The Layered Fabric of Cerebral Artery Fenestrations

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Background and Purpose Intravascular bridges, resulting from developmental anomalies of brain arteries, are now better known as arterial fenestrations. Their tendency to develop aneurysms, similar to arterial bifurcations, makes their anatomy and microstructure important for study.

Methods Six segments of artery, each including a fenestration (five from the verteobasilar junction and one from the middle cerebral artery), were pressure distended, fixed, and sectioned. We made three-dimensional orientation measurements of smooth muscle and collagen, stained to enhance their birefringence, using the polarized light microscope.

Results The general contour of the fenestrations is streamlined, with a thickened layered subendothelium at the trailing or distal edge, structurally similar to the region of convergence of major brain arteries. Defects of the medial layer were found at both proximal and distal edges of all the fenestrations. Results included regional mean orientations of individual layers, with circular SDs. The medial layer was found to be coherently aligned perpendicular to the direction of blood flow, with a mean circular SD of 12°. The adventitia was less coherent (mean circular SD, 16°) with the same average orientation, and the multilayered subendothelium had layers of obliquely oriented fibers with a wide range of coherence for individual fiber groups. Layers of the side regions were analogous to those in segments of brain artery and differed significantly from the proximal and distal edges of the fenestration structure.

Conclusions The plasticity of form of the fenestrations at both the proximal and distal edges is in response to hemodynamic forces and is analogous to branching regions of brain arteries. Medial defects, a common feature in both brain arteries and fenestrations, may predispose the arterial fenestration to aneurysm formation.

Key Words • cerebral aneurysm • cerebral arteries • collagen • muscle, smooth • fenestration

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the strength of birefringence (e.g., picrosirius red or James’ silver impregnation) and permit more precise measurement of orientation through the polarized light microscope. The universal stage has been the principal instrument for the present study. It extends the effectiveness of the polarizing microscope by enabling measurements of three-dimensional orientation directly from stained tissue sections. By means of these microscopic techniques we assessed the structural similarity between fenestrations and the fabric of the nearby artery and also examined the subendothelium, which is a hemodynamically adaptive part of the bridge and artery wall.

Materials and Methods

Six fenestrations, each from a different autopsy, were obtained as part of our studies on cerebral arteries. Five were located in the basilar artery near the junction of the vertebral arteries and the sixth in a middle cerebral artery. They were all found by examination of the external appearance of the vessels, where the existence of a fenestration is indicated by a small depression or dimple in the outer wall. Segments of artery including an individual fenestration were isolated from a circle of Willis, ligated, and fixed under a distending pressure of 14.7 kPa (110 mm Hg) in 10% neutral-buffered formalin. They were embedded in paraffin and sectioned at 7-μm thickness. Four of the structures were cut in cross section, which is the longitudinal plane for the artery, parallel to the plane of the joining vertebral arteries, and the other two were cut approximately longitudinally, as determined by the cross-sectional plane of the principal artery. In the case of the fenestration in the middle cerebral artery, the longitudinal axis was not parallel to the cross-sectional plane of the vessel, and the geometry of individual sections was variable. Several sections spanning the extent of individual fenestrations were stained with James’ silver impregnation to enhance the birefringence of muscle and collagen fibers or with picrosirius red, which is specific for collagen fibers. Slides nearby in the sequence to those studied for orientation were stained with Gomori’s trichrome to aid in differentiation of the areas of smooth muscle and collagen or with a modified Movat’s pentachrome for elastin.

The study was divided into two parts, the first focusing on geometric factors, including location, size, type, and number of the tissue layers of each fenestration, and the second focusing on the three-dimensional structure of the individual layers, relating them to the equivalent layers within the artery wall. Measurements of the location and dimensions of the fenestrations were made from traced sections obtained on a projection microscope and by the use of photomicrographs. The length, height, and width of each fenestration were calculated, as well as its position relative to the vessel wall, to the apex of the converging vertebral arteries, and to other vessel branches. We investigated the continuity of the layers around the fenestration regions by following the major tissue components identified on sections stained with Gomori’s trichrome.

