A Lamellar Unit of Aortic Medial Structure and Function in Mammals

By Harvey Wolinsky, M.D., and Seymour Glagov, M.D.

ABSTRACT
The close association of elastin, collagen, and smooth muscle in the mammalian aortic media results in viscoelastic properties that account for many of its static and dynamic mechanical features. The structural components of the media are precisely oriented in concentric layers, or lamellar units, of fairly uniform composition. A comparative study of the adult thoracic aorta in 10 mammalian species, including 15 canine breeds, showed that the number of lamellar units in the media of adult mammalian aortas is very nearly proportional to aortic radius regardless of species or variations in measured wall thickness. Estimated wall tensions ranged from 7,820 dynes/cm in a 28-g mouse to 203,000 dynes/cm in a 200,000-g sow, but the average tension per lamellar unit of an aortic media was remarkably constant regardless of species, ranging from 1,090 to 3,010 dynes/cm. The findings suggest that the elastin lamella and the contents of its adjacent interlamellar zone represent the unit of structure and function of the mammalian aortic wall.

ADDITIONAL KEY WORDS structure-function elastin collagen wall tension comparison of 10 species

The mammalian aortic wall contains smooth muscle cells and the fibrous proteins collagen and elastin. Smooth muscle proteins account for about 20% of the dry weight of the media; collagen and elastin together account for about 60% (1, 2). There is usually more elastin than collagen in the thoracic aortic segment and more collagen than elastin in the abdominal segment. However, the total fibrous protein concentration is nearly the same for both segments and is remarkably constant for adult mammalian aortas, regardless of species (1, 2). At physiological distending pressures, aortic elastin and collagen fibers and smooth muscle cells are precisely oriented and form well defined layers (3). Relatively thick elastin bands are seen as concentric plates or lamellae; finer elastin fibers form networks between lamellae. Collagen fibers are dispersed in the interstices and aligned circumferentially. Although the fine elastin fibers connect with elastin lamellae, no attachments between collagen and elastin fibers are demonstrable. Smooth muscle cells extend circumferentially between adjacent elastin lamellae among the finer elastin and collagen fibers. The close association of elastin, collagen, and smooth muscle in the aortic media results in viscoelastic properties that account for many of its static and dynamic mechanical features (4-7). However, the functional significance of segmental differences in aortic wall composition is not understood, nor is there any satisfactory explanation for the orderly uniform lamellar disposition of the structural components.

We undertook a comparative study of the adult mammalian thoracic aortic wall in an attempt to investigate relations between aortic diameter, medial thickness, medial architecture and vessel wall tension. Thoracic
aortas of 10 mammalian species including several different canine breeds were analyzed. The findings suggest that an elastin lamella and the contents of its adjacent interlamellar zone represent the unit of structure and function of the mammalian aortic media. The relation between the total number of these lamellar units in the media of an aorta and the aortic radius is very nearly proportional; the tension per aortic lamellar unit is nearly constant, regardless of animal weight, aortic diameter or medial thickness.

**Materials and Methods**

Aortas of 48 mammals, representing 10 species, were examined. Only animals whose body weight fell within the ranges commonly accepted as normal for adults were used (8). The species studied are listed in order of increasing weight in Table 1. The smallest animal was a mouse weighing 28 g and the largest a sow weighing approximately 200,000 g. Mice, rats, rabbits, guinea pigs, cats and dogs were anesthetized before removal of aortic segments. The vessels of the sheep and swine were obtained within 30 min after slaughter. Human aortas were removed from the bodies of patients who had died less than 6 hours before autopsy.

A segment of thoracic aorta was removed after intercostal artery branches had been ligated near their origins and the adventitial surface had been marked in situ by dots of gentian violet solution placed at 0.5-cm intervals. The center of each excised specimen was the midpoint between the left subclavian and celiac arteries. A cannula of suitable diameter was fitted and tied to each end of the excised vessel segment. Cannulas were held in a previously described adjustable fixing frame (3). The proximal cannula was attached to a closed pressure system; the distal cannula could be closed or opened by means of an easily manipulated clamp. Using the adventitial markings as reference points, the in situ length was restored by extension of the adjustable frame while the vessel was perfused under pressure with normal saline solution at 37°C. A warm solution containing 50% barium sulfate and 5% gelatin was then substituted and allowed to flow until the saline solution was washed out. The distal clamp was then closed and the vessel segment distended by the gelatin-barium sulfate mixture under constant pressure. After 3 min, the frame with the distended vessel segment was plunged into 10% buffered formalin at 2 to 4°C. The time lapse between exposure of the thoracic aorta of anesthetized animals and immersion of the distended segment in chilled formalin was 10 to 15 min. The mixture used for distending the vessel gelled in about 2 min. The cannulas and the attached vessel segment were transferred to a specific plastic frame that maintained the in vivo length of the vessel during subsequent processing.

