**Collagen Organization in the Branching Region of Human Brain Arteries**

Helen M. Finlay, BSc; Peter Whittaker, PhD; Peter B. Canham, PhD

**Background and Purpose**—Unruptured saccular aneurysms are relatively common, occurring in 4% to 9% of autopsies. Their development at the apex region of brain artery bifurcations is attributed to a combination of structural factors and the effect of blood pressure. Collagen is a primary tension-bearing fabric of the vessel wall, and our purpose was to examine its 3-dimensional alignment at arterial branches.

**Methods**—Sixteen segments of arteries from the circle of Willis, including bifurcations, were pressure distended, fixed, and sectioned in 1 of 3 orthogonal planes. We measured the 3-dimensional organization of collagen at the flow divider by using the polarized light microscope. An electron microscopy study performed in tandem provided measurements on the collagen fibril diameters and packing density.

**Results**—Orientation data of the collagen fabric were obtained from sections from 3 different cutting planes. The tunica media of all bifurcations had an alignment that was primarily circumferential, and the medial gap (medial defect) was distinguishable at the apex of all bifurcations. The subendothelial layer was thin at the apex but thicker and more disorganized distally. Adventitial collagen showed little organization except for a high degree of alignment along the apex. Results from the electron microscopy study showed densely packed collagen fibrils of uniform diameter at the apex, compared with slightly smaller and less densely packed fibrils nearby.

**Conclusions**—In the region of the medial gap, a narrow band of highly aligned tendonlike collagen running in the direction of the ridge of the flow divider was a consistent finding. This structure would provide strength and stability to the vessel and is inconsistent with the concept of an inherent defect in the structure of bifurcations. (Stroke. 1998;29:1595-1601.)

**Key Words:** biophysics cerebral aneurysms cerebral arteries collagen

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The branching region of brain arteries has long been known to be the site of formation of saccular aneurysms, balloonlike structures capable of gradual enlargement and catastrophic rupture.1-3 This region is mechanically and structurally complex, with curvatures of the vascular wall that must bear the tensions arising from the distending forces of blood pressure.4,5 We undertook mapping of the collagen organization as a 3-dimensional, layered fabric that provides the structural framework for the arterial bifurcations. By focusing on collagen, we are acknowledging its role as the strong component of the wall that may be a factor in the development of aneurysmal lesions if it yields to blood pressure forces.6-8

Brain arteries are muscular arteries, with the characteristic dominance of circumferentially organized smooth muscle cells in the tunica media and a distinct internal elastic lamina.9,10 The adventitia is a layered collagen fabric with a strong dominance of circumferential fibers adjacent to the media and a wide range of orientations in the outer layers.10 This characteristic structure of cylindrical segments of the artery continues into the junction region, with transitions in the tissue structure above and below the plane of the bifurcation, where the trunk vessel widens.4 At the curving ridge of the flow divider, the normal layered fabric of the wall becomes significantly altered. A well-established feature of the cerebral artery bifurcation is a region at the apex in which the media is absent. This is commonly known as the “medial defect,” terminology that may be misleading, because this region is almost always present in cerebral artery bifurcations and therefore is unlikely to be an acquired defect. Other terms used have been “medial gap” or “raphe.”12 In our study of fenestrations (regions of incomplete fusion of arteries at the embryological stage resulting in a length of duplication of the artery), we investigated the structure at the regions of divergence and convergence.13 We demonstrated how the influence of flow factors is revealed by the contrast of the built-up, layered subendothelium at the distal end of the fenestration compared with the minimal structure at the leading edge, or flow divider.

In the course of this work, we have demonstrated that there is a collagen band, or sling, that runs through the bifurcation region in the direction of the flow divider. This collagen reveals itself under polarized light as strikingly aligned, like tendon, with densely packed, uniform fibrils that may serve...
as a tendon of the bifurcation. The position of this band of collagen coincides with the medial gap, the region devoid of media.