Fabric organization was measured within each of the four fenestrations cut in cross section and from one longitudinally sectioned by the use of the polarizing microscope and universal stage. In each case one section was selected from near the geometric center of the fenestration. One set of readings was also taken from toward the end of one of the structures (bridge 2), in an area where its fabric was merging into the adjacent vessel wall.

Each measurement was made as a pair of orientation values, an azimuthal angle and an elevation angle, that uniquely define three-dimensional orientation (Fig 1a). An individual measurement of orientation can be made from any area of coherently aligned tissue of approximately 4 to 5 μm or from 8 to 10 μm of tissue if the alignment is out of the plane of the section. The number of readings from individual layers varied, being higher in number if the region was larger in size and less coherent. Fewer readings were taken for particularly coherent areas (shown by a sweeping band of extinction as the microscope stage is rotated) either because extra readings were not needed statistically or because measurements were not possible owing to low birefringence or lack of localized coherence. The data were plotted on a Lambert equal-area projection, as illustrated in Fig 1b, in which each point represents the orientation of a single reading. The Lambert projection is a circular graph on which are mapped the positions distributed over a three-dimensional hemisphere, with each position defining a line in space, through the center of the hemisphere, with the azimuthal angle lying in the equatorial plane of the hemisphere and the elevation angle the amount of elevation away from the equatorial plane. Initially the data were recorded relative to the azimuthal alignment of one edge of the microscope slide, and the distance from the outer “rim” of the Lambert projection is the elevation angle. Point 3, for example, lies at an azimuth of 344° with an elevation of +7° (and has the equivalent orientation of −16° azimuth and +7° of elevation). Rotation by computer techniques made it possible to align data relative to a biologically meaningful reference. The data on the projection of Fig 1c were rotated 90° around the east-west axis with the new elevation and azimuthal zero references at the center. The two single points appearing at the top in Fig 1b are seen when rotated to be part of the main population of data, which has a mean azimuth of −1° and elevation of −21°.

We have become familiar with this method of analysis, originally learned from methods in physical geology, through our research on the blood vessel wall. Analysis by circular statistics was undertaken to cluster our measurements obtained from a mean orientation and a circular standard deviation (CSD), which is a measure of the scatter about the mean and identifies the circular angle containing 63% of the data on the hemisphere (similar to the SD in conventional statistics).

A strength of the quantitative method for three-dimensional orientation data is that statistical measurements can be obtained. Error relating to a single reading depends on strength of...
birefringence, degree of coalignment in the region of the measurement, and elevation angle of the local fabric being measured, with the highest precision for fibers aligned parallel to the section plane. An estimate of the repeatability was obtained from one of the sections of bridge 1 by marking precisely, on a photomicrograph, the positions at which a first set of 67 readings was made; at a later time the section was remounted on the universal stage and the micrograph used to relocate the positions for a repeated set of measurements. Readings were taken and were analyzed from the media and subendothelium on both sides of the fenestration and the distal (or downstream) side of the subendothelium. The absolute difference between the mean orientations of each region was 1° or less, whether comparing the azimuth or the elevation angle, and the measure of dispersion (the CSD) was also nearly the same between the two sets. This was an important result because individual pairs did not each have the same values for azimuth or elevation, chiefly because it was not always possible to relocate the exact position for making the repeated measurement. (Three instances, for example, had differences between 10° and 14°, indicating that we had not relocated the exact fiber location of the first reading.)

An additional set of readings was made from another section of the same fenestration separated from the first by 140 μm to give an indication of the structural consistency within an individual fenestration (provided in "Results").

