Response of the Rat Aortic Media to Hypertension

MORPHOLOGICAL AND CHEMICAL STUDIES

By Harvey Wolinsky, M.D., Ph.D.

ABSTRACT

Clinical and experimental studies indicate that hypertension accelerates the development of arteriosclerosis. Morphological and chemical studies of the distended rat thoracic aorta were undertaken to define the structural and compositional alterations of the media which accompany hypertension and to relate these changes to increases in calculated medial stress. An 8-week period of hypertension was associated with significantly greater diameter, medial thickness, and cross-sectional area of the media of the thoracic aorta than in normotensive animals. Calculated wall tension was significantly higher in hypertensive animals, but the number of medial lamellar units was not greater than that usual in normotensive animals; this resulted in a strikingly elevated value for calculated tension per medial lamellar unit for the aortas of hypertensive animals. A highly significant linear relation was found between total tension and cross-sectional area of the media of the same segment. In addition, the absolute amounts of both medial elastin and collagen were increased in hypertensive animals; however, the percent of these elements remained essentially constant, indicating little change in the composition of aortic tissue. Increments in both fibrous proteins were linearly related to increases in calculated mural stress, and medial accumulations of elastin and collagen proceeded at similar rates. These findings demonstrate a linear relation among vessel dimensions and amounts of medial elastin and collagen and calculated wall tension.

ADDITIONAL KEY WORDS structure-function relations elastin collagen smooth muscle vascular disease cross-sectional wall area

- Evidence that hypertension is a significant accelerating factor in the development of arteriosclerosis has accumulated both from clinical (1-3) and experimental (4-7) studies. Since the effects of hypertension on cholesterol levels are minor (6), primary attention has been given to the direct metabolic and morphologic consequences of increased stress on the vessel wall itself. It is known, for example, that increased permeability of the intima of large vessels to circulating cells (8, 9), plasma cholesterol and serum albumin (10) under conditions of acute and chronic hypertension leads to their accumulation in the intima and inner media. Comparative studies of hypertensive and normotensive vessels of humans and other animal species have also shown that vessels of hypertensives are generally thicker and stiffer than those of their normotensive counterparts (11-16). Among the factors proposed to be responsible for these changes are increased amounts of collagen and hyperplasia of smooth muscle cells (11, 17-19), changes in cross-linkages of the fibrous proteins (18), smooth muscle hypertrophy (17, 19), altered electrolyte equilibrium (20), and “water logging” of the vessel wall (19, 20).

Earlier studies by others of elastin and collagen content of hypertensive vessels found no striking differences in percent of these
fibrous proteins in the vessels of normotensive and hypertensive humans (21) and dogs (18). The collagen-to-elastin ratio of these vessels, considered to be a useful index of distensibility, was generally slightly lower in hypertension, a paradoxical finding in view of the demonstrated increase in stiffness of these vessels (18). These results seem to indicate that the amounts of collagen and elastin, the two strongest elements of the vessel wall (22), do not change in the presence of hypertension. The present study of the rat thoracic aorta was undertaken to better quantify the structural alterations of the media which accompany hypertension and to determine the absolute amounts of elastin and collagen in anatomically defined, identical segments of the thoracic aorta of normotensive and hypertensive rats. Further, it was intended to ascertain if the morphological and chemical changes found could be quantitatively related to calculated values of wall tension.

**Methods**

Male Carworth (CF-N) rats of three different ranges of body weight and age were used. Mean initial body weights of the groups are shown in Table 1; corresponding approximate initial ages are 6 weeks for group III, 9 weeks for group II and 18 weeks for group I. All animals were housed two per cage and were given laboratory rat chow ad libitum.

Hypertension was produced with a subcutaneous deoxycorticosterone acetate pellet and 1% saline drinking water followed in 1 week by clipping of the left renal artery with silver clips of the following apertures: group III, 0.20 mm; group II and group I, 0.25 mm. Systolic hypertension of greater than 150 mm Hg generally ensued 1 week after clipping; it has previously been established that these methods also result in diastolic hypertension. Pressures invariably rose for 1 to 2 weeks, but stabilized by the thirdweek at levels which were maintained for the duration of the experiment. All treated animals were given 1% saline drinking water; control animals of each group were given ordinary drinking water.

