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Carotid Bifurcation Atherosclerosis

Quantitative Correlation of Plaque Localization with Flow Velocity Profiles and Wall Shear Stress

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SUMMARY. The distribution of nonstenosing, asymptomatic intimal plaques in 12 adult human carotid bifurcations obtained at autopsy was compared with the distribution of flow streamline patterns, flow velocity profiles, and shear stresses in corresponding scale models. The postmortem specimens were fixed while distended to restore normal in vivo length, diameter, and configuration. Angiograms were used to measure branch angles and diameters, and transverse histological sections were studied at five standard sampling levels. Intimal thickness was determined at 15° intervals around the circumference of the vessel sections from contour tracings of images projected onto a digitizing plate. In the models, laser-Doppler anemometry was used to determine flow velocity profiles and shear stresses at levels corresponding to the standard specimen sampling sites under conditions of steady flow at Reynolds numbers of 400, 800, and 1200, and flow patterns were visualized by hydrogen bubble and dye-washout techniques. Intimal thickening was greatest and consistently eccentric in the carotid sinus. With the center of the flow divider as the 0° index point, mid-sinus sections showed minimum intimal thickness (0.05 ± 0.02 mm) within 15° of the index point, while maximum thickness (0.9 ± 0.1 mm) occurred at 161 ± 16°, i.e., on the outer wall opposite the flow divider. Where the intima was thinnest, along the inner wall, flow streamlines in the model remain axially aligned and unidirectional, with velocity maxima shifted toward the flow divider apex. Wall shear stress along the inner wall ranged from 31 to 600 dynes/cm² depending on the Reynolds number. Where the intima was thickest, along the outer wall opposite the flow divider apex, the pattern of flow was complex and included a region of separation and reversal of axial flow as well as the development of counter-rotating helical trajectories. Wall shear stress along the outer wall ranged from 0 to −6 dynes/cm². Intimal thickening at the common carotid and distal internal carotid levels of section was minimal and was distributed uniformly about the circumference. We conclude that in the human carotid bifurcation, regions of moderate to high shear stress, where flow remains unidirectional and axially aligned, are relatively spared of intimal thickening. Intimal thickening and atherosclerosis develop largely in regions of relatively low wall shear stress, flow separation, and departure from axially aligned, unidirectional flow. Similar quantitative evaluations of other atherosclerosis-prone locations and corresponding flow profile studies in geometrically accurate models may reveal which of these hemodynamic conditions are most consistently associated with the development of intimal disease. (Circ Res 53: 502–514, 1983)

TRANSITIONS in artery wall configuration at bends and bifurcations, and about branch ostia, are associated with local modifications in rate and pattern of blood flow. These regions are also predisposed to the development of intimal thickenings, including the formation of atherosclerotic plaques. Attempts have therefore been made to assess the role of hemodynamic factors in atherogenesis by correlating the distribution of intimal lesions, usually in excised collapsed arteries, with presumed changes in flow conditions or with flow patterns visualized in idealized glass or plastic models. On the basis of such studies, elevations or variations in flow velocity and shear stress (Fry, 1968), decreased wall shear (Caro et al, 1971), flow separation (Scharfstein et al, 1963), and turbulence (Ferguson and Roach, 1972; Gutstein et al., 1973; Stehbens, 1975) have each been proposed as hemodynamic potentiators of lesion formation. Quantitative studies in which precise locations of intimal deposits in specimens restored to normal dimensions are correlated with flow phenomena measured in anatomically accurate models could help to identify which of the several possible hemodynamic variables is most consistently associated with the presence or absence of intimal disease. The human carotid bifurcation is particularly well suited for such an investigation. It is a site of early lesion formation (Peterson et al., 1960) and of clinically significant disease (McGill et al., 1968), and the division of the common carotid artery in
this region into two channels of unequal size and flow distribution results in a wide range of hemodynamic changes. Specimens suitable for controlled pressure-fixation are obtainable at autopsy, and dimensions of the carotid bifurcation determined from in vivo angiograms can be used to construct geometrically accurate scale models. We have utilized data derived from such preparations to relate the axial and circumferential distribution of early, asymptomatic, nonstenosing, intimal lesions to flow patterns, flow velocity profiles, and shear stresses. The construction of the model bifurcations and the flow patterns associated with them have been described elsewhere (Balasubramanian, 1979; Bharadva et al., 1982a, 1982b). In the present report, we furnish data concerning the precise axial and circumferential localization of intimal lesions in human carotid bifurcations and compare these with flow and shear stress data at the same locations in the models. The findings indicate that regions of reduced wall shear stress, flow separation, flow reversal, and departure from axial flow are associated with maximum intimal thickening and atherosclerotic plaque formation; regions where flow is axial and unidirectional, and flow velocities and wall shear stresses are high, tend to be spared.