Methods

For the collagen orientation measurements, 16 branching regions of major brain arteries from the circle of Willis were obtained from 13 autopsies (7 male and 6 female) between the ages of 42 and 77 years. Our intent was to obtain samples of bifurcations from those vessels known to be sites of aneurysm formation. Causes of death were post heart transplant in 1, post lung transplant in 1, myocardial infarction in 3, dementia in 3, pneumonia in 1, and liver cirrhosis in 2. For 2 of the 13 cases, we were not able to ascertain the cause of death. We saw no evidence of atherosclerosis on gross examination of the cerebral vessels that we used. Segments of artery, including the bifurcation, were cannulated, pressure distended, and fixed in 10% formalin at a physiological pressure of either 110 or 120 mm Hg. Twelve of the vessels used were bifurcations of the middle cerebral artery, vessels most frequently associated with aneurysms,14 and 4 were bifurcations of the posterior cerebral artery. The branching regions were embedded in paraffin and sectioned at either a 5- or 7-μm thickness. Three different sectioning planes were used, so that the orientation of the fibers within the bifurcations could be explored thoroughly (Figure 1). We sectioned 4 bifurcations in the plane of the cross section of the main proximal vessel (Figure 2a); 8 in the longitudinal direction perpendicular to the plane of the bifurcation, which would include the ridge of the apical divider in profile (Figure 2b); and 4 in the longitudinal transverse plane of the bifurcation (Figure 2c). Collagen has natural birefringence that can be enhanced by the use of appropriate stains. For most of the studies, we used picrosirius red, a strongly enhancing stain for collagen. Gomori’s silver impregnation, Masson’s trichrome, and Verhoeff’s elastin stains were used to highlight structures other than collagen.

The 3-dimensional alignment of collagen fibers was measured by using a Zeiss polarized light microscope with a rotating universal stage attachment.15,16 The universal stage is mounted on the main rotating stage of the microscope and permits the tissue slide to be tipped and rotated in oblique planes, with all rotational displacements calibrated. Thus, the 2 angles that define the orientation of a fiber of collagen in 3-dimensional space, ie, the azimuthal angle (in the plane of the stage) and the elevation angle (out of the plane of the stage), are recorded for each fiber measured within the tissue section. Key components of the universal stage are 2 glass hemispheres with an index of refraction of 1.55, which are located above and below the glass slide, so that the incident light is always normal to the glass. Interfaces between the slide and these hemispheres are coated with glycerol, with an index of refraction of 1.47, that serves to minimize any reflection and refraction. The fiber or group of fibers chosen for measurement is ~4×4 μm and must be centered between the 2 glass hemispheres.

Methods of analysis of the 3-dimensional orientation data are well established.10,11 For graphical presentation, the data were plotted on Lambert equal-area projections. These plots are similar to polar projection maps used to display entire hemispheres of the earth. Each data point on the Lambert projection represents the intersection, at the surface of the hemisphere, of the direction of a line originating at the center. Each region of concentration of data points on the projection shows organization of the collagen fibers, with the amount of coilignment indicated by the group concentration of the points. The projection may be rotated about any axis, so that data taken from the bifurcations from any of the 3 sectioning planes can be compared. We analyzed the 3-dimensional orientation data by using Fisher spherical statistics17,18 to provide values of the mean orientation of all sets of fibers and of their circular standard deviation (CSD). The CSD is a measure of dispersion of 3-dimensional alignments, analogous to the standard deviation in a gaussian distribution, and is defined as the solid angle about the mean orientation that encloses 63% of the data.

A complementary assessment was made of the apical collagen fibers by using transmission electron microscopy. For these measurements standard procedures were followed.19 Three middle cerebral artery bifurcations from 2 autopsies (additional to those used in the orientation studies) were pressure fixed at physiological pressure with Karnofsky’s perfusion fluid. The vessels were trimmed so that only the apex and some tissue from the daughter vessels remained. Samples were stained in 1% OsO4 in a 0.1 mol/L s-collidine buffer, pH 7.4, for 1 hour at 4°C; immersed in 2% aqueous uranyl acetate for 1 hour; dehydrated in alcohol; and embedded in Epon 812. Thin sections were cut on a Sorval MT 1 ultramicrotome equipped with glass knives. The sections were mounted on unfiltmed grids, stained with lead citrate, and examined with an AEI 801 or JEOL 100CX transmission electron microscope at 80 kV. The sections used were taken midplane longitudinally through the apex, with the intent that the collagen fibers would be cut in cross section.

