Transmural Organization of the Arterial Media
The Lamellar Unit Revisited

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When scanning electron micrographs of orthogonal transmural fracture surfaces of distended aortas were compared with appearances on corresponding semithin light microscopic and ultrathin transmission electron microscopic sections, medial lamellar units could be resolved into composites of overlapping, musculo-elastic fascicles lying parallel to tangential planes of section. Each fascicle consisted of a group of commonly oriented, elongated smooth muscle cells and an encompassing array of branching, similarly oriented elastic fibers. On longitudinal sections of straight segments of the aorta, fascicles appeared as closely packed, transversely transected smooth muscle cell groups within compartments formed by similarly transected elastic fibers. On transverse sections, fascicles appeared as groups of cells within cell strips or layers, each of which was bracketed on both its luminal and abluminal sides by straight elastic fibers. Fascicles were increasingly evident during growth as commonly oriented groups of cells within the already present concentric cell layers became demarcated by enlarging elastic fibers. Wavy collagen fiber bundles, distinct from the interlaced fibrils of the immediate pericellular matrix, were interposed mainly between the facing elastin systems within the fibrous regions between cell layers. Thus, the radial transmural disposition of cells and matrix fibers on transverse sections of the media in well developed aortas proved to be: elastin-cells-elastin — collagen bundles — elastin-cells-elastin — collagen bundles, etc. Medias of major branch arteries were also composed of musculo-elastic fascicles, but their encompassing elastic fiber systems were less prominent than in the aorta. In straight segments of aortas and arteries, fascicles were uniform in size at any given transmural level and oriented mainly circumferentially. At bends and branch points fascicles were smaller and less uniform in size and orientation. In relation to changes in vessel wall curvature, alignment of the fascicles was usually in the direction of presumed resultant tensile stress. The findings suggest that these subunits of medial organization correspond to the distribution and magnitude of tensile stresses.

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scanning electron microscopy of mural fracture surfaces with details observed by light microscopy of semithin sections and transmission electron microscopy of ultrathin sections, has permitted us to describe the organization and distribution of cells and fibers that comprise arterial medial layers. In a previous report, we described the immediate relationships and attachments among medial cells and between medial cells and matrix fibers of aortas fixed while distended at normal or elevated pressures. In the present communication we present evidence that the previously described aortic medial lamellar unit, as well as the layers of major branch arteries, can be resolved into musculo-elastic subunits, the size, orientation and composition of which appear to correspond to the distribution of tensile stresses.

Methods

Animals

Aortas and several major branch arteries of Sprague-Dawley rats, New Zealand white rabbits, and Yucatan pigs were fixed by perfusion in situ at controlled and monitored pressures in the physiologic range. Perfusion fixation was carried out in the rabbits and rats with the animals anesthetized. In each instance animals were anesthetized with sodium pentobarbital administered intraperitoneally or intravenously (50 mg per kg body weight). The pigs were killed before fixation by means of a lethal dose of the anesthetic. The rabbits were studied at birth (within 24 hours) and at 1, 2, 4, 8, and 52 weeks of age; rabbit fetuses at 20 and 25 days gestation were also examined. The pigs were newborn as well as 1, 2, 3, 10, and 25 weeks old. The rats were adults, weighing 250–300 g. Satisfactory preparations were judged distention to be complete and processing and taken of intercostal, mesenteric, renal, and femoral arteries at their ostia and at a distance of about two artery diameters from the ostia.