**Methods**

**Carotid Bifurcation Specimens**

**Fixation**

Carotid bifurcations were excised at autopsy from patients aged 27–73 years (mean equal to 53 years) who had no history of stroke or symptoms of cerebral disease. The external carotid artery and its branches were ligated and the cut ends of the common carotid artery at the base of the neck and the distal internal carotid artery at the base of the skull were cannulated. Care was taken not to dissect the connective tissue between the internal and external carotid arteries, in order to preserve the spatial geometry at the bifurcation. Each bifurcation with its attached carotid sinus was warmed to 37°C by immersion in a temperature-controlled bath containing buffered, balanced salt solution. The cannulas then were attached to an apparatus designed to distend vessels at selected intraluminal pressures (Glagov et al., 1963). Each bifurcation was flushed and distended with the warm buffered solution at a luminal pressure of 100 mm Hg, which restored both length and diameter of the branches, and then were flushed and redistended with warm buffered formalin (3.8%) at the same pressure. Fixation was carried out for 60 minutes with the distended bifurcation immersed in warm buffered formalin, while luminal pressure was monitored and maintained at 90–100 mm Hg. This procedure rendered the vessels rigid, so that there were no discernable changes in diameter, length, or configuration of the bifurcation when pressure was reduced after fixation. The fixed vessels were then flushed and filled with a radiopaque mixture consisting of a barium sulfate suspension (Micropaque: Damancy and Co. Ltd.) in a 5% solution of gelatin (Grayslake Gelatin Co.) in normal saline at 37°C and then maintained at 90–100 mm Hg luminal pressure while the gelatin mass was permitted to cool to room temperature and solidify.

**Angiographic Study**

Radiographs of the barium-filled specimens were taken in three projections at 45° rotation intervals with a radiopaque rod of known diameter in the plane of the bifurcation angle to permit correction of projected measurements. The initial indexing projection was that which produced the maximum bifurcation angle. The films were used to assess irregularities of lumen contour, and to determine length of the carotid sinus, inner (lumen) and external (outer wall) diameters at standard levels, as well as the angle formed by the center axes of the internal and external carotid, and the angle of inclination between the internal carotid and the common carotid arteries. In order to confine our attention to the hemodynamic effects of bifurcation geometry on intimal thickening and plaque localization and minimize possible confounding secondary effects of flow disturbances attributable to superimposed stenosis, only those carotid bifurcations with no x-ray evidence of lumen stenosis were included in the present study. Of a large series of bifurcations being gathered for correlative studies, 12 of the first 14 pressure-fixed specimens fulfilled this criterion and were sectioned for histological evaluation.

**Histological Preparations and Measurements**

After radiography, rows of sutures were placed through the adventitia and outer media of the common carotid and internal carotid arteries to mark the position of the flow divider and the plane of the bifurcation angle. These served to establish the position of the flow divider on the histological sections which were used for subsequent morphometric quantification by polar coordinate mapping. The apex of the flow divider was taken as the point of bifurcation and the reference point from which measurements for section locations were made. Transverse rings were removed at five standard levels, and the precise location of each section was marked on the corresponding postmortem angiogram (Fig. 1). The standard levels were defined as follows: A—the common carotid at one vessel diameter proximal to the bifurcation; B—the proximal internal carotid immediately distal to the bifurcation; C—the carotid sinus at its midpoint; D—the distal internal carotid beyond the sinus; and E—the proximal external carotid distal to the bifurcation. The same anatomical definitions were used to establish the axial positions for flow velocity measurements in the model carotid bifurcation described below. Tissue samples were processed through paraffin, sectioned at 5–7 μm, and stained with hematoxylin and eosin, or with the Weigert van Gieson or Gomori trichrome aldehyde-fuchsin procedures for differential staining of connective tissue fibers and cells. Typical sections at levels A through D are shown in Figure 2. Each section was projected onto a digitizing tablet by means of a microprojector, and the contours of the lumen, the internal elastic lamina, and the outer limit of the media were traced (Glagov et al., 1981). These data were stored in a microcomputer and processed to display the traced contours and to compute the cross-sectional area of the lumen, intima, and media, as well as lumen radius, intimal thickness, and media thickness at 15° intervals around the vessel circumference using the position of the center of the bifurcation flow divider as projected along the inner...
Model Flow Studies

The models of normal human carotid bifurcations, in which the flow studies were conducted were constructed from measurements obtained from 57 biplane carotid angiograms of patients from 34 to 77 years old, as described previously (Balasubramanian, 1979; Bharadvaj et al., 1982a, 1982b). A blown glass model was produced for flow visualization and a machined plexiglass model was utilized for velocity measurements with laser Doppler anemometry. Vessel dimensions were scaled upward to allow accurate resolution of velocity profile measurements. The actual diameters of the model at the levels corresponding to the standard sampling sites of the post-mortem specimens were as follows: A—common carotid, 31.0 mm; B—proximal internal carotid, 32.3 mm; C—mid-carotid sinus, 34.4 mm; D—distal internal carotid, 22.0 mm; E—proximal external carotid, 17.6 mm. There was close agreement between the dimensions measured on the 12 postmortem angiograms of the cadaver carotid bifurcations and the dimensions derived from the in vivo angiograms which were used to develop the scaled model bifurcations (Table 1). The maximum carotid sinus diameter (level C) for construction of the models was slightly larger (6.8 mm compared to 6.2 mm) than the postmortem samples. In both the model and the specimens, the internal carotid artery narrowed markedly distal to the sinus, with a decrease in diameter of 40% and a decrease in cross-sectional lumen area of 65% for each. The bifurcation angle derived from the angiograms from which the model was constructed was 50 ± 21° (mean ± SD) and the bifurcation angle of the cadaver carotids examined in this study was 46 ± 25°.