Fibril diameters were measured from high-contrast photomicrographs (magnification ×60 000) of 2 regions of the bifurcations—the apical ridge, which was also the region of the medial gap, and the nearby tunica adventitia at a distance of ~0.4 mm from the gap. From photomicrographs of 3 bifurcations, a total of 1100 fibril diameters were measured from each of the 2 regions. The volume fraction of the fibrils was determined by point counting20 by using a transparent overlay with a 4-mm grid. Measurements were taken

Figure 1. Schematic of bifurcation region of cerebral artery indicating 3 orthogonal sectioning planes. Upper diagram shows characteristic cross section immediately distal to the apex.

Figure 2. Profile of representative sections showing 3 main layers of the artery wall and how they appear at the apical region of the bifurcation with the sectioning planes used.
from 3 bifurcations by using 3 photomicrographs taken at the medial gap and 7 taken a small distance away from the gap (magnification ×35 000).

Results
Examination of variously stained sections from the region of the bifurcations revealed the following. An internal elastic lamina was present in all vessels, including the apex; this was seen most clearly from the 2 longitudinal cutting planes. It was sometimes wavy and in all of the sections had an appearance of having small discontinuities. There was no external elastic lamina. The tunica media was composed mostly of smooth muscle, with a small amount of collagen. In most cases there was little tunica intima, but regions of thickened intima were seen locally in 7 sections, some from all 3 cutting planes. These are probably indications of the intimal pads frequently seen in association with bifurcations.

Cross Sections
When the main proximal vessel is cut in right cross section, the initial sections are round. As a cut toward the bifurcation is made, the sections widen into an oval and then in quick succession to a figure 8 and to 2 round sections belonging to the daughter branches closest to the apex (Figure 1). Subsequent sections are at various angles of oblique cross section for the 2 daughter vessels, depending on their branch angle and curving path away from the junction region. Four bifurcations were sectioned in this way at 7-μm thickness, and we focused on those sections just proximal to just distal to the apical region (Figure 2a). From each bifurcation, 20 measurements of the 3-dimensional alignment of collagen fibers were made in the region of the medial gap, the apex, and 20 measurements were made from the adventitial collagen spaced around the 2 daughter vessels. In all cases, the mean orientation of the collagen was in the circumferential direction around the daughter vessels and was close to the plane of the microscope section. The CSDs of these data about the mean direction for the apical region and the adventitia away from the apex are given in the Table, indicating a striking difference in coherence. The photomicrographs of Figure 3a show the flow divider just before the edge of the apex, with the intensely bright collagen visible at both sides as it approaches the apical ridge. Figure 3b, ~40 μm farther along the bifurcation, shows the highly aligned tendonlike collagen of the apex, with the much less birefringent subendothelium above and below, toward the lumen of the daughter vessels. The values of CSDs for the media and the subendothelium in cross section in the Table are from a previous report.21

Longitudinal Perpendicular
Sections cut in this plane are able to show the true profile of the apical flow divider (Figure 2b). The positioning for sectioning of these bifurcations in the paraffin blocks was important for ensuring that the section plane was along the apical ridge. This goal established a challenge for sectioning because many of the bifurcations have daughter vessels that are dissimilar with respect to their diameters and the angles of divergence from the axis of the parent artery. As a consequence, we were not equally successful at revealing the apical ridge for all bifurcations. We made measurements from 8

