Fenestrations in the Internal Elastic Lamina at Bifurcations of Human Cerebral Arteries

GORDON J. CAMPBELL, P. ENG., M.A. SC., AND MARGOT R. ROACH, M.D., PH.D.

SUMMARY Measurements of fenestrations (or windows) in the internal elastic lamina at the bifurcation of human cerebral arteries, were obtained from photomicrographs (scanning electron microscope). Thirteen of 28 bifurcations revealed regions of enlarged fenestrations among the normal fenestrations in the vicinity of the apex. The mean diameter of the enlarged fenestrations (7.0 ± 0.34 SEM μm) was significantly greater than the mean diameter (2.1 ± 0.13 SEM μm) of the normal fenestrations. The number of fenestrations per sq mm was less (2606 ± 284 SEM per sq mm) for the enlarged fenestrations than for the normal fenestrations (4518 ± 397 SEM per sq mm). The proportion of the area of internal elastic lamina comprised of fenestrations increased to 15.0 ± 1.1 SEM percent for the enlarged fenestrations from a mean of 1.8 ± 0.16 SEM percent for the normal fenestrations. Fenestrations from bifurcations without enlarged fenestrations, demonstrated characteristics similar to the normal fenestrations. More than 80% of the specimens exhibited a gap in the internal elastic lamina in the apical region of the bifurcation. Based on a comparison of stress concentration factors, we propose that the presence of enlarged fenestrations represents a weakness in the internal elastic lamina at the bifurcation apex which may contribute to the initiation of microaneurysms.

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THE STRUCTURE of the internal elastic lamina of cerebral arteries has never been clearly delineated. In addition, the role of the internal elastic lamina in the formation of intracranial saccular aneurysms, known to form predominantly at bifurcations, remains an enigma.

Dees first described the internal elastic lamina of human and bovine aortas and renal arteries as a continuous sheath penetrated by small windows. However, Nystrom described the elastic lamina of human arteries as a fibrous structure. Lang and Kidd stated that the elastic lamina of human cerebral arteries is split into layers with the luminal surface a meshwork of fibers which transforms into a sponge-like layer, and finally forms a solid mass in the layer adjacent to the media.

Hassler examined the fenestrations, which he described as windows, contained within the elastic lamina of cerebral arteries of humans, from newborns to the age of 90. He presented data to describe relevant characteristics of fenestrations in the internal elastic lamina of straight cylindrical segments isolated from the intracranial portion of the internal carotid and anterior cerebral arteries. Cook, Salmo, and Yates examined the “length” and “number of gaps” in the internal elastic lamina of the external iliac artery from humans aged 20 to 70 years. Other authors have made reference to fenestrations, but did not provide data concerning their characteristics. A subsequent paper by Hassler showed large fenestrations at the neck of a saccular aneurysm obtained at autopsy. Nevertheless, specific information on the size of these fenestrations was not presented.

The present study was undertaken in order to: 1) establish numerical values for the fenestrations in the apical region of bifurcations; 2) resolve whether the enlarged fenestrations observed by Hassler existed prior to the development of the aneurysm, or whether they had evolved as a consequence of distention of the internal elastic lamina during enlargement of the aneurysm.

Methods and Materials

Cerebral arteries (designated series I, II, III and IV) which did not exhibit any visible atherosclerotic disease were obtained at autopsy and stored at 4°C for 4 to 17 days in isotonic saline. Series I was a circle of Willis obtained from a 62-year-old male. The other cerebral arteries were obtained in situ: series II, from a 62-year-old female; series III, from a 65-year-old male; and series IV, from a female aged 58. Subsequently, the complete circle of Willis and larger peripheral branches were carefully removed in toto. The complete arterial tree from series III and IV was photographed in vitro and stored in isotonic saline at 4°C.