A single pressure was used to distend the aortic segments of all individuals of a given species. For this purpose, mean blood pressure values

| TABLE 1
Range of Body Weights and Distending Pressures in 10 Mammalian Species |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Species</td>
<td>No. animals</td>
<td>Weight range (g)</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>28-30</td>
</tr>
<tr>
<td>Rat</td>
<td>3</td>
<td>400-450</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>3</td>
<td>960-1420</td>
</tr>
<tr>
<td>Rabbit</td>
<td>8</td>
<td>2925-3475</td>
</tr>
<tr>
<td>Cat</td>
<td>3</td>
<td>3100-3400</td>
</tr>
<tr>
<td>Monkey</td>
<td>3</td>
<td>3300-4150</td>
</tr>
<tr>
<td>Dog</td>
<td>15</td>
<td>5500-28,800</td>
</tr>
<tr>
<td>Human</td>
<td>4</td>
<td>52,000-76,000</td>
</tr>
<tr>
<td>Sheep</td>
<td>2</td>
<td>83,000-91,000</td>
</tr>
<tr>
<td>Pig</td>
<td>4</td>
<td>Approx. 200,000</td>
</tr>
</tbody>
</table>
for each species were obtained from several sources and averaged. These included mean physiological pressures reported by other investigators (8, 9), determinations made previously in our laboratories on rats (10), rabbits (11, 12), and sheep and on all of the dogs and cats used in the present experiments. Dogs and cats were anesthetized by intravenous injection of pentobarbital (Nembutal) in doses of 30 mg/kg body weight.

Roentgenograms of the distended aortic segments were made after 48 hours of fixation in formalin. The plastic frame holding the cannula and artery was mounted in a support fitted with a rotating bar. A short fine metal wire was placed on the adventitia at the previously marked dot indicating the midpoint of the segment. Four different radiographic projections were taken of each vessel by rotating the vessel 45° between successive exposures using a nonscreen technique. The distance between specimen and high contrast film (Kodak-Royal blue; medical X-Ray type) was 2.5 cm and the distance between tube and specimen 100.0 cm; under these conditions the projection of the intraluminal barium column was extremely sharp. Vessel lumen diameters were measured at the central reference mark on the x-ray films; the known inside diameter of the shafts of the cannulas was used to calculate a correction factor for projection distance.

Transverse sections of each vessel segment were made at the reference point, and blocks were embedded in paraffin for histologic study. Care was taken to keep the gelatin core in place throughout embedding and sectioning procedures. Sections 7 µ thick were stained with hematoxylin-eosin and Weigert-Van Gieson stains. Configuration of the components of the wall was studied on several adjacent sections of each vessel. Medial thickness, considered to be the perpendicular distance between the innermost and outermost medial elastin lamellae, was measured on microscopic sections by means of an eyepiece micrometer.

Measurements were made at four positions, the first randomly chosen and the others at approximately 45°, 90°, and 135° around the vessel perimeter; the average of the four measurements was taken as medial thickness. The number of medial elastin lamellae at each of the measurements was counted; the average of the four counts was considered to be the total number of medial elastin lamellae.

Three aortas, one from a guinea pig, one from a dog, and a third from a man were x-rayed at three different stages of the procedure in order to estimate shrinkage resulting from fixation and subsequent processing. These distended segments were first chilled in cold saline rather than formalin and x-rayed. Then, after 48 hours in cold formalin, they were x-rayed again. A third exposure was made of the paraffin-embedded segment in its tissue block. Diameters before fixation, after fixation, after embedding, and after microtomy and staining were compared. Fixation reduced the diameter an average of 14%; paraffin embedding and histologic processing resulted in a further reduction of about 16%. In some of the dogs comparisons could be made between in vivo aortograms made for other purposes and measurements from histologic sections. Since the dimensions before fixation corresponded most closely to in vivo dimensions, diameters measured from fixed specimens and medial thicknesses measured from histologic sections were corrected to correspond to probable unfixed values.

Total tangential wall tension, T, in dynes/cm, was calculated from the expression T = Pr (Law of Laplace), where P is the distending pressure in dynes/cm² and r the vessel radius in cm. Average tension per lamellar unit in dynes/cm was calculated for each specimen by dividing the total tensions by the number of medial elastin lamellae.