### Mean Dispersion (CSD) of Collagen Alignments Within Each Region

<table>
<thead>
<tr>
<th>Section Orientation</th>
<th>Apex</th>
<th>Adventitia</th>
<th>Media</th>
<th>Subendothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross section</td>
<td>7.3</td>
<td>24.4</td>
<td>7.7*</td>
<td>15.0*</td>
</tr>
<tr>
<td>Longitudinal perpendicular</td>
<td>7.8</td>
<td>...</td>
<td>10.5</td>
<td>35.8</td>
</tr>
<tr>
<td>Longitudinal planar</td>
<td>4.5</td>
<td>37.6</td>
<td>13.0</td>
<td>11.6</td>
</tr>
</tbody>
</table>

All values are in degrees.
*These data are from an earlier study.21

![Figure 3a](http://stroke.ahajournals.org/)
![Figure 3b](http://stroke.ahajournals.org/)

Figure 3. Photomicrographs of cross-sectioned bifurcation, taken with circularly polarized light. 3a and 3b show region just before the flow divider with a brightly birefringent band of collagen on each side. Complete band is shown at the apex in 3c and 3d, ~40 μm distally.
bifurcations in this plane and found considerable variability in the appearance of the medial gap. Two of the vessels had a region \( \approx 1 \) mm long in which the media was absent, and in 3 cases the gap was extremely short, \(<100 \) \( \mu \)m, giving the appearance of an intrusion of the adventitia toward the subendothelium. These features may have been due to misalignment of the section plane. In all of the bifurcations there was a segment of the adventitia at the apex, coinciding with the region of the medial gap, in which the collagen was seen in the plane of the section to be straight and highly coaligned. Using the sections in this plane, we made measurements from the adventitia, tunica media, and subendothelium. For each of these layers, 5 readings were taken from each of 10 separate regions that were evenly spaced around the ridge of the flow divider. Spherical statistics were used to analyze the combined 50 orientation measurements of each layer, and the results are summarized in the Table.

**Tunica Adventitia**
The mean CSD of the 8 vessels was 7.8° for the adventitial layer, with the mean azimuthal orientation tracking the planar curvature of the section, and the mean elevation angle being within 3° of the plane of the section for 5 of the bifurcations. The other 3 had mean elevation angles of 8°, 20°, and 28° out of the section plane. We interpreted these sections as having been cut at an angle that was oblique to the plane of the flow divider.

**Tunica Media**
The same statistical analysis was applied to the data from the tunica media, and in this case, 6 of the vessels had a mean CSD of 10.5°, whereas the other 2 had considerably greater CSD values (29.3° and 38.4°). These latter 2 were the vessels containing the very large area in which the media was absent, so that the regions of measurement were at a distance from the apex.

**Subendothelium**
The mean CSD for all 8 vessels was 35.8°, indicating a large scatter in the fiber orientations, with a weak central tendency. Results from this layer were very inconsistent in both the CSD values and the mean orientations. In some vessels, the orientations of the subendothelial fibers separated into 2 main directions, having 1 group of fibers aligned along the direction of the apical ridge and the other concordant with the main alignment perpendicular to the ridge, i.e., in the direction circumferential to the daughter vessel.

**Longitudinal Planar**
Our third sectioning plane was the longitudinal planar (Figure 2c), which is the one most commonly used to reveal branching vessels. The main artery, branches, and bifurcation are seen in profile. We took readings from 4 of these bifurcation sections, all of which had a region of medial gap at the apex. The sections selected for measurements were those in which the gap was the most pronounced, to be as close as possible to the midregion of the divider. Twenty orientation measurements were made from each of the 3 main layers adjacent to the region of the apex. Readings were made from both sides of the apex in the subendothelium, which in 3 of the vessels was very thin, and from both sides of the medial gap in the media. The readings from the adventitia were made from the region close to the apex. In addition to these measurements, we detected a small region of highly aligned collagen that stood out at the apical edge of the adventitia, i.e., in the medial gap. This region is \( \approx \) 10 to 25 \( \mu \)m across and consists of collagen oriented perpendicular to the section plane. A set of 20 measurements was made from each of these 4 regions (subendothelium, media, adventitia, and apical ridge). Analysis by spherical statistics from 4 bifurcations gave mean CSDs as shown in the Table. The CSD value of 4.5° at the apex in this plane is lower than the values of 7.3° and 7.8° obtained from the other 2 planes, for which measurements were made over a greater length of the apical collagen band. With polarized light, the very highly aligned, birefringent collagen of the apex region, if oriented perpendicular to the microscope axis, is shown as an area that remains dark when the stage is rotated through 360°. If the alignment is off by as little as a few degrees from the perpendicular, then the region appears bright when seen with polarized light. In 1 of the sections examined, the dark region of perpendicular collagen was visible with the microscope stage in its horizontal position, and in the other 3, the region could be identified only when the universal stage was tilted. Lambert projections of the data from 1 of the planar sections are shown in Figure 4. The data are presented so that fibers oriented perpendicular
to the microscope section appear at the center of the plot. The strong coalignement of the apex collagen is shown by the tight grouping of fiber orientations (CSD = 3.8°), whereas in the adventitia and the media, there is a greater dispersion of fiber orientations (CSD = 37.7° and 7.8°, respectively). In the subendothelium, 2 main directions were often observed.

