by perfusion. Qualitatively, the JCT region appeared more expanded in the perfusion-fixed eyes, particularly in the space between the outermost corneoscleral lamellae and the JCT (this space was not included in the analysis of the JCT). In these three pairs of eyes, the perfusion-fixed eyes had approximately 6% more empty space than the immersion-fixed eyes. This difference was also found when comparing the entire group of eyes fixed by each method (Table 4; note unequal numbers of eyes in the two groups).

Sample Size Calculations
As shown in Tables 1 and 2, most parameters measured had similar variabilities, approximately 20% (as expressed by the coefficient of variation). Using this as an estimate, sample size calculations can estimate the number of quadrants that must be examined from an eye to yield a valid representation. The two methods of calculation yielded similar values. To estimate the true mean within 50%, three quadrants should be examined, assuming that no significant inherent differences exist (α = 0.05, β = 0.8). Attempts to obtain more accurate estimates require examination of proportionately more samples (Table 3, Fig. 3). The greater variability of the empty space touching Schlemm’s canal results in a significantly larger sample size: 21 areas from around the circumference of the eye would be needed to estimate the true mean within 50% (α = 0.05, β = 0.80).

Expressed in a different fashion using the confidence interval approach, the uncertainty in the estimate of a mean can be determined and related to sample size (Fig. 3). This calculation shows that the ability to estimate the true value of a mean improves dramatically as sample size increases from two to six. A sample size of two yields an estimate within 180% of the true mean, whereas a sample size of six yields an estimate within 20% of the true mean. A sample size of one, as occurs when examining trabeculectomy specimens, yields an estimate of the true mean that may differ by 200% or more from the true mean (Fig. 3).

DISCUSSION
The juxtacanalicular tissue is the most likely site of outflow resistance in normal eyes and in eyes with primary open-angle glaucoma. Quantitative studies of this region have recently been performed in attempt

<table>
<thead>
<tr>
<th>Desire to Know True Mean Within x %</th>
<th>20%</th>
<th>40%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>50%</td>
<td>3</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>30%</td>
<td>6</td>
<td>16</td>
<td>51</td>
</tr>
<tr>
<td>20%</td>
<td>10</td>
<td>33</td>
<td>128</td>
</tr>
</tbody>
</table>
to understand the mechanism of normal outflow resistance and the mechanism of the abnormally high resistance present in glaucoma.4-7 An issue basic to these studies, and to any study of the meshwork, is the variability of these tissues around the circumference of the eye. Without knowledge of this variability, it is difficult to know how representative studies based on trabeculectomy specimens or single samplings of the meshwork are.

The variability of most tissue components around the circumference of the eye was similar for normal and glaucomatous eyes, approximately 20% (coefficient of variation). The thickness of the JCT region was somewhat more variable, approximately 35%. The largest variability was found in the measurement of empty space touching Schlemm’s canal endothelial cells, approximately 80%. This is the one parameter found to be related to outflow facility in a study of monkey eyes by Lütjen-Drecoll.16 Recent work indicates that the pores in the giant vacuoles of the inner wall, in conjunction with the adjacent juxtacanalicular tissue, may create a significant outflow resistance.7 If aqueous egress to Schlemm’s canal, and hence the pores and giant vacuoles, is limited to the clear spaces touching Schlemm’s canal, the variability we found in this component makes adequate sampling of the juxtacanalicular tissue especially important.

For most tissue components, sample size calculations indicate that three quadrants per eye should be studied to estimate a tissue component within 50% of its “true” value, assuming that no significant inherent differences are present (considering a 20% variability of the tissue component; α = 0.05. β = 0.8). The more variable parameter, empty space touching Schlemm’s canal, would require study of 21 areas, a Herculean task with current techniques. These calculations also indicate that caution should be used in studying single samples from one eye, as occurs with trabeculectomy specimens, because a single sample may underestimate or overestimate the mean by more than 200%.

Light microscopic analysis underestimated the amount of empty space by 20% when compared with analysis of the entire juxtacanalicular tissue on a slot grid. Although rapid and inexpensive, light microscopy does not seem to be accurate enough for quantitative analysis of the juxtacanalicular tissue.

The actual values of the amounts of most tissue components found in this study were similar to those reported by others. The length of Schlemm’s canal is often cited as 300 μm,7,22 similar to the values we found