Results

Relation of Aortic Diameter to Body Weight. Aortic diameter increased with body weight from approximately 1.2 mm in the mouse to 23.0 mm in the pig. Diameters are plotted against adult body weight in Figure 1. The curve of best fit consists of two nearly linear components of very different slope. For relatively small mammals, weighing 20,000 g or less, aortic diameter increases rapidly with increasing body weight; a difference of 1,000 g in body weight corresponds to a 0.50 mm difference in diameter. Aortic diameters of animals weighing more than 50,000 g increase much less rapidly with increased body weight, being only 0.04 mm greater for each 1,000 g. Large dogs weighing approximately 25,000 g are represented by points on the inflected portion of the curve; however, points corresponding to dogs of low adult weight lie near points corresponding to other species of comparable adult weight.

Relation of Medial Thickness to Body Weight. Aortic medial thickness ranged from 0.03 mm in a mouse to 1.12 mm in a human. Thickness is plotted against body weight in three different stages of the procedure in order to estimate shrinkage resulting from fixation and

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Abbott Laboratories, Veterinary Nembutal, 60 mg/ml.

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Diameter of the thoracic aorta in 10 mammalian species. Diameter increases more rapidly with adult body weight for small mammals than for large.

Figure 2. The points again fall into two main groups. Aortic walls of mammals weighing 20,000 g or less were 0.05 mm thicker per 1,000 g additional body weight. However, little or no increase in thickness occurs with increasing body weight in animals heavier than 50,000 g. The points representing canine aortas are located along the upper half of the linear steep portion of the curve; medial thickness corresponded to adult body weight for the various dog breeds.

Relation of Medial Thickness to Aortic Diameter. Medial thickness was very nearly proportional to aortic diameter (Fig. 3). Wall thickness was approximately 0.05 mm greater for each 1.0 mm increase in aortic diameter, regardless of species or adult body weight.

The Lamellar Unit of Aortic Structure. The medial lamellar arrangement of elastin and smooth muscle is easily observed on sections made from pieces of aorta fixed without taking precautions to conserve or restore in vivo aortic length or diameter. However, under such conditions, the striking uniformity of the lamellar pattern and the orientation of the interlamellar components are obscured. Fixation during distention with restoration of length resulted in considerable straightening of the elastin lamellae and the delineation of concentric interlamellar zones of relatively uniform thickness and composition. For each specimen, the distance between elastin lamellae was fairly uniform along the circumference and throughout the depth of the media (Fig. 4A). The average interlamellar distance at the central reference point of the descending thoracic aortic segment in the adult mammals examined was 0.015 mm; values

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1.0
0.8
0.6
0.4
0.2
0

WEIGHT (kilograms)
0 20 40 60 80 100 120 140 160 180 200

WALL THICKNESS (mm)

 MOUSE
 RAT
 GUINEA PIG
 CAT
 RABBIT
 MONKEY
 DOG
 HUMAN
 SHEEP
 PIG

FIGURE 2

Thickness of the media of the thoracic aorta in 10 mammalian species. Medial thickness increases rapidly with adult body weight in the relatively small mammals; there is little or no increase with body weight in the large mammals.

ranged from 0.006 in the mouse to 0.018 in man. Within the interlamellar zones were fine elastin fibers making up the interlamellar elastin net and circumferentially arranged smooth muscle cells. Generally, a single layer of cells lay between and nearly parallel to adjacent elastin lamellae (Fig. 4B). Collagen fibers were uniformly dispersed about the smooth muscle cells and the fine elastin fibers.

An aortic media may therefore be considered to be composed of concentric layers of fairly uniform composition clearly bounded by thick elastin lamellae. An elastin lamella and the oriented elements contained in the immediately adjacent interlamellar zone may be called a lamellar unit.