Every bifurcation from the 4 series was isolated from the arterial tree and then sectioned along the lateral borders from the daughter branch to the parent artery (in order to minimize damage to the apex). The specimen was then floated on to a cork or balsa wood backing, spread and pinned (as illustrated in figure 1) in an unstretched condition with the adventitial surface exposed.

The specimens were next treated to remove the adventitia and smooth muscle coats by a method similar to that of Stephen, Minns and Thomas. The pinned specimens were placed in a solution of NaOH maintained at a concentration of 0.1 N for 70 minutes, at a temperature of 75°C. These specimens were next immersed in 0.1 N NaOH at room temperature for 5 minutes, followed by a 5 minute immersion in distilled water, neutralization in 0.1 N HCl (2 minutes) and a final immersion for 5 minutes in isotonic saline. The specimens were fixed in phosphate buffered 2.5% glutaraldehyde for a minimum of 48 hours. All specimens were treated and fixed within 18 days of death, since preliminary studies showed no degradation of

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Series I and II specimens were processed through graded acetones (30%, 50%, 70%, 90%, 95%, 100% and 100%) followed by critical point drying in a Polaron Model E 3000 Critical Point Drying Apparatus. Series III and IV specimens were immersed in distilled water for one hour. Upon removal, excess water was eliminated and specimens stored overnight in a freezer maintained at —15°C. The specimens were then freeze-dried in a Virtis Model #10-030 Freeze Drier at -50°C and 0.1 torr for 3 hours.

A complementary study (in preparation) of the fenestrations using straight arterial segments showed no significant difference in the characteristics of the fenestrations regardless of whether the specimens were critically point dried or freeze dried. A comparative study by Boyd et al.9 revealed that the net shrinkage in embryonic and brain tissue produced by freeze drying was only 4.5%. Similarly, Grut et al.10 demonstrated that the shrinkage of purified porcine aortic elastin was essentially zero for freeze drying, compared to 60% for similar air-dried specimens.

Subsequently, all specimens were sputter coated with gold in a Technics, Hummer II Sputter Coater and the external surface of the internal elastic lamina was examined with a Philips scanning electron microscope model SEM 500.

The surface of each specimen was scanned initially at magnifications of 40 and 160 to provide a general impression of the surface topography and as well to delineate areas of interest. Ten photomicrographs at magnifications of 640 or 1250 were obtained of areas with fenestrations of "normal" size when judged in relation to adjacent straight segments. Only areas free of debris and containing a minimum of at least 30 fenestrations with distinct borders were selected.

Photomicrographs containing at least 30 fenestrations of the same bifurcation, in regions with "enlarged" fenestrations, were also obtained. In this manner each bifurcation acted as its own control for a later comparison of regions of normal and enlarged fenestrations. All photomicrographs were obtained without tilting the specimen and a constant focal plane was maintained by adjusting the stage rather than the focus adjustment in order to maintain the focus. The purpose of this method was to minimize both distortions and measurement error.

The films from all 4 series were mounted in a Simon Omega B-22 enlarger and the image projected on the platen of a Hewlett Packard Digitizer (Model 9804A). The image represented a final magnification of either 1150 or 2250. Six of the photomicrographs were selected from the "normal" group for measurement. All photomicrographs of reasonable quality from the region of enlarged fenestrations were selected for measurement.

Since the fenestrations were generally ellipsoidal in shape, 2 points representative of the borders of the major axis and of the minor axis for the internal diameter of each fenestration were digitized and the values entered directly into a Hewlett-Packard 9830A microcomputer for further processing and storage. Only fenestrations which appeared to pass completely through the internal elastic lamina were measured.

The area for each fenestration was computed with the use of the equation for an ellipse \( A = \pi \times \frac{a \times b}{2} \), where \( a \) and \( b \) represent the major and minor diameters.

Three geometric characteristics were computed:

1) Diameter — the diameter of a circle of equivalent area.
2) Density — the number of fenestrations per square mm.
3) Percentage Area — percentage of the surface area comprised of fenestrations (computed by the summation of the area for each fenestration) with respect to the total area of the field of view in the photomicrograph.