Results

Qualitative Observations

The two contrasting images of a fenestration either as a separate structure dividing blood flow, much like an island in a stream, or as the side-by-side region of two adjacent parallel arteries were highlighted by our construction of two three-dimensional models. The reconstructions in Fig 2 convey representations of the true three-dimensional structure in vivo (made possible by the tissue preservation at arterial distending pressure of 110 mm Hg). The rheological impact of the fenestration as a tapered slender bridge is revealed in the model of reassembled longitudinal sections (Fig 2a). Our subsequent use of the term "bridge" emphasizes the concept of a distinct structure within the lumen of the parent vessel, as opposed to the term "fenestration," which puts more emphasis on the perforation of the vessel as visualized from outside.

Tracings reproduced here of bridges 1 and 2 (Fig 3) convey the general shape and positioning relative to the basilar artery entrance. The adjacent low-power photomicrographs reveal (1) the similarity of the points of flow convergence (the trailing edge of the bridge and the flow convergence of the vertebral arteries), (2) the contrast in amount of subendothelium between the leading and trailing edges of the fenestration, and (3) the underlying medial gap or medial defect, which has been recognized as characteristic of the apex regions of brain arteries. The "fineness ratio," ie, the ratio of length to width of structures in a fluid flow field, varied widely, being 8.2 for bridge 2, 2.4 for bridge 1, and 9.5 for bridge 5. (The ratio for bridge 5 was calculated by means of section thickness and the number of serial cross sections of the basilar artery needed to extend through the bridge in the midregion.) Bridge 1 is longitudinally aligned with the apex of the convergence of the feeder arteries and therefore off center in the basilar artery. It has a very substantial buildup of subendothelium, with an outer profile aligned approximately with the estimated direction of blood flow.

However, the "structural" axis, identified by the muscle cells of the tunica media bordering the tunica adventitia at the inner core of the fenestration, is aligned off axis, at approximately 20°.

Polarized light micrographs can be used to indicate general organization, with local regions of birefringent fabric appearing alternately bright and dark, under the microscope as the stage is rotated through 360°. Extinction (blackness) occurs when the optical axis of the localized region of tissue lies parallel to either the polarizer or the analyzer filter axis, and thus extinction is repeated four times in each 360° cycle. The narrowness of the extinction band relates to the way one perceives relative light intensity, which is on a logarithmic scale, with maximum brightness occurring midway between two orientations identifying extinction. The positions of maximum transmitted light are broad, with an uncertainty of approximately 10° to 15°, and alignment for extinction can be very precise (approximately 2° to 3°). (As part of an earlier study we made a graphic demonstration of this principle on collagen fibers in tissue sections of porcine heart valve.) Examination of the sections stained with picrosirius red under polarized light revealed the adventitia to have speckled brightness at all orientations, consistent with layered wavy fibers that are without localized regions of coherence. The generally dark tunica media at all orientations has two interpretations: either a region lacking birefringence or
tissue aligned approximately perpendicular to the sectioning plane; the latter was found to be true when the section was assessed on the universal stage. The subendothelial build up in the "outer" layers of bridges 1 and 2 is relatively strongly birefringent, and the threadlike local zones of birefringence are indicative of more organized alignment than the collagen in the adventitia. The layered appearance at the convergence points of the vertebral arteries and at the trailing edge of the fenestrations is caused partly by abrupt changes in general organization and changes in birefringence.

Analysis of the transversely sectioned basilar artery that includes bridge 3 revealed the same microscopic structure with an absence of continuous media at both ends, ie, medial defects, and a very thickened subendothelium distally. (Medial defects have been noted at the proximal end of fenestrations by Crompton 29 and at both ends by Black and Ansbacher. 30) Reconstruction of the sixth fenestration from the middle cerebral artery was not attempted. The appearance was that it passed obliquely across the vessel wall, with a broader attachment at one end than the other. There was still the impression of two separate vessels being formed, although they were of unequal size and with an irregular shape, and in this case the main vessel had a smaller branch distal to the fenestration.