Relation of the Total Number of Medial Lamellar Units to Body Weight, Aortic Diameter, and Aortic Wall Thickness. The number of concentric lamellar units in an aortic media was easily determined on microscopic sections of vessels fixed while distended. The smallest aorta, that of an adult mouse, had 5 medial lamellar units; the largest, that of the sow, had 72. The relation of the number of medial lamellar units to adult body weight is shown in Figure 5. The fitted curve resembles the one which relates aortic diameter to body weight (Fig. 1). For small mammals, up to approximately 20,000 g body weight, the total number of lamellar units increases rapidly with increased weight or diameter; about 4 units are added for each 1,000 g of body weight or 0.50 mm in diameter. For large animals, differences in adult weight correspond to much smaller differences in number of lamellar units, only 0.30 units being added for each 1,000 g of body weight or
each 0.04 mm in diameter. Thus, the total number of medial lamellar units is very nearly proportional to aortic diameter regardless of species body weight; aortic diameter is plotted against total number of medial lamellar units in Figure 6. The distribution of points corresponding to dogs of various adult weights along a large segment of the curve lends further support to this observation. Medial thickness is plotted against total number of medial lamellar units in Figure 7. The relation is nearly linear, but plotted points appear to show somewhat greater scatter than those relating aortic diameter to lamellar units, particularly for large species. Micrometric comparisons of the components of the aortic media in many mammalian species indicates that departures from a linear relation are associated with differences in both width and composition of the lamellar units in aortas of large animals. These findings form the subject of another report and will be presented elsewhere.

Relation of Aortic Mural Tension to Medial Architecture. Calculated total mural tension and tension per lamellar unit at physiological mean pressure are plotted against body weight in Figure 8. The total mural tension increased markedly with body weight and aortic diameter, ranging from 7,820 dynes/cm in the mouse to 203,000 dynes/cm in the pig (Fig. 8, continuous line). Total tension in the aortic walls of small animals increased about 5,000 dynes/cm for each 1,000 g increase in body weight; for large animals tension changed only 500 dynes/cm for each 1,000 g. Compared to the rise in total mural tension with adult weight and aortic diameter, the average tension per lamellar unit of aortic media was remarkably independent of species
Microscopic appearance of the media of the thoracic aorta after fixation during distention by physiological intraluminal pressure. Lamellar units consist of relatively straight elastic bands and intervening fine elastin fibers, collagen fibers, and smooth muscle cells. In A, elastic fibers appear black in a photograph of a section stained by the Weigert-Van Gieson method. In B, cell nuclei appear black and elastin fibers appear white in a photograph of a section stained with hematoxylin-cosin.

Comparison of the Ranges of Body Weight, Aortic Diameter, Medial Thickness and Tension per Lamellar Unit. The ranges of body weight, aortic diameter, medial thickness, total number of medial lamellar units, total medial tension and tension per lamellar unit are presented in Table 2. Minimum and maximum values are the smallest and largest values obtained for each of the variables; the range is also expressed as a ratio of the maximum to the minimum values. Tension per lamellar unit showed the smallest range, with a maximum-to-minimum ratio of only 2.75 in mammals whose body weights differed by a factor of 7,000. Aortic wall thicknesses differed by a factor of 43, diameters by 20,
TABLE 2

Range of Values in 10 Mammalian Species

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Ratio (maximum/minimum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>28</td>
<td>200,000</td>
<td>7,000</td>
</tr>
<tr>
<td>Aortic diameter (mm)</td>
<td>1.2</td>
<td>23.5</td>
<td>20</td>
</tr>
<tr>
<td>Medial thickness (mm)</td>
<td>0.027</td>
<td>1.12</td>
<td>43</td>
</tr>
<tr>
<td>Number of lamellar units</td>
<td>5</td>
<td>72</td>
<td>14.5</td>
</tr>
<tr>
<td>Medial tension (dynes/cm)</td>
<td>7,820</td>
<td>203,000</td>
<td>25.9</td>
</tr>
<tr>
<td>Tension/lamellar unit (dynes/cm)</td>
<td>1,090</td>
<td>3,010</td>
<td>2.75</td>
</tr>
</tbody>
</table>

FIGURE 5

Number of lamellar units in the media of the thoracic aorta in 10 mammalian species. The number of lamellar units increases with adult body weight less rapidly for large animals than for small.

Discussion

The elastin and collagen content of the media of the mammalian thoracic aorta is nearly constant, regardless of species (1, 2) or wall thickness, and the medial structural components are precisely oriented in concentric layers, or lamellar units, of fairly uni-
Relation between aortic diameter and the number of medial lamellar units. The number of units is very nearly proportional to aortic diameter.

Form composition. Species differences in medial tangential tension are due primarily to differences in aortic radius, for species variation in mean arterial blood pressure is quite small compared with the range of aortic radii. Among the mammals that formed the basis of the present study, adult body weights ranged from 28 to 200,000 g, aortic radii ranged from 0.06 to 1.20 cm, and mean arterial pressure ranged only from 75 to 130 mm Hg. Although aortic radii of small mammals were much larger for a given increase in adult body weight than were those of relatively large animals, the number of lamellar units in an aortic wall was very nearly proportional to aortic radius regardless of species or medial thickness. Total wall tension ranged from 7,820 to 203,000 dynes/cm, but the tension per lamellar unit was remarkably constant by comparison, ranging between 1,090 and 3,010 dynes/cm.