The methods used to characterize flow patterns have been described in detail elsewhere (Balasubramanian, 1979; Bharadvaj et al., 1982a, 1982b). In brief, the visualization studies were conducted using bubble and dye injection techniques. For the bubble studies, a cathode wire was installed in a coupling between the inlet tube and the bifurcation model, so that bubbles formed at the wire by electrolysis were convected into the test section by the flow. The coupling could be rotated so that the wire was aligned along any desired diameter. The bubbles were photographed against a dark background. For the dye injection studies, black ink was injected rapidly through a catheter inserted from the downstream end of the internal carotid branch. Successive photographs were taken against a white background as the dye was transported downstream. The velocity measurements were made with a DISA 55L Laser Doppler Anemometer (LDA) system. The laser beam was split into two beams of equal intensity, and one beam was passed through a Bragg cell which provided a 40 MHz frequency shift. The beams then were focused into an approximately ellipsoidal sample volume with a length of 1.08 mm and diameter of 0.12 mm. The interference fringes within this sample volume translated due to the frequency shift of the beam passing through the Bragg cell, thus allowing both positive and negative velocities to be measured. Light scattered by 1.5-μm silicon carbide particles traversing the sample volume was collected by a photomultiplier tube and amplified, and the velocity of the particles was related to the Doppler frequency, fD, of the photomultiplier signal by the expression

\[ V = \frac{fD}{2} \sin \left( \frac{\Theta}{2} \right), \]

where \( V \) is the component of particle velocity normal to the bisector of the beam intersection angle, \( \Theta \). The signal...
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**Figure 2.** Histological preparation of transverse sections through a typical carotid bifurcation at four of the standard locations indicated on Figure 1. A—common carotid artery, B—proximal internal carotid artery, C—midpoint of carotid sinus, D—distal internal carotid. The 0°, 90°, 180°, and 270° degree reference locations are indicated. Sutures (arrows) were used for orientation. The thickest portions of the eccentric plaques in B and C are located at the outer wall (180°) of the artery. Black masses in lumen in sections B and C are remnants of the contrast medium and gelatin used for angiography. Gomori trichrome-aldehyde fuchsin stain. 9X.

From the photomultiplier was introduced into a frequency tracker to determine $f_0$. Since $\lambda$ and $\theta$ are known, the velocity component $V$ is proportional to the Doppler frequency. The laser and its associated optics system were mounted on a traversing device which allowed three degrees of freedom in translation and two in rotation. The least counts for translation and rotation were 0.025 mm and 0.5°, respectively. Corrections were made for differences in model wall thickness and differences in the index of refraction of the various media in relation to mechanical movement of the LDA system and the location of the sample volume within the model (Balasubramanian, 1979). A Hewlett-Packard 5451B Fourier analyzer was employed to process the analog output of the frequency tracker. Averaging times from 25 to 30 seconds were used in calculating the velocity at each of the selected points across the flow field at each level. Accuracy checks on the velocity data were performed by integrating the velocity profiles in the common carotid artery to obtain volume flow rates. Comparison of these values with flow rates measured independently by electromagnetic flow meters was within 5%.

Wall shear stress was determined at each level in the model corresponding to the standard levels of section shown in Figure 1 using the velocity profile technique. Velocities were measured at three radial stations, with the first station as close to the wall as possible while retaining an adequate Doppler signal. The remaining two points were located at positions corresponding to a laser movement of 0.13 mm in air. A least-squares-fit straight line was passed through the velocity data at these three points, and its slope was assumed to be the same as the velocity gradient at the wall. This method of determining the wall velocity gradient was checked in the common carotid artery by comparison with the Hagen-Poiseuille value and was found to be within 15% of the theoretical result. Flow velocity profiles were measured and wall shear stresses calculated in orthogonal planes, chosen to correspond to the 0, 90, 180, and 270° positions on the tissue sections, as described above. Wall shear stress was determined by scaling the measured velocity gradient at the model wall to actual vessel conditions, assuming that the viscosity of blood is 0.035 poise, and using the relation $\tau_w = \mu (du/dy)_w$, where $\mu$ is the blood viscosity and the shear rate $(du/dy)_w$ is the scaled value of the slope of the line obtained from experiments. For values at polar coordinates 0° and 180°, the velocity gradient was found from the measured axial velocity profile, since there is no tangential velocity component due to symmetry about the bifurcation plane. At 90° and 270°, however, it was necessary to measure, in addition, the circumferential velocity profile, since the axial velocity component does not completely describe the velocity direction.