Specimen Preparation

The methods used for in situ controlled pressure fixation with buffered isosmolal 2.5% glutaraldehyde and the subsequent processing of perfusion-fixed tissues have been described and justified in previous reports. In brief, rings of unopened vessels were removed at the standard sampling levels and postfixed in 1% osmium tetroxide. Whole rings or parts of rings were embedded in Epon and sections 1–2 μm thick (i.e., semithin sections) were stained with toluidine blue or by a polychrome method for differential staining of elastin and cells and were viewed directly by light microscopy (LM) at magnifications up to 2000 X. Several blocks were sectioned serially or studied in steps at 10- to 20-section intervals in order to establish the boundaries of structures which extended continuously for distances greater than 0.5 mm. The thickness of semithin plastic embedded sections (1–2 μm) was less than the distance between successive aortic medial layers or the width of large elastic fibers. Details could therefore be observed with less superimposition than is usually present in paraffin-embedded material. For transmission electron microscopy (TEM), ultrathin sections of selected areas of corresponding blocks were stained with lead citrate and uranyl acetate, or with phosphotungstic acid, and viewed and photographed in a modified RCA-EMU 3F electron microscope or in a Siemens Elmiskop 1A. For scanning electron microscopy (SEM), specimens were frozen and fractured as described in detail elsewhere. In brief, ethanol-substituted tissue or tissue infiltrated with glycerol was frozen in liquid nitrogen and broken. Glycerol-impregnated material was dehydrated in graded ethanol; critical-point drying was carried out through 40°C and 1300/psig in a Bomar-SPC-50 apparatus. Dried specimens were coated with gold palladium (40:60) in an Edwards vacuum coating chamber and viewed in a modified Hitachi HF-5-2 scanning electron microscope equipped with a field emission tip. SEM of fracture surfaces permitted the study, directly in three dimensions, and at relatively high resolution, of much larger specimens than could be prepared for TEM.

In addition, structures not rendered electron-dense by the usual TEM-intensifying reagents, and not removed by processing, were revealed by the metal coating of SEM preparations. Immediately adjacent samples of each vessel were also embedded in paraffin, sectioned at 7 μm and stained with hematoxylin and eosin, and with the Weigert-van Gieson or Gomori trichrome aldehyde-fuchsin procedures for LM examination. Fresh and fixed samples of thoracic aorta of two adult rabbits and pigs were subjected to autoclaving in sodium hydroxide at 30 psi for 1.5 hours. This procedure left only the amorphous component of elastin and afforded a view by SEM of transmural elastic fiber distribution. This procedure was equally effective on fresh and glutaraldehyde-fixed material.
Orientation of Samples

Attempts were made to direct fractures, orient specimens on SEM stubs, and orient blocks for LM or TEM sectioning, so as to provide transverse, longitudinal, or tangential planes for study. Ideal planes of fracture and section are shown diagrammatically in Figure 1. Transverse planes were exposed when fracture surfaces or planes of sections were perpendicular to the long axis of straight vessel segments. Exposure of longitudinal (axial) planes required fractures or sections which passed through the wall parallel to the long axis of the vessel and coincided with a radius. Tangential planes were revealed when a fracture or section was made through the media parallel to the long axis of the vessel but perpendicular to a radius. Although fractures usually occurred in desired planes, the relationship of exposed planes to vessel geometry could nearly always be ascertained on SEM specimens, for these were large enough to permit observation of the fracture plane in relation to the preserved adjacent vessel wall. Occasionally, when some of the specimens were fractured while thawing, separations occurred along tangential planes, thereby exposing cell and fiber surface contours. This appearance has been described elsewhere.4 Transverse and longitudinal LM and TEM samples were reoriented when necessary to provide full thickness views of the media as nearly perpendicular to the intimal surface as possible. Sections that required reorientation provided supplementary material cut in planes at small angles to the final acceptable orientation. The features of medial micro-architecture that emerged from these studies is presented here in detail. The plane of fracture or section with respect to vessel geometry is stated for each illustration.