**Quantitative Results**

Measurements of three-dimensional orientation were made from the four bridges that were sectioned transversely. Since each has a different shape and structural composition, we were unable to average the results and instead presented the data in tabular form for each midsection analyzed (Table). In each case, the section judged to be at the middle of the bridge was selected for detailed measurement. We grouped sets of results according to their "radial" position around the bridge within the individual layers, and in some cases the...
subendothelial part was also subdivided into areas of distinct coalignment. We kept separate the readings from the two opposite sides of the fenestration (micrographs of Fig 3), since each side is associated with a different one of the two duplicated vessels. Each direction measurement was calculated relative to the tangent taken at that point of the outer profile of the fenestration. Relatively high values of CSD for the media result from the pooling of measurements along the whole fenestration length. Much lower CSD values were obtained when measurements were from narrow zones, as in Fig 4.

In all measured sections, the elevation angle in the medial layer was high, indicating mean fiber orientations toward the bridge axis (or the direction labeled H in Fig 6). The high azimuth angles associated with these show a radially oriented component because of the plane of sectioning. The low angles of the side subendothelial regions of bridges 1 through 3 reveal mean alignments oriented around the perimeter of the bridge structure. In these three fenestrations the measurements from the distal end were aligned obliquely, with a high CSD in each case. The distinct differences in values seen in bridge 4 indicate that the plane of sectioning was oblique to the bridge axis.

In "Materials and Methods" we reported the results of repeating measurements from the same section, which was a midsection, S1, for bridge 1. We also compared those measurements with similar regions of the same fenestration on section S2 (140 μm away) included in the Table. The angular separations of the mean orientations were 14° and 20° in the two medial regions, 6° and 7° in the side subendothelium, and 37° in the end subendothelium regions, and the CSD was almost identical in both layers on one side but approximately 50% higher on side 2 and the end subendothelium than those shown in the Table (bridge 1). Both sections appeared to be close to the middle of the bridge, but it may be that section S2 of the repeated readings was in fact toward one end. The inference drawn from these comparisons is that the fenestration microstructure is much more varied than arterial microstructure revealed in earlier studies along straight segments of brain artery.

Additional measurements were taken from one of the fenestrations to explore the transition region where the bridge merges with the main artery, in the area that would be seen as a dimple on the artery wall. The section used was 380 μm from the midsection of bridge 2 and had the appearance of a slightly flattened vessel turned inside out, with the adventitia on the inside and the subendothelial layer on the outside. Results from this end region, when compared with bridge 2 from the Table, showed little change in the mean orientation of the side subendothelial layer. At the distal end of the subendothelium, however, the mean elevation angle was 37° lower, with a similar decrease in elevation in the side medial regions, consistent with the flaring out at the attachment of the bridge to the artery wall. In this region it was also possible to make measurements of the media at the distal end, revealing a much higher elevation angle there than at the sides (69° compared with 17°).

In Fig 4 the Lambert projections and statistical summary of the readings from some individual data points are shown, with the locations of those points labeled on the schematically reproduced section for bridge 1. The Lambert projections included illustrate one set of measurements across a complete wall of one of the duplicated vessels. In this midfenestration region (as well as at both the proximal and distal ends), the medial and adventitial mean orientations are at high elevation angles relative to the section plane, with the adventitia having larger CSD values. The three subendothelial layers vary from being aligned in the plane of the section (along the vessel axis), in the layer adjacent to the media, to almost perpendicular in the outer layer, next to the blood flow. The thickened distal subendothelium shows a reversal of this pattern, with mean elevation angles of 50°, 31°, and 23° from media to lumen, and the proximal edge has an elevation of only 10° in the outer layer. This is in contrast to more circumferential orientations seen in this region of the normal artery subendothelium.