**Electron Microscopy**

The electron microscopy study was conducted in 2 parts. The first was the measurement of the diameter of collagen fibrils at the apex of the bifurcation and at a distance (≈0.4 mm) from the apex. Although small, this distance was far enough from the apex so that there was a layer of tunica media underlying the adventitia, the dimension of the apical collagen band being ≈50 μm. The cutting plane was longitudinal planar, so that at the apex the direction of the fibrils would be perpendicular to the section plane. The mean diameter of the fibrils of the apex from a total of 1100 measurements from 3 bifurcations was 49.9 ± 7.2 nm and of the adjacent adventitia was 52.3 ± 8.4 nm, a difference that was not significant.

The second part of the transmission electron microscopy study was the comparison of fibril density, or volume fraction, in the 2 regions. The total number of test points was 1368 at the apex and 3287 in the adjacent adventitia. Fibrils of the apical region occupied a volume fraction of 62%, whereas fibrils in the nonapical region occupied 46%. In the adventitial region, the range of measurements (variability of the packing density) was ≈3 times greater than in the apex. The transmission electron photomicrograph of the apical region in Figure 5 shows aligned fibrils that are very densely packed. In the photomicrograph of the near-apex adventitia, variations in the fibril diameters and orientations as well as in the packing density are displayed. It was noted that the entire area shown in Figure 5 is smaller than the zone of measurement for a single orientation reading from the universal stage.

**Discussion**

The striking feature of collagen in the medial gap is its highly aligned organization, much like that of tendon under load. Fibers and fibrils run parallel to each other and, at the apex, track through the perpendicular to the long axis of the parent artery. Polarized light microscopy is possibly the best microscopic technique for studying apical collagen. The method is ideally suited for detection of the region of coaligned fibers and also for making comparative measurements on their directional organization. A complementary study uses the high resolution of transmission electron microscopy to compare the fibril diameters and packing density of the apical and near-apical collagen.

The bifurcation region bears the same transmural pressure as does a straight length of artery, yet there appears to be little structural accommodation of the added loading in the wall. In the straight lengths, the principal stress is circumferential (hoop) stress, \( \sigma_C \), and the key structural layers, the media and adventitia, have mainly circumferentially directed collagen and smooth muscle fibers.\(^{10,22}\) The lesser presence of longitudinal fibers in the outer adventitia is able to bear the lower
longitudinal stress, \( \sigma_z = \sigma_c / 2 \). The challenge of providing a stable bifurcation is illustrated in Figure 6. Laplace’s law for thin, convex vessels with principal radii of curvature \( R_1 \) and \( R_2 \) provides the relationships among wall tension \( T \), transmural pressure \( P \), and curvatures \( R_1 \) and \( R_2 \): \( P = T(1/R_1 + 1/R_2) \).\(^{23}\) For a right cylinder, \( 1/R_z = 0 \) and \( P = T \), which relates to circumferential stress by the equation \( \sigma_c = T/t \), \( t \) being wall thickness. Not only is \( R_2 \) negative at the apical region of a bifurcation but also for brain arteries, \( R_2 \) is smaller in magnitude than \( R_1 \), thus making the Laplace relationship untenable. The modified Laplace’s law for unequal tensions \( P = T/R_1 + T/R_2 \) brings out an important factor for brain artery branch regions. Because \( R_2 \) is negative, \( T_z \) must therefore be substantially higher than \( T_z \), possibly by a factor of 5 or 10 times that for the branches sectioned in the plane of the bifurcation. This argument of basic mechanics illustrates 2 points: (1) there is a requirement for extra strength at the apex, a need that is satisfied by a tendonlike structure of collagen running along the apical ridge and (2) the high tension of this structure would straighten any natural waviness of unloaded fibers. (The other load-bearing fabric, elastin, is only a minor contributor to wall mechanics in the region of bifurcations, mainly because in comparison to collagen, its stiffness is less by a factor of \( \approx 1000 \) and its tensile strength less by a factor of \( \approx 100 \).\(^{24,25}\)