The flow data which were compared with lesion loca-
tions in the human bifurcation specimens were conducted under steady flow conditions corresponding to Reynolds numbers \( \text{Re}^* \) of 400, 800, and 1200 for the common carotid artery inlet of the bifurcation. Based upon data available on carotid flow rates, a Reynolds number of 400 was taken to be representative of mean flow conditions and \( \text{Re}^* \) equal to 1200 was selected as representative of conditions at peak systolic flow (Kristiansen and Krog, 1962; Tindall et al., 1962). Velocity measurements were made with flow division ratios between internal and external carotid branches of 60:40, 70:30, and 80:20. Velocity data obtained in the model were scaled to provide appropriate values for real vessel dimensions using similarity principles common in fluid dynamics (Schlichting, 1968).

### Results

#### Lesion Localization in Specimens

Although none of the postmortem bifurcation specimens examined in this study were from patients with symptoms of cerebrovascular disease and none showed evidence of lumen stenosis on postmortem angiograms, each of the specimens showed intimal thickening or plaque formation on histological examination, including the bifurcation obtained from the youngest subject, a 27-year-old man. Lumenal intimations of the adjacent wall. None of the lesions were ulcerated or surmounted by thrombi. Lumens showed only minimal intimal change. Mean intimal thickness at the proximal internal carotid sinus was more than three times greater than in the common carotid artery \( (P < 0.01) \) and five times greater than in the distal internal carotid artery \( (P < 0.001) \). Similarly, intimal thickness at the midpoint of the carotid sinus was significantly greater than in the common carotid or distal internal carotid sections \( (P < 0.01) \). Intimal cross-sectional area was greater at both the proximal internal carotid and mid-sinus sampling sites than in the proximal common carotid \( (P < 0.01) \) or distal internal carotid samples \( (P < 0.001) \). In 23 of the 24 proximal internal carotid and mid-sinus sections examined, intimal cross-sectional area was greater than in the corresponding common carotid artery, and in no instance was distal internal carotid intimal cross-sectional area greater than that in the carotid sinus. There were no significant differences in lumen cross-sectional area between the common carotid and carotid sinus sampling-sites, indicating that no effective lumen stenosis was present despite the marked difference in plaque thickness. There was, however, a 2.5-fold increase in the cross-sectional area of intima encompassed within the internal elastic lamina in the carotid sinus as compared to the common carotid \( (P < 0.01) \).

Polar coordinate mapping at each standard sampling site established the circumferential location of intimal plaques with respect to the apex of the flow divider. Results are shown in Table 3. In the common carotid artery \( (A) \), there was no consistent circumferential localization of intimal thickening. In the carotid sinus \( (\text{levels B and C}) \), intimal thickness along the inner wall was no different from that present in the common carotid. Along the outer wall of the sinus, however, intimal thickness was 4.5 times greater than at the inner wall \( (P < 0.01) \). Analysis of variance at levels B and C revealed the intimal thickness of inner wall to be consistently less than

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Postmortem specimens ( (n = 12)^* )</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A—Common carotid</td>
<td>6.1 ± 0.2</td>
<td>6.1</td>
</tr>
<tr>
<td>B—Proximal internal carotid</td>
<td>6.0 ± 0.4</td>
<td>6.3</td>
</tr>
<tr>
<td>C—Midpoint of carotid sinus</td>
<td>6.2 ± 0.3</td>
<td>6.8</td>
</tr>
<tr>
<td>D—Distal internal carotid</td>
<td>3.5 ± 0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>E—External carotid</td>
<td>4.0 ± 0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Length of internal carotid bulb (mm)</td>
<td>13.9 ± 0.5</td>
<td>13.0</td>
</tr>
<tr>
<td>Bifurcation angle (degrees)</td>
<td>46 ± 7°</td>
<td>50°</td>
</tr>
</tbody>
</table>

* Values are means ± SEM.

---

\( \text{Re}^* = \frac{uD}{v}, \) where \( u \) is the mean velocity in the artery, \( D \) is its diameter, and \( v \) is the kinematic viscosity.
TABLE 2
Dimensions in Postmortem Specimens*

<table>
<thead>
<tr>
<th>Location</th>
<th>Intimal thickness (mm)</th>
<th>Intimal area (mm²)</th>
<th>Lumen area (mm²)</th>
<th>% Stenosis (fraction of IEL area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A— Common Carotid</td>
<td>0.12 ± 0.01</td>
<td>2.2 ± 0.3</td>
<td>21.1 ± 0.7</td>
<td>9.3 ± 1.2</td>
</tr>
<tr>
<td>B— Proximal internal carotid</td>
<td>0.40 ± 0.06</td>
<td>7.1 ± 1.5</td>
<td>22.5 ± 2.9</td>
<td>23.1 ± 3.0</td>
</tr>
<tr>
<td>C— Midpoint of carotid sinus</td>
<td>0.31 ± 0.04</td>
<td>4.9 ± 0.8</td>
<td>20.8 ± 2.7</td>
<td>19.5 ± 2.3</td>
</tr>
<tr>
<td>D— Distal internal carotid</td>
<td>0.08 ± 0.01</td>
<td>0.9 ± 0.4</td>
<td>11.3 ± 1.1</td>
<td>6.1 ± 1.8</td>
</tr>
<tr>
<td>E— External carotid</td>
<td>0.15 ± 0.04</td>
<td>1.4 ± 0.3</td>
<td>10.4 ± 1.3</td>
<td>11.3 ± 2.0</td>
</tr>
</tbody>
</table>