A similar composite of results was assembled for bridge 5, which is from a fenestrated basilar artery cut in cross section (Fig 5). Although the bridge is not presented in cross-sectional profile, the region is directly comparable to straight sections of artery. Sets of measurements were taken across all the layers at six different locations, three from each of the duplicated vessels. Lambert projections of the results from one of the regions are included in Fig 5 and are similar for the other five regions. The tunica media, which served as the reference layer for many of our arterial studies on muscular arteries, is nearly perfectly aligned (CSDs between 4° and 6°, indicating a tightly coherent group of fibers as seen on the Lambert projections), with an average orientation that is almost exactly circumferential (elevation angles and tangent referenced angles are low, shown as centrally positioned on the Lambert projection). Because of the different section plane, a high-elevation angle for this vessel represents a fiber longitudinally oriented along the vessel axis. The adventitia is aligned mainly in the circumferential direction...
regions of measurement

two layers of subendothelium

media

adventitia

media

adventitia

FIG 5. Tracing of midfenestration area of bridge 5, from which readings were made across the complete wall in the six regions indicated. In this section plane the appearance of the fenestration is of two separate vessels. Lambert projections are of three layers from one of the regions and are in the rotated projection (as in Fig 1c) so that a fiber oriented circumferentially along the vessel wall would be at the center of the graph, and one oriented out of the plane of the section would appear at the north or south pole. Az indicates azimuth; Elev, elevation, and CSD, circular SD.

around both the duplicated vessels, but with a higher scatter shown by CSDs from 8° to 22°. Subendothelial measurements reveal these data as part of circular helices at different degrees of pitch. (Fibers aligned longitudinally appear at either the north or south pole, as in the subendothelium, and circumferential fibers would have readings at the center.) One of the duplicated vessels revealed two layers of subendothelium: the mean elevation (helical) angle of the layer next to the media, from the three measured regions, was 75°, and that next to the lumen was 16°. These agree well with the helical angles of the comparable region of bridge 1 in Fig 4.

Discussion

Arterial development in the cerebral circulation has been thoroughly described by Padget, a description that makes clear the potential for incomplete fusions and tissue bridges across the luminal channel. Fusion of the two bilateral neural arteries to form the basilar artery takes place at a fetal age of 29 to 32 days, when the embryo length is 4 to 6 mm; the vertebral arteries form at 32 to 35 days (embryo length, 7 to 12 mm) and anterior communicating artery (which completes the circle of Willis) by 44 days (embryo length, 21 to 24 mm). Both the basilar and communicating arteries are the more common sites associated with intravascular bridges.

Fenestration aneurysms, although infrequent at the site of these fusion anomalies, have become recognized clinically by means of the improved diagnostic imaging of the cerebral circulation. The location of fenestration aneurysms varies widely, with the most common of all sites being the basilar artery. Earlier references focused on the hemodynamic impact of the bridges on blood flow and the fact that they may be sites of thrombosis leading to arterial occlusion. Hypertrophy and thrombus formation appear again as themes in recent publications. The shape and structural composition of the fenestration combine either to result in a stable and benign structure or to have an intrinsic weakness and susceptibility to aneurysm formation.

Structural composition is revealed in three dimensions by the measurements from the universal stage. The central area of the fenestration is similar to the normal brain artery wall, with a multilayered, loosely organized tunica adventitia, a highly aligned circumferential media, and a discretely layered subendothelium. All layers of the subendothelium are helically oriented, with the more longitudinally oriented fibers adjacent to the media and more circumferentially oriented fibers next to the lumen, as in brain arteries. Tissue sections cut longitudinally through the fenestration (the fenestration appearing as an island in the flow stream) reveal the full spectrum of microstructure from the leading to trailing edges, with the midsection appearing like normal artery wall. The media is completely absent locally at both edges, corresponding, for the leading edge, to the medial gap of brain artery bifurcations, but the trailing edge medial gap occurs despite the substantial buildup of subendothelium. The trailing edge of the fenestration, corresponding hemodynamically and anatomically to the junction of two arteries, has a subendothelium aligned quite differently from the central region. There is less variation in collagen orientation from layer to layer and a continuously changing mean orientation over the saddle shape of the trailing edge (both from differing regions around the same section and from measurements on nearby sections).