Results

Organization and Sequence of Elements Comprising the Aortic Media

Medial cells in all of the aortic sections were markedly elongated, and extended circumferentially or obliquely within tangential planes, but never lay with their long axes spanning radially from one fibrous layer to the next. Medial layers were not organized as elastin sheets forming single, continuous lamellae alternating with cells. Instead, in well-oriented transverse planes of fractures or in transverse sections of straight portions of the aorta (Figures 2 A, 3 A, 3 C), each cell layer appeared, for the most part, to consist of groups or strips of overlapping, elongated cells, bracketed on either side by closely associated elastic fibers. In longitudinal planes of fracture or section (Figures 2 B, 3 B, 3 D), cells were cut mainly in cross section (i.e., in their narrowest dimension, or slightly obliquely), and the cell groups appeared as closely packed short rows, each of which was slightly offset from neighboring groups. Each group was encompassed by an array of uniformly spaced round or oval profiles of elastic fibers also cut in their narrow dimension. Views of tangential fractures of autoclaved specimens (Figure 4 A) as well as of tangential semi-thin sections of intact media (Figure 4 B) revealed that the elastic fibers surrounding individual cell groups consisted of more or less uniform, interconnected, cylindrical structures, similar in overall appearance to those visualized by others.11-13 These were oval or circular on cross section and showed some variation in diameter, but were never hollow. In general, well-formed elastic fibers surrounding cell groups appeared in the form of bars. When elastic fiber systems of adjacent cell groups appeared to be interconnected (Figures 3, 4, and 5), sequential sectioning usually revealed that these were foci of close approximation rather than single continuous fibers or connecting bridges. Manipulation of auto-claved preparations such as shown in Figure 4 A revealed that connections did exist between the elastic fibers of successive layers, but that these were much more easily disrupted than the interconnections of the elastin systems bracketing the layers. Thus, steady radial stretching resulted in irregular separation of successive layers, more in the manner of pastry layers than in the manner of onion layers. On SEM views of tangentially fractured preparations, and in TEM sections, the cell groups associated by surrounding elastic fibers were further associated by the common basal lamina sheaths and basketworks of fine collagen fibrils that have been described in detail elsewhere.4

Successive layers of cells and their surrounding elastic fibers were separated from one another by a narrower, intervening acellular zone containing thick, crimped or wavy, collagen fiber bundles, distinct from the interwoven fibrils of the pericellular matrix. The collagen bundles extended in the same direction as immediately adjacent elastic fibers but
Figure 2. A. Scanning electron microscopic view of a transverse fracture through the media of the descending thoracic aorta of an 8-week-old rabbit. In such preparations, cell layers (C) appear more or less continuous and sharply transected, while the intervening fibrous layers tend to fracture and tear irregularly. Elastic fibers (white arrows) within each fibrous zone form two separate systems. Both transected and projecting wavy collagen bundles (black arrows) are visible mainly between the separate facing elastic fiber systems of the fibrous layers. Bar = 2 μm. B. Scanning electron micrograph of adult pig descending thoracic aorta fractured in a longitudinal plane. An artifactual separation (X) of cells (C) from an adjacent elastic fiber (E1) exposes cell surface contours (S) covered by a mat consisting of fine collagen fibrils and basal lamina. Facing elastin systems (E1 and E2) are typically demarcated by interposed wavy-collagen fiber bundles, the broken ends of which (F) are seen here. When both facing elastin systems are well developed (i.e., in mature animals), the thick bundles of collagen fibers are tightly wedged between the elastin plates and identify the boundary between the two systems. The level of a tangential plane of fracture such as that shown in Panel C is indicated by the double lines and asterisk. Bar = 1 μm. C. Tangential plane of fracture that passes through a fibrous zone (plane of such a section is indicated in B). Wavy collagen fiber bundles (F), many of which are frayed by the fracturing process, course in and out of the plane of section among the relatively straight elastic fibers (E). Bar = 1 μm.
Figure 3. Light microscopic appearance of semithin plastic-embedded sections of the thoracic aorta of a 26-week-old pig. All magnifications are the same (bar = 15 μm). In each panel, sets of two oblique arrows identify the typical facing elastin fiber systems within fibrous zones between successive cell layers. The position of the corresponding collagen fiber bundle for each of the identified facing elastic fiber systems is indicated by a vertical arrow. Collagen fiber bundles are barely seen in these photographs because of staining and photographic techniques that accentuate elastin fibers. (Collagen fibers are more easily seen in Figure 6). Cell layers of the media are most easily identified in transverse sections in straight portions of the aorta, but fascicles comprising the layers are most evident in longitudinally sectioned preparations due to their stacked and offset appearance in this section plane. A. Transverse section of descending thoracic aorta. Cell layers (C) are bracketed on either side by elastic fibers. B. Longitudinal section at the same level as A. Cells are now transected in their narrow dimension and lie side by side within the cell layers (C). The associated branching elastic fibers are also sectioned in their narrow dimension, or slightly obliquely, and appear as rows of dots or ovals. Subgroups of cells can be discerned within each layer. At this level, where the aorta is straight, cell layers are fairly uniform and cells in adjacent groups are similarly oriented. C. Transverse section at the level of the arch. Cell layers are less uniform than in the descending aorta and cell orientation varies much more from layer to layer and from subgroup to subgroup (compare with A.) Three subgroups of different cell orientation are identified (C1, C2, C3).
did not intertwine with one another or traverse cell layers or cell groups, i.e., the collagen fiber bundles clearly marked a plane of separation between the facing elastic fiber systems associated with the successive cell layers. The thickness and abundance of the collagen bundles did, however, depend largely on vessel diameter. In the aortas of 25-week-old pigs, for example, large collagen fiber bundles were numerous and were wedged tightly between closely approximated, relatively thick, closely packed, facing elastic fiber systems (Figure 2 B), while in the aortas of rabbits, facing elastic fiber systems of successive layers were not as closely approximated and interposed collagen bundles were not as prominent. Light microscopic connective tissue stains that distinguish collagen from elastin in paraffin-embedded sections revealed the relationships among collagen and elastin fibers most distinctly when sections were precisely oriented. Thus, the previously identified aortic lamellar units \(^2\) proved, on comparison of extensive SEM fracture surfaces with transverse and longitudinal semi-