* All values are means ± SEM.

that of the anterior (90°) lateral (180°) or posterior (270°) wall (P < 0.05). There was no significant difference in intimal thickness about the circumference at levels A, D, or E. Maximum intimal thickness in the proximal carotid sinus (level B) was 0.9 ± 0.1 mm and occurred at 161 ± 16°, whereas minimal intimal thickness was 0.05 ± 0.02 mm and occurred at 20 ± 14° (where 0° is at the center of the inner wall and 180° is at the center of the outer wall). Minimum thickness was within 15° of the center of the flow divider in 11 of the 12 sinus specimens. Thickness at the anterior and posterior wall positions of the carotid sinus (90° and 270°) was always greater than at the inner wall. In all but one specimen, anterior and posterior wall thickness was less than at the outer wall. In that instance, a patient 44 years old with the largest atherosclerotic plaque, thickness of the intima was slightly greater at the inner wall (0°) than at the outer wall (180°) but was even greater anteriorly than posteriorly (at 90° and 270°). In the distal internal carotid (D), intimal thickness was once again uniform about the circumference. Intimal thickening in the proximal portion of external carotid artery (E) was less than in the corresponding internal carotid sinus section and tended to be somewhat greater on the inner than on the outer wall of the artery. However, the differences never attained statistical significance. Thickness was on the average 0.27 ± 0.15 mm at the inner wall and 0.12 ± 0.05 mm at the outer wall of the single cross-section examined on each external carotid sample.

TABLE 3
Circumferential Distribution of Intimal Thickness

<table>
<thead>
<tr>
<th>Location</th>
<th>Polar coordinate position (deg)</th>
<th>Intimal thickness* (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A— Common carotid</td>
<td>0</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>B— Proximal internal carotid</td>
<td>0</td>
<td>0.14 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.47 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.63 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>0.37 ± 0.10</td>
</tr>
<tr>
<td>C— Midpoint of carotid sinus</td>
<td>0</td>
<td>0.19 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>0.31 ± 0.07</td>
</tr>
<tr>
<td>D— Distal internal carotid</td>
<td>0</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.08 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>E— External carotid</td>
<td>0</td>
<td>0.27 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>0.12 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>270</td>
<td>0.15 ± 0.05</td>
</tr>
</tbody>
</table>

* Values are means ± SEM.

† 0° is at center of flow divider; for common carotid 0° is at centerplane of external carotid, 180° is at centerplane of internal carotid.

‡ P < 0.01 analysis of variance.

§ P < 0.05 analysis of variance.

Relation of Lesion Localization to Flow Dynamics in the Models

Flow Visualization

As flow from the common carotid enters the bifurcation, changes in streamline direction occur. Central lines of rapid flow are compressed toward the flow divider and inner wall of the internal carotid artery (Fig. 4). Rapid laminar flow was present along the inner wall of the carotid sinus at 0° where intimal thickening was least in the tissue sections. Along the outer wall of the sinus (180°), where lesions were thickest, a large area of flow separation develops and is characterized by a reversal of axial flow and slow fluid movement upstream. Particles of dye are therefore carried rapidly along the inner wall but are cleared very slowly from the outer region of flow separation and low velocity (Fig. 5, level B and C). The region of separation is not simply a zone of stasis and recirculation but is rather a zone of complex secondary flow patterns including counter-rotating helical trajectories (Fig. 6). The zone of separation along the outer wall of the sinus existed throughout the range of Reynolds numbers and flow division ratios studied. Along the inner wall of the sinus (0°), where the flow divider intercepts the inlet stream, the streamlines were directed predominantly longitudinally, whereas, at
FIGURE 4. Hydrogen bubble flow visualization in the glass model. The hydrogen-generating wire was oriented in the plane of the photograph. Flow is rapid, laminar, and longitudinal along the inner wall of the carotid sinus (black arrow), and a large area of flow separation is formed along the outer wall of the sinus (white arrows). The Reynolds number is 400 and the flow division ratio between internal and external carotid artery is 70:30. The levels corresponding to the tissue sections (A-E) are indicated. The anterior and posterior walls (90°, 270°) where lesion thickness is intermediate, the angle of near-wall streamlines depended strongly upon specific flow conditions. The flow patterns are more sensitive to changes in branch flow division ratio than to changes in Reynolds number. Flow reattaches before leaving the carotid sinus, and the distal internal carotid (level D) experiences relatively rapid axial flow throughout its cross-section with relatively small tangential components remaining from helices generated within the sinus.