The prominent layering that occurs at the trailing edge of the bridge, along with the contrasting negligible subendothelium at the leading edge of each bridge in our study, is a strong indicator of blood flow being the causative factor. One quantitative hemodynamic difference between the leading and trailing edges is the fluid shear stress adjacent to the wall, which is generally higher around the leading edge than the trailing edge. (This difference exists despite the thin line or point of flow stagnation at both the leading and trailing edges where fluid shear stress is zero because blood flow divides around the bridge.)

Elastin is a minor component of the brain artery wall, appearing primarily in the internal elastic lamina. A deficiency has been noted particularly at regions of bifurcations. Using tissue sections stained with Movat's pentachrome, we investigated the continuity of the internal elastic lamina of bridges 1, 2, and 3 in both the fenestration and the region of convergence of the vertebral arteries. In each case there was a marked discontinuity of the elastin at the leading edge, coinciding with the absence of media; at the trailing edge where the subendothelium is thickened, the elastin divided into several lamellae that followed the contours of the subendothelial tissue. Each lamella was continuous except in regions of the apex, where discontinuities were sometimes observed. The elastin of the pressure-fixed arteries appeared regular and smooth, and the region of the vertebral junction in our samples showed a division into lamellae at the apical region similar to the fenestration trailing edge.

Geometric data that are retrievable from serial sections enabled us to explore implications of the saddleshaped leading and trailing edges of the fenestrations. The bridge wall sustains transmural arterial blood pres-
sure (except in regions where the adventitial core layers may be in direct physical contact). These edge surfaces, like the apex or concurrence points at junctions of arteries, have one principal radius of curvature that is positive and able to bear the load of transmural pressure, and one that is negative \((R_1\) and \(R_2\) in Fig 6). Because the negative radius of curvature is clearly smaller than the positive curvature, the direction of tension must be primarily in the direction of the positive curvature. An expression for relating transmural pressure \((P)\), the principal radii \((R_1\) and \(R_2\)), and unequal wall tensions \((T_1\) and \(T_2\)) along those curvatures is a modification of the law of Laplace:\(^6\): \[ P = T_1/R_1 - T_2/R_2, \] with \(R_1\) negative relative to \(R_2\) (and thus responsible for the minus sign). From the serial section reconstructions we have one point for which both the principal radii of curvature can be estimated: \(R_2\) directly from the end curvatures of the middle tissue section of the longitudinally cut fenestration (eg, micrograph of midssection in Fig 3) and \(R_1\) from dimensions of the serial section reconstructions. We made an estimate of the average positive curvature \(R_1\) of the leading and trailing edges by plotting the half length of the bridge as a function of the height and chose the best-fitting local curvature by eye (Fig 6). As the principal radius of curvature, \(R_1\) was expected to be approximately equal to the radius of the basilar artery. From Fig 6 we obtained values for \(R_1\) of 0.61, 0.56, and 0.91 mm, compared with approximate values for \(R_2\) of 0.1 to 0.2 mm. From the fenestration in the cross-sectioned basilar artery (bridge 5), information is available to measure curvature of both ends separately. Calculated \(R_1\) values for the leading and trailing edges were both 1.7 mm. The inference structurally is that one might find a collagen backbone running along the direction of the curvature of the leading or trailing edge of a bridge, because that is the direction of principal load bearing.

In conclusion, we note that arterial fenestrations are relatively common and have been recognized for a long time; however, knowledge of their structure has only recently gained importance because of the clinical awareness of fenestration aneurysms. Our stereological measurements reveal that the layered multidirectional fabric of the subendothelium has clearly been molded by the mechanical forces of blood flow. This contrasts with the stable circumferential order of the tunica media and adventitia, similar to that of the arterial wall, which provides the structural backbone of the fenestration.

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