Figure 3 (continued). D. Longitudinal section at the same level as Panel C. Layering is less distinct than in the transverse or the longitudinal sections of the straight portion of the aorta (compare with A and B), but smooth muscle subgroups (C) or fascicles are more prominent due to differences in staining intensity. Each is surrounded by its own elastic fiber system. The differences in intensity of staining of cells in adjacent fascicles at this level is due to differences in cellularity, size, orientation, and plane of section through individual fascicles. This appearance is characteristic of transitional regions such as the aortic arch.

Figure 4. Medial elastic fiber systems of adult pig descending thoracic aorta. A. Tangential fracture after autoclaving to remove collagen and cells. View through the media reveals successive transmural elastic fiber systems (E1, E2, E3), corresponding to the successive medial layers. Bar = 15 \(\mu\)m. B. Tangential semithin section of plastic-embedded specimen shows successive cell layers and their corresponding elastic fibers. Superimposition is minimal in such sections so that subgroups or fascicles of commonly oriented cells (C) and fibers (arrows) can be seen within each cell layer. Cell and fiber orientation differ slightly from fascicle to fascicle. The spindle shape of medial cells with their long dimensions lying within tangential planes is characteristic of the aortic media. Bar = 30 \(\mu\)m.
thin sections of straight portions of the aorta, to be resolvable into closely fitted musculo-elastic subgroups or fascicles packed side by side (Figures 3 D, 4 B, 6 B). The radial disposition of cells and fibers across the media on transverse sections at most locations along a radius was not an alternation of cells and coaxial elastic fiber sheets, but a sequence of elastin-cells-elastin — collagen bundles — elastin-cells-elastin — collagen bundles — elastin-cells—elastin, etc. Once the presence of this transmural pattern was seen from the SEM fracture preparations and from the semithin plastic embedded sections, it was readily discerned in differentially stained 7-μm paraffin sections (Figure 6), despite the overlap of structures, providing the samples were suitably oriented and obtained from pressure-fixed aortas.