Flow Velocity

Figure 7 presents an overview of axial velocity profiles, drawn to scale, in the plane of the bifurcation. Levels corresponding to the sites of morphologic study shown in Figure 1 are indicated on Figure 6. In the common carotid artery (A), there is a fully developed laminar velocity profile. At the bifurcation (B), the axial velocity profile becomes skewed toward the apex of the bifurcation and remains skewed within the carotid bulb (C). A negative flow velocity develops along the outer wall of the carotid sinus (180°), where lesions in the specimens were thickest. The dotted line in Figure 7 indicates the locus of points of zero velocity in the profiles, and outlines the area in the sinus where flow velocities were low and directed back toward the common carotid artery. As flow entered the distal internal carotid (D) after leaving the sinus, it accelerated due to the decreasing cross-sectional area. In axial veloc-

FIGURE 5. Flow visualization with dye injection in the glass model of the carotid bifurcation illustrating slow clearance from the separated flow region along the outer wall of the carotid sinus (arrows). Dye remains along the outer wall and within the separation zone long after it has been convected away from the region of the flow divider and distal internal carotid artery. The Reynolds number is 400 and the flow division ratio is 70:30. The letters A through E indicate the levels which correspond to the standard sampling sites shown in Figure 1.
Hydrogen bubble visualization in the glass model with the bubble-generating wire oriented in a plane perpendicular to the photograph. Streamlines are skewed towards the apex of the bifurcation, and complex helical flow patterns occupy the separated flow region of the sinus. The Reynolds number is 800 and the flow division ratio is 80:20. The letters B and C indicate the proximal internal carotid and mid-sinus levels corresponding to the standard sampling sites shown in Figure 1.

Figure 7. Axial velocity profiles at positions (letters and arrows) in the carotid bifurcation model which correspond to the sites of sampling for morphological study in the human bifurcation specimens shown in Figure 1. Dotted line indicates the locus of points of zero velocity. The Reynolds number is 800 and ratio of flow in the internal to external branches is 70:30. Arrowheads indicate positions at which velocity profiles have been measured to provide shear stress values shown in Figure 8.

Along the outer wall of the carotid sinus, at the 180° location, where lesions were thickest, flow velocity was greatly reduced and attained negative values. Distal to the sinus (level D), where flow reattachment occurred, flow velocities at each polar measuring position are comparable to values noted in the common carotid artery corresponding to the near absence of lesions in this location in the specimens.

Wall Shear Stresses

Since wall shear stress is related to the velocity gradient at the wall, the shifts in flow velocity maxima indicate that shear stress is higher along the inner wall of the carotid sinus than at the outer wall, that shear stresses were greater along the flow divider than in the common carotid, and that shear stress values were much greater in the distal internal carotid than in the common carotid artery. This...
distribution of shear stress was inversely related to the distribution of intimal thickness in the specimens. Calculated values of wall shear stresses are given in Table 5. In the common carotid artery (level A), shear stress was relatively low at all Reynolds numbers. In the carotid sinus (levels B and C), shear stress was high along the inner wall, i.e., at the 0° index point where lesion thickness was least, and increased 5-fold (31 to 160 dynes/cm²) from Re 400 to Re 1200. By contrast, along the outer wall, at 180°, where lesions were thick and where the zone of separated flow occurred, shear stress was quite low at all Reynolds numbers, with values ranging from 0 to −6 dynes/cm². The negative value for shear stress reflects a reversal of the directional vector relative to centerline flow. In the distal inter-
nal carotid (level D), where diameter decreased abruptly, where flow reattached and accelerated, and lesions were least evident, shear stress increased markedly, reaching 600 dynes/cm² at Re = 1200. Wall shear stress variation in a longitudinal plane along the outer wall of the carotid bifurcation at 180° for Re values of 400, 800, and 1200 and a flow division ratio of 70:30 is presented in Figure 8. The arrowheads in Figure 7 correspond to points plotted along the abscissa in Figure 8. The levels corresponding to the standard tissue sections are indicated in both Figures.

Discussion

Preferential localization of advanced atherosclerosis along the outer wall of the carotid sinus has been noted in postmortem anatomic specimens (Heath et al., 1973; Solbert and Eggen, 1971), on angiograms of patients with severe carotid stenosis (Bauer et al., 1962), and in carotid bifurcation plaques removed during carotid endarterectomy (Imparato et al., 1979). Static zones and boundary layer separation have been noted radiographically at the outer wall of the carotid sinus in patients with cerebrovascular symptoms, and Fox and Hugh (1976) have suggested that atherosclerosis develops in such regions of “stasis.” These observations, made on bifurcations with advanced disease, are consistent with our quantitative findings in carotid bifurcations with intimal thickening and early atherosclerosis. By comparing the location of non-stenosing intimal disease in human bifurcations with flow field distribution in corresponding axial and circumferential positions in anatomically accurate models of the entire bifurcation, we were able to associate atherogenesis with hemodynamic variations related mainly to bifurcation geometry at several levels and without the complicating secondary effects of stenosis on flow.