Results

Although 54 bifurcation specimens were prepared, only 28 were deemed acceptable for analysis (table 1) either because they were not flat, or more commonly, because a layer of fibrous tissue obscured the surface even after 4 hours of treatment with NaOH. A recent study by Merei et al. showed that these are elastic fibers buried in the media and adventitia.

Figure 2 illustrates a typical example of the topography of the internal elastic lamina as viewed in the scanning electron microscope. The fenestrations penetrating the internal elastic lamina are generally round or slightly ellipsoidal, with smooth contours. There are remnants of fibrils on the surface and, in addition, there appear to be fibers buried in the matrix.

Thirteen of the 28 bifurcation specimens analyzed (table 1) had regions in the vicinity of the apex where the fenestrations were noticeably enlarged as shown in figure 3. The shape of these enlarged fenestrations was again either round or ellipsoidal with smooth contours. Although 2 of the 6 analyzed specimens from series II demonstrated possible regions of enlarged fenestrations, the quality of these regions was inadequate for analysis. Nevertheless, they have been included in the group of bifurcations with enlarged fenestrations.

The remaining 15 specimens did not demonstrate enlarged fenestrations in the regions analyzed. Nevertheless, enlarged fenestrations may have been present but were obscured by the partial covering of fibrous material. The fenestrations from these 15 specimens were either round or ellipsoidal, similar to the group of normal fenestrations surrounding the regions of enlarged fenestrations.

A group of straight arterial segments prepared currently, revealed enlarged fenestrations in only 2 of the 34 specimens analyzed (table 1). The scarcity of regions of enlarged fenestrations in the straight segments suggest that the enlarged fenestrations are almost exclusively located in the apical region of the bifurcation. This factor, together with the absence of distension of the specimens, virtually eliminates drying artifacts as the cause of the enlarged fenestrations. The regions of enlarged fenestrations generally formed clusters or circumferential bands in the region of the apex of the bifurcation and on the medial aspect of the daughter branches.

During examination of series II specimens with the scanning electron microscope, large elliptical gaps were observed at the center of most of the specimens (85%) corresponding to the apical region of the bifurcation. Visual examination of a few untreated specimens from series III and IV did not usually reveal the gaps until a slight tension was applied between the daughter branches. A split oriented across the saddle of the bifurcation became enlarged to form an elliptical gap. The presence of these gaps was confirmed during subsequent examination with the scanning electron microscope in about 80% of the specimens from series III and IV (table 1). The regions of enlarged fenestrations were usually adjacent to the gaps and the circumferential bands mentioned previously were oriented in the same direction as the long axis of the gap. In most cases, the lip of the gap could not be examined since it had been reflected under the specimen, obscuring it from observation by the scanning electron microscope. However, in at least 3 cases the split in the internal elastic lamina had propagated through a region of enlarged fenestrations. The internal elastic lamina in the apical region of some specimens appeared disrupted with a fibrous matrix forming the major structural element.

Table 2 lists mean values for diameter, density, and percentage area. The definitions and methods for computation of these characteristics have been outlined previously.

Figure 4 shows a typical histogram of fenestration diameters for a bifurcation with regions of both enlarged and normal fenestrations. A least squares polynomial has been computed and the curve overlaid.

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<td>Number with enlarged fenestrations</td>
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to demonstrate each distribution. The distribution curve of the diameters for the normal fenestrations was very peaked, with few fenestrations less than 0.3 μm or larger than about 7 μm. The distribution curve for the enlarged fenestrations diameters was less peaked (due to the greater dispersion) and distinctly skewed to the right. The peak for the enlarged fenestrations was shifted to a larger value and there are many fenestrations that are greater than 10 μm in diameter, with some as large as 30 μm.