**Distribution and Orientation of Musculo-Elastic Fascicles**

Fascicles tended to be relatively long and uniformly oriented where the aortic wall was straight, shorter, and more varied in orientation near bends or branch angles. Regardless of species or location, fascicles of the aortic media were usually one or two cells thick in their radial dimension (i.e., in the radial direction across the vessel wall) (Figures 3 and 6).

The *width* of cell fascicles, i.e., their size in the direction perpendicular to their long dimension (in the longitudinal or axial direction in straight portions of the vessel) varied considerably depending on the vessel diameter and location (Figures 3 and 5). In the rabbit aorta, for example, sequential step-sections revealed that fascicles were only five or six cells wide (i.e., approximately 100–200 μm in total width), while many of the groups in the straight portion of the thoracic pig aorta proved to be 1 to 3 mm in width when followed in sequential sections.

The *length* of a fascicle in the direction of the long axes of its cells and elastic fibers (i.e., mainly in the circumferential direction in straight portions of the aorta) was usually impossible to estimate from single sections, because fascicles were frequently transected slightly obliquely on transverse sections and curved out of the plane of section, particularly in the ascending aorta and at other sites of changing configuration. In the relatively small and uniform descending rat thoracic aorta and in the comparatively small aortas of young rabbits, fascicles that lay entirely within a single transverse section frequently occupied the entire circumference. In the larger aortas of adult rabbits, however, and in those of young and mature pigs, repeated series of step-sections revealed that fascicles occupied only a segment of the aortic circumference and attained maximum estimated lengths of 4 to 5 mm.

In all three species, aortic fascicles deviated from a predominantly circumferential toward a more axial orientation near the adventitia, particularly in pigs and adult rabbits. Fascicles on the luminal side, particularly in the ascending aorta, also tended toward axial orientation (Figure 5). Where bends occurred, at the arch of the adult pig aorta for example, fascicles appeared to criss-cross or twist over one another. Fascicle orientation also changed consistently in relation to modifications of vessel configuration about the ostia. Small, but complete, rings of cells forming circumferential fascicles were found at the
Figure 6. Light microscopic paraffin-embedded (7 μm) sections of adult pig aorta. Gomori trichrome-aldehyde fuchsin stain. A. On a well-oriented transverse section of a normally distended aorta, red medial cell layers appear to be bracketed on either side by relatively straight purple elastic fibers transected parallel to their long dimensions. Intervening wavy, green collagen fiber bundles are easily seen, despite considerable overlap of structures in the thick sections. B. A well-oriented longitudinal (axial) section of the same vessel shows the juxtaposed cell groups that form the cell layers. Purple elastic fibers are transected in their narrowest dimension, and green collagen fiber bundles are prominent between fascicles. Bar = 15 μm.

Development of Musculo-Elastic Fascicles during Growth

Layering of cells was distinct in the aortic media at birth (Figure 8) and remained a prominent feature of aortic organization throughout growth. Concentric layers of cells were progressively augmented, but

Figure 7. Descending thoracic aorta of an adult rabbit. A. A longitudinal semithin section passes through the center of the ostium of an intercostal artery. Three fascicles of medial cells that form the transition from aorta to branch are labeled B1, B2, B3. The adjacent aortic medial fascicles are marked A. B. A diagrammatic reconstruction shows the plane of transection evident in the histological section and the corresponding encircling fascicles at the transition between intercostal branch and aorta. Bar = 20 μm.
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Figure 8. Transverse section of a fetal rabbit aorta. Cell layers are quite distinct despite the paucity of elastin at this stage of development. Due to the relative lack of matrix fibers, gentle manipulation causes separations (arrows) between layers, emphasizing the continuity of the cell layers. Cells tend to be narrower and layers more compressed at the adventitial side of the media (MA). Mitoses (circles) are easily identified at this stage. L = lumen. Bar = 25 μm.