These findings suggest that in mammalian species, selective adaptation to increased aortic radius and therefore to increased medial tension has been accomplished mainly by the addition of well-defined structural layers of fairly uniform composition. Each layer, bounded by relatively thick elastin bands contains circumferentially oriented collagen fibers, a nearly circumferential network of fine elastin fibers, and a layer of smooth muscle.

Elastin lamellae have been enumerated in several mammalian species by other investi-
Relation between aortic medial thickness and the number of medial lamellar units. Though the relation is nearly linear, the points representing large animals show greater scatter than corresponding points in Figure 6.

gators (13-18); their figures are in accord with the data presented in this report. Corresponding measurements of aortic diameter or medial thickness on distended specimens have not been made previously. Discussions of the significance of the lamellar structure of the aorta are few. Muller (19) analyzed a theoretical model of a bovine aorta consisting of 10 coaxial thin-walled rubber tubes with fluid between them. He concluded that a series of coaxial tubes with intervening fluid would have a lower modulus of elasticity than a homogeneous rubber wall, resulting in relatively small changes in pressure per unit change in volume for a wall of given thickness.

Karrer (20) has noted that new elastin lamellae are formed at the adventitial boundary of the aortic media in the chick embryo; this is presumably the zone of maximum tension gradient. The media of the mammalian main pulmonary artery is distinctly lamellar at birth, resembling the aorta, but is replaced by a less organized musculofibrillar arrangement after intraluminal pressures fall during growth and maturation. If, however, systemic pressures are maintained in the pulmonary circulation after birth, the distinct lamellar structure persists (21). These observations lend support to the concept that lamellar structure is related to medial tension and suggest that more detailed analysis of aortic medial differentiation during fetal and postnatal periods, when wall tension augments rapidly because of increasing blood pressure.
and aortic diameter, could provide insight into the physical factors governing lamellar formation.

Peterson and his co-workers (22, 23) have argued that the thickness of the aortic wall should not be ignored in estimating its modulus of elasticity and that tension per unit of wall thickness is physiologically more meaningful than total wall tension. They have, therefore, included the ratio of aortic radius to wall thickness in their calculations. They obtained a value of 6.0 for this ratio for the adult canine thoracic aorta; this value was about 10.0 in our material. These authors measured aortic diameter in situ on anesthetized animals and wall thickness on histologic sections prepared from aortic segments distended by a balloon during fixation; diameters thus measured were similar to those we found, but our methods of fixation and measurement, including correction for processing, resulted in smaller values for medial thickness. Wagenvoort (24) stated that in human pulmonary arteries average wall thickness is 4.5% of radius; this is equivalent to a radius-to-wall thickness ratio of 22.0. In pulmonary arteries, the relatively thin walls correspond to wall tensions much smaller than those in the wall of an aorta of comparable radius because of the comparatively low pulmonary artery pressures.

In the present study, the relation between the number of medial lamellar units and medial thickness or aortic radius was nearly linear. However, determinations of medial thickness are subject to greater experimental

FIGURE 8
Aortic medial tension at physiological pressure in 10 mammalian species. Total medial tension increases markedly with body weight or aortic diameter (continuous line) but tension per medial lamellar unit is remarkably constant regardless of species (broken line). The insert is an enlargement of the portion of the graph about the origin.
Aortic medial thickness is plotted against number of medial lamellar units for unfixed, fixed, and embedded specimens.

The uniformity of composition of aortic lamellar units and the relatively constant tension per lamellar unit, regardless of species, indicate that the proportion of collagen, elastin, and smooth muscle in the media are optimal for the stresses to which the aorta is subjected. The intimate association of an elastin network with collagen fibers and of elastin bands with smooth muscle cells results in a wall of relatively high tensile strength and adequate but not excessive distensibility, with uniform distribution of stresses and appropriate viscoelastic responses to pulsatile oscillations. The relative proportions of the structural elements determine the elastic modulus; the smooth muscle is probably a major factor in the viscoelastic response to pressure pulsation; the elastin net distributes stresses throughout the wall. The aortic media has been compared to two-phase materials such as fiberglass (25) and its response to pulsatile stress has been analyzed in terms of viscoelastic models of three or more phases (7, 26). The lamellar unit of structure and function described in this report is consistent with these analyses of the aortic wall.

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References

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