Figure 5 illustrates a typical graph of density versus diameter. Two distinct groups are evident. A greater variation of density was apparent for the normal fenestrations, whereas the enlarged fenestrations demonstrated more variability of the average diameter. The graph of percentage area and diameter illustrated in figure 6, again demonstrates 2 groups. The increase in percentage area with an increase in diameter has been attributed to the influence of the product of the diameters for the computation of the percentage area. A similar graphical representation of percentage area and density (fig. 7) did not reveal a similar increase in area with increased density, even in the case where the largest density was a factor of 4 greater than the smallest density.

In order to assess the validity of the assumption that there are 2 distinct classifications (enlarged and normal fenestrations), a single factor analysis of variance was performed on the data and the Newman-Keuls multiple range test, at the 0.01 level of significance, was utilized to isolate any specimens which did not conform to an assigned classification. The mean values for the diameter, density and percentage area for both the normal and enlarged fenestrations are es-

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<td>Normal</td>
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FENESTRATIONS IN LAMINA OF CEREBRAL ARTERIES/Cam>6e//

and Roach

493

FIGURE 3. Illustration of enlarged fenestrations near the apex of the bifurcation. (Short white marks represent 10 μm).

sentially the same within each group, except for a limited number of specimen comparisons made between extreme values.

It is evident from the data presented in table 2 that the mean diameter for the enlarged fenestrations (7.0 ± 0.34 SEM μm) is greater than that for the normal fenestrations (2.1 ± 0.13 SEM μm). The density for the enlarged fenestrations (2606 ± 284 SEM per sq mm) is lower than that for the normal fenestrations (4518 ± 397 SEM per sq mm). In addition, the percentage area has increased from 1.8 ± 0.16 SEM percent for the normal fenestrations to 15.0 ± 1.1 SEM percent for the enlarged fenestrations.

Since the characteristics for both the enlarged and normal fenestrations have been computed for each bifurcation, a paired sample t-test was employed to establish whether the differences in the characteristics were significant. The paired sample t-test showed that

FIGURE 4. The distribution curve and histogram for the diameter of the normal (solid lines) and enlarged fenestrations (broken lines).
the enlarged and normal fenestrations were significantly in diameter ($p < 0.0005$) and area ($p < 0.005$) but not different in density ($p > 0.20$).

Discussion

The characteristics of diameter, density and percentage area measured in this study showed remarkable similarity among the 4 subjects. The average fenestration diameter of the normal fenestrations was about 2.1 µm which is considerably less than the values of 6.8 and 3.5 µm, for the cylindrical segments of the anterior cerebral artery and internal carotid artery respectively, reported in the study by Hassler for this age group. However, the range of the diameters for the normal fenestrations in this study is in close agreement with the 1 to 2 µm quoted by Lang.
and Kidd. The discrepancy between the values in this study and that of Hassler could possibly be attributed to a difference in the measurement of the diameters. Hassler measured the “maximum diameter of the windows,” whereas in this study the internal or minimum diameter was measured. Examination of photomicrographs from this study, as well as the study by Lang and Kidd, revealed that the fenestrations in cross-section are funnel-shaped at both entrances which could result in large differences depending upon whether the measurements were made at the lip or throat of the fenestration. Alternatively, the enhanced resolution capabilities of the scanning electron microscope may have resulted in the measurement of many smaller fenestrations, not discernible with the light microscope. There is also a distinct difference for the percentage area between the 2 studies which again could be accounted for by the difference in diameters.

There is reasonable agreement for the same age group, between the average density of 4518 per sq mm for the normal fenestrations observed in this study and the value of 3800 per sq mm in the study by Hassler. Variation again could be attributed to a difference in technique, since the technique employed by Hassler may not have removed the debris embedded in the fenestration which would have obscured some of the fenestrations. The enhanced resolution of the scanning electron microscope, again, could also account for the variation.