not replaced, by the deposition of distinctly aligned collagen and elastin structures on either side of each layer. The central position of the cell layers in the developing fascicular architecture of the media was evident in aortas of immature animals, and the progressive organization of the forming elastic fibers about the cell groups and layers could be reconstructed from the complementary planes of section and fracture. Earlier findings that the amorphous component of aortic elastin accumulates at foci of microfibril aggregation between cell layers were confirmed, but fixation in distention revealed that the niduses of amorphous elastin were interconnected by microfibrillar skeins alongside both the luminal and abluminal surfaces of each cell layer, and that both the rows of elastin niduses and their interconnecting microfibrils were aligned with the cells. Elastogenesis therefore usually appeared as two separate rows between successive cell layer groupings across the media (Figure 9). As cells became progressively elongated and fusiform during growth, adjacent elastin niduses coalesced along the microfibrillar skeins to form continuous elastic fibers, and the parallel orientation of developing elastic fibers and immediately associated cell groups within cell layers became increasingly evident. Where foci of elastogenesis were immediately apposed to a cell surface, the cell membrane and the immediately adjacent cytoplasm showed increased electron density and the previously described cell surface attachment ridges became increasingly prominent.

Medial Cell Layers and Musculo-Elastic Fascicles In Branch Arteries

The media of the distended major branch arteries were not as clearly stratified as those of aortas. Despite the relative paucity of connective tissue fibers, however, fibrocellular groups, similar to the fascicles comprising the aortic layers, could be discerned. These could be identified by the same criteria used to distinguish aortic fascicles, i.e., common orientation of their component cells and of their immediately associated elastic fibers (Figure 10). The elastic fiber systems were composed of narrower and more widely spaced elements than in the aorta. The interstices among cells within fascicles also tended to be somewhat wider than in the aorta and appeared to contain small, isolated collagen bundles as well as scattered small elastic fibers. Both elastic fibers and wavy collagen fiber bundles between adjacent fascicles were consistently more prominent than were elastic fibers or collagen fibers between cells within the fascicles. Since the prominent elastic fibers were associated with individual fascicles, as in the aorta, elastic fibers between adjacent fascicles often appeared as double layers (Figure 10). The fascicles in the media of branch arteries were usually thicker in their radial dimension that those of the aorta. Many were three to five cells thick. As in the aortic media, their size, distribution, and orientation appeared to differ in relation to vessel size, curvature and branching sites. For any particular homologous vessel, such as the intercostal or renal arteries of the three species examined, the greater the vessel diameter, the more prominent were the elastic fiber systems related to the muscle fascicles.

Discussion

The microarchitecture of the mammalian aortic media and of the media of branch arteries that is most consistent with our observations is depicted in Figure 11. The layers of the arterial media are actually composites of relatively discrete subgroups or fascicles of commonly aligned, elongated smooth muscle cells. Each group is reinforced and demarcated

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Figure 9. Formation of elastic fibers in descending thoracic aorta of rabbit. 

A. Oblique plane of fracture through the descending thoracic aorta of a 7-day-old rabbit. Islands of elastogenesis (arrowheads) appear adjacent to a layer of transected cells (C). Connecting microfibrils (arrows) are difficult to demonstrate in scanning electron microscopic fracture preparations due to their fragility and to the thickness of the metal coating. Bar = 0.2 μm. 

B. In a newborn rabbit, a longitudinal semithin section reveals islands of elastogenesis (E) close to the surface of a medial cell (C). The microfibrils (arrows) are seen around the islands but not between the successive islands in this plane of section. Bar = 0.8 μm. 