The microscopic studies of this research provide strong evidence for an apical “tendon” in 4 ways: (1) The transmission electron microscopy sections, though unsuited for quantitative orientation studies, emphasize the uniformity of size of the cross-sectioned fibrils and their high volume fraction. The measured fibril sizes are similar in mean value and variation to those of Merrilees et al.,\(^{26}\) who reported fibril diameters of 50 and 53 nm in the inner and outer adventitia, respectively, of human coronary artery. (2) We viewed the bifurcation in the cross-sectional plane, which shows the daughter vessels joined at the apical ridge (or flow divider). Polarized light microscopy revealed the collagen to be coherent and parallel to the line of contact between the daughter vessels. In this plane, any unexpected transverse fibers would stand out microscopically; however, none were observed. (3) Collagen, when viewed in longitudinal perpendicular sections, had straightened fibers tracking along the apical ridge. (4) When viewed in longitudinal sections parallel to the plane of the bifurcation (the conventional view for bifurcations), the coherent alignment of the thin band of collagen was striking. With the correct tilt on the universal stage, the collagen is aligned vertically and can then be visualized as a small black region within the adjacent brightly birefringent adventitia, which remains dark as the universal stage is rotated. This confirms that all of the fibers of this region are highly co-aligned in the direction parallel to the optic axis of the microscope. A summary of the CSD values from the different cutting planes (the Table) shows how much more organized the apical collagen is than that of the other regions.

Our results, in combination with the findings of other studies, provide a fuller understanding of wall mechanics in the region of brain artery bifurcations. We observed collagen fiber structure in the medial gap to be similar to that of tendon and quite unlike that of the rest of the adventitia. Because of this band of collagen, at any given pressure the medial gap will be stiffer than the rest of the adventitia. Macfarlane et al.\(^{3,27}\) reported that the apex of the bifurcation was able to maintain its general shape even at negative pressures and that the radius of curvature at the apex changed only moderately as the pressure increased over a wide range. Evidence for a strong band of collagen in the bifurcation apex, rather than indicating a region of weakness, points to its being a region of high strength; however, the immediately adjacent vessel wall may be left vulnerable to forces of hemodynamics and blood pressure. The formation of aneurysms is therefore most likely to be initiated at the discontinuity at the edge of the band, where distension of the vessel could occur. Forbus\(^1\) reported on the mechanical failure of middle cerebral artery bifurcations exposed to excessive pressure and found that in no case did rupture occur at the apex but was always nearby in the general region of the bifurcation. Stehbens\(^{27}\) had noted that the site of formation of aneurysms in human cerebral arteries was often in the daughter vessel, just distal to the medial gap. More recent studies by Hazama and colleagues\(^{28,29}\) have a complementary theme. Their work on experimentally induced cerebral aneurysms in animals showed that the vessel wall developed grooves directly adjacent to the apex, some of which later developed into saccular aneurysms.

Our quantitative morphological investigation provides new information about the bifurcation region of brain arteries that, in the vast majority of cases, are mechanically stable. The mechanical vulnerability of this region to the formation of aneurysms has still to be explained.

Acknowledgments

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References

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