It has not yet been established whether the gap observed at the apex of many of the bifurcations existed in vivo or was accidentally produced during removal of the arterial tree from the brain. In either case, the existence of the gaps suggests that there is a distinct weakness in the internal elastic lamina at the apex of the bifurcation. The presence of enlarged fenestrations in this region and their absence in the trunk specimens suggest that they may contribute to this weakness.

Distension of the fenestrated internal elastic lamina by transmural pressure is analogous to a system of holes in a flat plate under stress. It is an established fact, from the analysis of stress in flat plates, that holes introduce localized areas of stress in the vicinity of the holes, that may be many times higher than the stress in the adjacent solid material. This phenomenon is termed stress concentration and is represented by the Stress Concentration Factor. The significance of stress concentration is dependent upon the number, shape, size, and distribution of the holes, as well as the type of stress (uniaxial or biaxial) and orthogonal proportion of the stress.

The parameter “ligament efficiency” is used to describe the spatial geometry of holes in flat plates. It is defined as the minimum distance of solid material between 2 adjacent holes divided by the center-to-center distance. As a result, a high ligament efficiency represents a combination of small fenestrations and greater center-to-center distances, whereas a low value represents a larger fenestration diameter and/or that the fenestrations are closer. Furthermore, lower ligament efficiencies imply that the solid material between the fenestrations must bear an increased load resulting in an increased stress. It is evident from figure 3 that the ligament efficiencies in regions with ellipsoidal fenestrations could be less in the direction of the major axis than in the direction of the minor axis.

A review of the stress concentration factors from the available literature for a number of different hole sizes and configurations, and as well variations of stress, revealed an increase for the regions of enlarged fenestrations. However, none of the configurations reviewed was completely representative of the apparent random distribution of enlarged and normal fenestrations observed in cerebral arteries.

In several regions of enlarged fenestrations depicted in the photomicrographs, the computed ligament efficiency was less than 0.55, which could create an average stress concentration of about 1.3 to 2.2 times the stress in an adjacent cylindrical branch. An additional factor, which would be expected to increase the stress concentration in the internal elastic lamina, is the corner formed at the apex of the bifurcation. The stress concentration factor derived analytically at the edge between a side branch and the main cylinder (analogous to the apex of the bifurcation) of a closed system under internal pressure is 3.94 times the stress in an adjacent cylindrical segment. As a consequence of the combined effects of the enlarged fenestrations and apical geometry, the stress sustained by the internal elastic lamina in the apical region could be as much as an order of magnitude greater than the stress in an adjacent straight segment. A further decrease in the ligament efficiency or degradation of the tissue could result in an excessive stress concentration causing rupture of the internal elastic lamina.

It has been stated by several authors that degeneration, fragmentation, and splitting of the internal elastic lamina is associated with the initiation of aneurysms. Since the 2.1 μm diameter of the normal fenestrations is substantially less than the standard 7 μm sections prepared for light microscopy, the fenestrations would not be visible and the internal elastic lamina would appear to resemble a solid sheet. However, if serial sections were prepared through the regions of the enlarged fenestrations where the average diameter is greater than 7 μm, then the intervening ligaments would appear as fragments of the internal elastic lamina.

It is, therefore, proposed that the micro-aneurysms formed by evagination of the internal elastic lamina into the smooth muscle as observed by other authors are actually regions of enlarged fenestrations with low ligament efficiencies. This would result
in an excessive distension of the internal elastic lamina in this region which would be observed as a bulging of the internal elastic lamina and underlying tissue.

The presence of enlarged fenestrations in the internal elastic lamina of normal intracranial arteries, without the development of an intracranial aneurysm, has been verified in this study. Since these regions of enlarged fenestrations may represent areas of weakened tissue and since they have been found almost exclusively in the apical region of the bifurcation, they could be considered a defect in the internal elastic lamina. Whether or not the regions of enlarged fenestrations could influence the development of an intracranial aneurysm has not been conclusively verified.

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