C. Transverse section of the material in B reveals the usual separate elastin systems (E1, E2) developing between adjacent cell layers (C1, C2). This tissue has been processed with ruthenium red to increase the electron density of the microfibrils (arrows). Bar = 1 μm.
Figure 10. Musculo-elastic fascicles in muscular arteries. A. Renal artery of adult pig in transverse semithin section. Elastic fibers are seen as septa, often in two layers (white arrows) between adjacent fascicles. The position of collagen bundles is indicated by black arrows. Most of the fascicles are oriented circumferentially, but there are longitudinally oriented fascicles (MFL) in both the inner and outer media. L = lumen. Bar = 7 μm. B. Transmission electron micrograph of adult rabbit coronary artery in transverse section. Individual fascicles may be difficult to recognize in small transmission electron microscopic fields, but two fascicles (MFa and MFb) can be distinguished in this section by the different orientation of their component cells, the investment by basal lamina bridging between and among cells within each fascicle (circles), and the greater abundance of elastin fibers outside of the fascicles (arrows) than within fascicles. The fascicle (MFa) near the internal elastic lamina is oriented obliquely and the cells therefore show irregular profiles on this transverse section. The deeper fascicle is oriented more circumferentially and the cells have more regular profiles. Bar = 0.8 μm.
by a surrounding array of elastic fibers, oriented in the same direction as the component cells of the associated cell group. When the aortas are fully distented, this configuration results, on carefully oriented transverse sections, in a more or less orderly transmural appearance of smooth muscle bands or layers, each bracketed on both its luminal and abluminal sides by straight elastic fibers. Compact, crimped collagen bundles, distinct from the interlaced fine collagen fibers of the immediate pericellular matrix, are located mainly between the facing elastic fiber systems of the fibrous regions between successive aortic layers and between adjacent fascicles in branch arteries. The impression from the usual paraffin-embedded light microscopic preparations of undistended aortas that the media consists of a succession of wavy, coaxial, elastic lamellae alternating with rows of obliquely or radially oriented smooth muscle cells corresponds to deformations introduced by the recoil of the elastic fibers and by the juxtaposition and overlap of the facing elastic fiber systems of adjacent layers of fascicles. The deviations from circumferential orientation of smooth muscle cells in our material were usually associated with bends and branches and also occurred near the intimal and adventitial sides of thick medias, but cells regularly lay with their long dimensions parallel to tangential planes of section. In studies of intraoperative biopsies of the human ascending thoracic aorta, Dingemans et al. suggested that the aortic microarchitecture of humans differs from that of other mammals. We found all of the features described in the present communication in normal human aortas at all levels of sampling when these were studied in suitably oriented sections of distended vessels.

We have referred to the smooth muscle cell group and its intimately associated elastic fiber system as a musculo-elastic fascicle (MEF), with the implication that this complex is the organizational and functional structural unit of the arterial media. Although earlier observers studying teased and specially stained light microscopic preparations described some of the features of aortic matrix fiber organization presented here, our observations of medias of normally distended vessels tend to emphasize the central structural role of the cell groups rather than the fibers. Since the elastic fibers between the aortic medial cell layers actually consist of two closely apposed systems, utilization of elastin lamellae for counting aortic transmedial structural units may lead to errors. Previously demonstrated close positive correlations among total medial tangential tension, depth of penetration of vasa vasorum, and the number of transmedial aortic layers remain valid, but the muscle lay-

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**Figure 10 (continued).** C. Scanning electron microscopic view of adult pig renal artery fractured in a longitudinal plane shows transected smooth muscle cells (C) in fascicles delineated by large intervening collagen fiber bundles (F) and elastic fibers (E). Occasional artifactual fissures (X) tend to occur between fascicles. Within fascicles, finer collagen fibrillar mats (arrows) surround individual cells. Bar = 0.8 μm.
Figure 11. Representation of the organization of cells and matrix fibers in mammalian aortas (Panel A) and muscular arteries (Panel B). C indicates the transverse (circumferential) plane of section, while L indicates the longitudinal or axial plane. Cell surface projections and other details of cell-to-elastin attachments have been omitted for clarity. Each organizational subunit (musculo-elastic fascicle) consists of a group of commonly oriented cells (Ce), invested by a matrix mat (M) consisting of basal lamina and a fine meshwork of collagen fibrils, and surrounded by a closely associated system of elastic fibers (E) oriented in the same direction as the long axes of the component cells. Wavy collagen bundles (F) course between the successive facing elastic fiber systems within the fibrous zones. In the aorta (Panel A) the fascicles form well defined layers in transverse planes of section. The close proximity of the individual musculo-elastic fascicles within each cell layer gives the impression of extensive, continuous elastic lamellae between cell layers. In muscular arteries (Panel B), layering is less well defined because of the paucity of matrix fibers between fascicles.
ers or their component fascicles, rather than the intervening elastin lamellae should prove to be a more accurate indexing feature, especially for curved portions of arteries and about branch sites.