We found that intimal thickness was moderate and uniform around the circumference in the common carotid artery sample. In this location, the flow profile is axisymmetric, flow streams are unidirectional and axially aligned, and wall shear stress ranged from 9 to 29 dynes/cm², depending on the Re number. In contrast, intimal thickness was 3 to 5 times greater in the proximal internal carotid section and in the mid-carotid sinus location than in the common carotid sample and was consistently eccentric. On the average the intima was 4 to 5 times thicker along the outer wall than at the inner wall. Minimal thickness determined from polar coordinate maps of the internal carotid sections nearly always occurred within 15° of the 0° center point of the flow divider. In this region, flow remains axially aligned with displacement of the maxima toward the flow divider, and corresponding shear stress was about 30 dynes/cm² at Re = 400. The region of maximum intimal thickening in the sinus, opposite the flow divider, corresponds to the complex flow pattern with evidence of separation, vortex formation, reduced velocity, and retrograde flow which develops in this region and with shear stress reduced to 0 dynes/cm². Distal to the sinus, where the diameter of the internal carotid narrowed abruptly, intimal thickening was minimal and uniform around the circumference. At this level, flow accelerates and is once again axially aligned and unidirectional; shear stress increased markedly, ranging from 53 to 600 dynes/cm² about the circumference at the standard test site. On the basis of these data, we conclude that low levels of shear stress in regions of flow separation and departure from axially aligned flow is associated with the enhancement of intimal thickening and the formation of atherosclerotic plaques, and that relatively high levels of shear stress in regions of rapid unidirectional laminar flow tend to inhibit lesion formation.

The mechanisms responsible for these effects are not clear. Caro et al. (1971) have suggested that low wall shear rates retard the transport of circulating particles away from the wall, resulting in increased intimal accumulation of lipids. Low shear stress could also interfere with adequate turnover at the endothelial surface of substances essential both to artery wall nutrition and to the maintenance of optimal endothelial metabolic function (Robertson, 1968). Although the region of flow separation in the model studies does not appear to be a closed and static recirculation zone, flow through this area is definitely markedly delayed and the residence time of circulating blood elements in such a region in a
human bifurcation would be expected to be prolonged. Flow separation has also been shown to favor deposition of platelets in vitro (Parmentier et al., 1981). Under such conditions, the release of a platelet growth factor could presumably stimulate cell proliferation and induce intimal thickening and plaque formation (Ross, 1981). Although we saw no evidence of thrombus formation or platelet aggregation in our material, postmortem human specimens are not suitable for evaluating either endothelial integrity or platelet adhesion.

In contrast to the implications of our findings and those of others (Kjaerem et al., 1981) that low shear stress is associated with intimal thickening and atherogenesis, several investigators (Fry, 1968; Joris et al., 1982; Reidy and Bowyer, 1977) have suggested that elevated shear stress favors intimal disease by engendering endothelial injury and disruption, thereby potentiating exposure of the underlying artery wall to atherogenic agents. It has been shown by means of a mechanical device placed in the canine aorta that sudden elevations in shear stress to levels of about 400 dynes/cm² may cause endothelial injury (Fry, 1968), and that denudation of the intimal surface may result in increased intimal cellularity (Stemerman et al., 1977). There is, however, no direct evidence that local endothelial injury or disruption attributable to shear stress occurs in usual flow situations, either in man or in experimental animals. We estimated that for Re = 1200, a peak shear rate of approximately 600 dynes/cm² occurs at the inner wall of the human lesion-free distal internal carotid. This value exceeds the shear stress which resulted in endothelial injury in the canine aorta. In experimental studies, we found no evidence for endothelial disruption at sites of high shear or at sites of plaque formation in monkeys (Taylor et al., 1978; Zarins et al., 1980; Zarins et al., 1981). Neither is there direct evidence that endothelial removal results in eventual sustained lesion formation. On the contrary, evidence has been advanced that the formation of experimental intimal plaques may require the presence of a continuous endothelial covering (Bomberger et al., 1980; Chidi et al., 1979; Minick et al., 1979).

The finding that sudanophilic lesions about the intercostal ostia of cholesterol-fed rabbits tend to occur distal to the flow divider edge (Cornhill and Roach, 1976) has been offered as evidence that plaques occur in regions of elevated shear stress. Similar findings have been reported about the ostia of aortic branches in human infants, but the sudanophilic area did not correspond to areas that were prone to subsequent plaque deposition in adults (Sinzinger et al., 1980). In neither of these studies were the arteries studied after pressure-fixation. Longitudinal opening and flattening of arteries create distortions, especially at branch points, which limit the accuracy of determinations of lesion location (Roach et al., 1978). Furthermore, a clear relationship between sudanophilic staining lesions and human atherosclerotic plaques is not well established, and further studies are needed.

It has also been suggested that transport of cholesterol between blood and the artery wall is shear dependant (Caro and Nerem, 1973). However, the relationship was not statistically significant, and no relationship was demonstrated between shear rate-enhancing cholesterol flux and lesion formation. It has also been suggested that variations in shear stress direction associated with pulsatile flow may lead to modifications of endothelial permeability, whereas even relatively high shear stresses which remain unidirectional may not be injurious (Fry, 1976). Such a suggestion is not inconsistent with our findings, for variations in direction occurred where the complex patterns of vortex formation and retrograde flow developed and intimal disease was greatest. However, it is striking that turbulence did not occur in our models over the range of flow conditions reported here. Although stenosing plaques of sufficient size may induce turbulence, the findings associated with non-stenotic disease reported here suggest that the initiation of atherosclerosis at the human carotid bifurcation is not a consequence of turbulence.