### Meshwork Variability

#### TABLE 4. Morphometric Studies of Juxtacanalicular Tissue

<table>
<thead>
<tr>
<th>Author</th>
<th>Specimen</th>
<th>n</th>
<th>n†</th>
<th>Fixation</th>
<th>Circum Study?</th>
<th>JCT: % Empty Space</th>
<th>JCT: % Solid Tissue</th>
<th>Tendon and Sheath: % of JCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lütjen-Drecoll15</td>
<td>M W</td>
<td>16</td>
<td></td>
<td>Im</td>
<td>2 Eyes</td>
<td>16.2*</td>
<td>83.8*</td>
<td></td>
</tr>
<tr>
<td>Lütjen-Drecoll2</td>
<td>H GL T</td>
<td>104</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H N W</td>
<td>35</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Segawa19</td>
<td>H GL T</td>
<td>11</td>
<td>11</td>
<td>Im</td>
<td>No</td>
<td>70.0*</td>
<td>30.0*</td>
<td></td>
</tr>
<tr>
<td>McMenamin20</td>
<td>H N W</td>
<td>20</td>
<td>8</td>
<td>Im?</td>
<td>No</td>
<td>25*</td>
<td>75*</td>
<td>17.7</td>
</tr>
<tr>
<td>McMenamin†</td>
<td>H N W</td>
<td>36</td>
<td>20</td>
<td>Im</td>
<td>15 eyes; 2+ areas</td>
<td>27.9*</td>
<td>72.1*</td>
<td>18.5</td>
</tr>
<tr>
<td>Lindenmayer21</td>
<td>M W</td>
<td>12</td>
<td></td>
<td>P</td>
<td>Yes</td>
<td>59.2</td>
<td>40.8*</td>
<td></td>
</tr>
<tr>
<td>Alvarado†</td>
<td>H N W</td>
<td>36</td>
<td>14</td>
<td>Im</td>
<td>No</td>
<td>–</td>
<td>–</td>
<td>22.9‡</td>
</tr>
<tr>
<td>Murphy6</td>
<td>H N W</td>
<td>2</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>55.9 (gel fraction)</td>
<td>44.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H GL T, W</td>
<td>11</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>63.4 (gel fraction)</td>
<td>36.6</td>
<td></td>
</tr>
<tr>
<td>Ethier4</td>
<td>H N W</td>
<td>2</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>23.0</td>
<td>77.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H GL T</td>
<td>2</td>
<td></td>
<td>Im</td>
<td>No</td>
<td>24.3</td>
<td>75.7</td>
<td></td>
</tr>
<tr>
<td>Miyazaki9</td>
<td>H N W</td>
<td>17</td>
<td>3</td>
<td>Im</td>
<td>Yes</td>
<td>26.1*</td>
<td>73.9*</td>
<td>40.4‡</td>
</tr>
<tr>
<td>Urakawa†</td>
<td>H C W</td>
<td>23</td>
<td></td>
<td>Im</td>
<td>1 eye</td>
<td>52*</td>
<td>48*</td>
<td>31.3‡</td>
</tr>
<tr>
<td>Buller</td>
<td>H N W</td>
<td>9</td>
<td></td>
<td>Im, P</td>
<td>Yes</td>
<td>32.8 (Im)</td>
<td>67.2 (Im)</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>H GL W</td>
<td>8</td>
<td></td>
<td>Im, P</td>
<td>Yes</td>
<td>36.8 (Im)</td>
<td>63.2 (Im)</td>
<td>24.7</td>
</tr>
</tbody>
</table>

* Value calculated from data presented in study; † number of eyes (ages 60 to 80) from published study used in generating comparative percentages; ‡ includes type I plaque.

H = Human; M = monkey; N = normal; GL = glaucomatous; C = cultured eyes; Im = immersion; P = perfusion; T = trabeculectomy specimen; W = whole eye; JCT = juxtacanalicular tissue.
in normal eyes (257 μm) and glaucomatous eyes (299 μm). Recent studies have found values of 210 to 260 μm² and 272 μm² for normal eyes, and 206 μm² for glaucomatous eyes. The mean thickness of the trabecular lamellae in normal eyes was 4.8 μm in the current study, thinner than that reported for normal eyes 60 years of age and older by McMenamin and colleagues (6.3 μm) although the mean variability we found was less: 12.4% versus 36.6%. The thickness of the juxtacanalicular region we found in all immersion-fixed normal eyes, 9.1 ± 1.5 μm, was similar to that reported by Murphy and colleagues, 8.96 ± 3.38 μm, although the value in all immersion-fixed glaucomatous eyes was slightly different, 9.7 ± 1.6 μm versus 7.8 ± 1.1 μm. Values for the amount of empty space or various tissue components in the juxtacanalicular tissue reported in the literature are quite variable, depending on fixation method and measurement techniques, and are summarized in Table 4. The variability of the components of the JCT around the circumference of the eye has rarely been addressed. In a paper by Miyazaki and colleagues, the coefficient of variation of the amount of empty space was 22.3% (calculated from the authors' data on 17 eyes). This is similar to our values of 31.2% in normal eyes and 20.3% in glaucomatous eyes. In an early study by McMenamin and colleagues, the coefficient of variation of "electron dense material" (presumably tendon and sheath material) was 51% (10 eyes), and 62% in a later study by McMenamin and Lee (calculated from authors' data, using eyes 60 years of age and older). These values are greater than our findings for the variability of tendon and sheath material of 32.1% in normal eyes and 26.6% in glaucomatous eyes. Values reported for the reproducibility of the JCT analysis technique using hand tracing, 7.5%, and using a point counting method, <10%, are similar to our values of 7% to 14%.

A question for further study involves quantitation of the changes in configuration of the juxtacanalicular tissue with different methods of fixation. When perfused with fixative at the normal, physiologic intraocular pressure of 15 mm Hg, Schlemm's canal is smaller, giant vacuoles are more common, and the juxtacanalicular tissue is looser and more expanded than when the eye is immersed in fixative with an intraocular pressure of 0 mm Hg. Nevertheless, most quantitative studies of the juxtacanalicular tissue have been performed on immersion-fixed eyes, which may not be representative of the physiologic state (Table 4). In our preliminary comparisons, we were surprised to find that perfusion-fixed eyes had only approximately 6% more empty space than did immersion fixed eyes.

Future quantitative studies of the JCT should include at least three quadrants per eye and should sample several regions of the JCT from each section. Attention should be given to the empty spaces directly in contact with the endothelium of Schlemm's canal, because they are the most variable and may represent potential flow channels for aqueous entry into the canal.

**Key Words**

trabecular meshwork, glaucoma, electron microscopy, morphometry, image analysis

**References**

14. Tschumper RC, Johnson DH. Trabecular meshwork...