The MEFs and their corresponding cell layers appeared to be most uniform in size, composition, and orientation in straight portions of arteries and least uniform, smallest, and most numerous in regions where contour changes were greatest, i.e., where vessels curved, bifurcated, or branched. Layering and branching of elastic fibers about artery ostia have been considered by several investigators to correspond to local patterns of stress distribution. Our observations tend to support these contentions with the added qualification that the focal modifications in elastic fiber organization actually reflect changes in alignment of the corresponding MEFs.

If our suggestion that MEFs are aligned in the direction of tensile stress is correct, the deviation from circumferential orientation of cells in the intima and of fascicles in the inner and outer media may reflect a significant axial component of tensile stress in these regions. Axial shear stress could be transmitted to the intima and inner media by deforming forces associated with blood flow, and to the outer media and adventitia by organ movement and by stresses associated with tethering by adventitia and branches. These stresses are probably dampened rapidly as they are transmitted across the media, so that the resultant stress is mainly circumferential, in keeping with the usual predominantly circumferential orientation of MEFs within the aortic media. The observation that aortic strips generate maximum force when cut perpendicular to the vessel long axis is consistent with this interpretation. The persistence of a wavy or crimped configuration of the collagen bundles in medias of fully distended aortas supports suggestions that the stiffness contributed by such fibers is probably due to resistance to deformation of these corrugations.

Postnatal formation of MEFs appeared to result from the segregation of cells into functional subgroups as they elaborate elastic fibers, presumably in response to increasing mechanical stress. In keeping with this impression is the finding that the media is sharply demarcated from the adventitia by the time of birth and that the widening of the aortic media at birth appears to be due to widening of already present layers. Furthermore, the rate of medial cell proliferation during early postnatal growth, at least in the ascending aorta and pulmonary trunk, has been shown to be independent of circumferential stress, while differences in matrix fiber production per cell in each of these vessels correlate positively with differences in the rate of increase in mean circumferential tension. A relationship between matrix synthesis and mechanical stress is also supported by morphologic studies of elastogenesis in relation to pulsation and by the demonstration that cyclic stretching of aortic smooth muscle cells in vitro increases the rate of matrix protein synthesis. The adaptive role of these biosynthetic responses is indicated by data which suggest changes in the composition and organization of the rat aortic media, and human vessels during growth correspond to changes in mechanical properties which tend to reduce or dampen tensile stimulation of the cells. It is therefore likely that the appropriate response of the media to increases and/or redistributions of medial stress depends on the capacity of medial cells to proliferate and/or to increase their rates of extracellular matrix fiber production. Segregation into MEFs provides an efficient structural mode for this adaptation, for it allows for the formation of fibrocellular groups of different size and composition in relation to the point by point distribution of stresses.

Abnormal increases in medial tension, changes with age in matrix fiber distribution and cell response, hormonal and metabolic status, elevations of blood lipids, the presence of intimal lesions, and compromise of medial nutrition may all be significant modifiers of medial biosynthetic and morphogenetic responses. It may also be possible that some cell groupings in atherosclerotic plaques are instances of modified fascicle formation, for local smooth muscle cell proliferation accompanied by matrix fiber accumulation is an important feature of atherogenesis, and individual atherosclerotic plaques may contain subgroups of cells, distinguishable by a common isoenzyme content. Characterization of the distribution, size, and composition of MEFs in normal and diseased vessels could provide insights into mechanical and metabolic factors that may modulate the morphogenesis of atherosclerotic plaques or contribute to the pathogenesis of medial sclerosis and aneurysm formation.

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