A complete description of blood flow through the human carotid bifurcation must take into account not only the complex and variable geometry of this region, but also the distensibility of the arterial wall, the non-Newtonian characteristics of blood, and the pulsatile nature of blood flow. Although the geometry of the model human carotid bifurcations used for the flow measurements (Balasubramanian, 1979; Bharadvaj et al., 1982a, 1982b) was based on measurements of human angiograms, rigid undistensible materials were utilized, as well as a Newtonian fluid and a steady flow system. Of these factors, bifurcation geometry has the greatest influence on the flow field, whereas the non-Newtonian characteristics of the blood have the least effect. Vessel distensibility is an important factor in pulse wave propagation, but its effect on local velocity profiles is small. Thus, use of a distensible structural material and a non-Newtonian fluid would not greatly alter the principal findings in the models which we compared with the distribution of lesions in the specimens.

The assumption of steady flow could, however, lead to inaccuracies. To address this problem, a range of Reynolds numbers and flow division ratios were used in order to approximate the conditions to be expected during the cardiac cycle and over a range of flow rates (Stein and Sabbah, 1980). Although a region of flow separation and complex flow develops in the carotid sinus at each of the Reynolds numbers that were used, the size of this region increases with increasing Reynolds number and with decreasing flow through the internal carotid. We have assumed that a similar range of flow behavior would occur if actual pulsatile conditions were introduced. Flow visualization studies in the
model referred to in the present study, but, utilizing a pulsatile velocity waveform (Ku and Giddens, 1983), have indeed revealed an area of flow separation in the carotid sinus in the same neighborhood as was noted in steady flow. There are, however, certain differences between the steady and pulsatile flow patterns. The separated flow zone does not appear to exist during the brief period of early systole in the model, for forward flow is observed throughout the sinus during this time. As the flow waveform reaches the decreasing velocity phase, however, separation occurs rapidly and its location migrates along the outer wall, becoming stable during diastole. In a similar study, LoGerfo et al. (1981), reported little qualitative difference in carotid bifurcation flow fields between steady and pulsatile flow. In a study of flow and plaque localization in a cast of a human aortic bifurcation, Friedman et al. (1981), found a negative correlation between intimal thickness and wall shear rate. They report, however, that the strongest negative correlation existed between intimal thickness and pulse shear rate, i.e., the cyclic variation in shear rate. They propose that large excursions of interfacial shear may protect against atherosclerosis. In view of these and our own recent findings, we believe that further examination of the detailed differences between steady and pulsatile flow, using quantitative methods to describe the flow field, are warranted, for the factors responsible for the appearance of the initial lesion may be quite focal. It is, however, unlikely that additional refinement of these studies will alter our general conclusions that plaques tend to develop in areas of flow separation and low wall shear stress where wall shear stresses change direction, and that plaques do not develop readily in areas of rapid, unidirectional laminar flow and high shear stress.

Contrasts between our hemodynamic findings and those of investigators who have used idealized branching models are noteworthy. Friedman et al. (1981) have made calculations based on branches with sharp ostial corners in two-dimensional models. Their results indicate that wall shear stress in the parent vessel increases as the branch point corner is approached. Our data for a three-dimensional model with normally rounded corners reveals decreasing wall shear stress at the lateral branch orifice angle followed by flow separation immediately distal to it. It is also apparent from our data that wall shear stress directions and magnitudes cannot be predicted unless the computational methods are extended to three-dimensional flows. In a study of flow in models of human aortic bifurcations, no separation was detected at branch corners when there was an equal division of flow between the branches (Friedman et al., 1981). This finding, as well as similar studies using cylindrical glass tubes with right angle branches (Houle and Roach, 1981), provide additional evidence that the existence of separation is dependent not only upon geometry, but also on flow division, and that hemodynamic studies in models must take this variable into account.

Carotid lesions were seen in all of our specimens, but individual variation in degree was considerable and was not consistently related to age in the specimens studied. The localized increase in the cross-sectional area of intima in the carotid sinus suggests that this region may be particularly predisposed to progressive luminal occlusion. However, this increase in intimal area was offset by a greater area encompassed by the internal elastic lamina, compared to the other locations. Thus, there was no resultant lumen stenosis. It is often assumed that the internal elastic lamina outlines the cross-sectional area of the original lumen before intimal thickening or plaque formation occurred. However, if arteries tend to enlarge as intimal thickening or atherosclerosis develop (Bond et al., 1981), the relative rates of intimal thickening and vessel dilation may be important in determining individual differences in the consequences of intimal disease in the sinus. Thus, since bifurcation geometry, flow ratio and pulse rate would appear to be major determinants of the flow profile in the sinus, individual differences in localization, extent, and rapidity of lesion enlargement could be related at least in part to individual differences in these variables. Quantitative studies of relationships among lesion size, bulb configuration, branch angle, and relative branch diameters must be performed in pressuredxed human specimens, since substantial errors are introduced by studying collapsed vessels (Zarins et al., 1983). In addition, experimental models of atherosclerosis will help illuminate the precise manner in which the features of the flow environment identified here condition the distribution of blood-borne elements in the immediate vicinity of the endothelium.

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References


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