Each group consisted of 12 animals; 8 were used for the morphological studies and 4 for the chemical studies. The systolic blood pressure of each animal was taken prior to any treatment and weekly thereafter using a tail-cuff and amplifier with the animal under light ether anesthesia. Systolic hypertension of greater than 150 mm Hg was documented in all treated animals for 8 weeks, after which all were killed. The hypertensive animals did not gain as much weight as their corresponding controls, so that final body weights were considerably lower in the treated groups (Tables 1 and 2). Additional evidence for the presence of hypertension was obtained after death by calculating the ratio of the heart weight to final body weight. In each group this ratio was significantly increased in hypertensive animals (Tables 1 and 2).

**Morphological**

After killing each animal by exsanguination, the segment of thoracic aorta located between the left subclavian artery and celiac artery was removed after carefully marking in-vivo length, tying intercostal branches, and cannulating both ends. The segment was then transferred to a

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body wt (g)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Heart wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>III C</td>
<td>4</td>
<td>102 ± 10</td>
<td>204 ± 25</td>
<td>115 ± 10</td>
</tr>
<tr>
<td>III H</td>
<td>4</td>
<td>100 ± 10</td>
<td>193 ± 25</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>II C</td>
<td>4</td>
<td>214 ± 15</td>
<td>344 ± 26</td>
<td>113 ± 7</td>
</tr>
<tr>
<td>II H</td>
<td>4</td>
<td>217 ± 18</td>
<td>241 ± 40</td>
<td>109 ± 23</td>
</tr>
<tr>
<td>I C</td>
<td>4</td>
<td>425 ± 18</td>
<td>525 ± 35</td>
<td>114 ± 11</td>
</tr>
<tr>
<td>I H</td>
<td>4</td>
<td>422 ± 25</td>
<td>427 ± 50</td>
<td>100 ± 18</td>
</tr>
</tbody>
</table>

N = Number of animals. C = Normotensive. H = Hypertensive. Values are means ± SD.
TABLE 2
Characteristics of Normotensive and Hypertensive Animals Used for Chemical Studies

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Body wt (g)</th>
<th>Average systolic blood pressure (mm Hg)</th>
<th>Heart wt/body wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
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<td>2</td>
<td>109</td>
<td>291</td>
<td>115</td>
</tr>
<tr>
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<td>105</td>
</tr>
<tr>
<td>III H</td>
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<td>96</td>
<td>206</td>
<td>195</td>
</tr>
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<td></td>
<td>102</td>
<td>180</td>
<td>200</td>
</tr>
<tr>
<td>II C</td>
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<td>437</td>
<td>350</td>
<td>200</td>
</tr>
</tbody>
</table>

N = Number of animals. C = Normotensive. H = Hypertensive.

special frame which held the cannulas so as to permit restoration of in-vivo length. The segment was first flushed with normal saline at 37°C and distended for a moment; the segment was then distended with a perfusion mixture containing gelatin, carbon, and barium sulfate at 40°C. The details of the pressure system and perfusion mixture have been described elsewhere (23). The pressure used to distend the aorta of each animal was the mean systolic blood pressure recorded in that animal over the final 4 weeks of the 8-week period. The average systolic blood pressure of each group of animals is shown in Table 1.

After allowing the segment to stabilize for a moment, the frame with segment still attached to the pressure system was immersed in 10% buffered formalin at 4°C. The perfusion mixture gelled in a moment or two, and the segment was removed from the pressure system without loss of distention. Special holders were then used to hold the segment at the appropriate length during fixation in formalin over the next 48 hours.

X-rays of the segment were then taken to determine the diameter of the segment at its midpoint. This technique has already been described in detail (23). Cross sections of the fixed, still distended, segment were then carefully taken from the midpoint of the segment and prepared for light microscopic examination. Histological sections, 4μ thick, and stained with hematoxylin and eosin and Weigert-van Gieson stains were used for measurements.

Micrometric techniques used to determine wall thickness, internal diameter and medial lamellae have been described elsewhere (23). The aortic media was defined as that portion of the wall which contained complete, concentric elastin lamellae. Cross-sectional area of the media was calculated according to the formula: the area of a ring between two circles of radius r1 and r2 is equal to \( \pi (r_1 + r_2)(r_1 - r_2) \). Tangential tension was calculated according to the law of LaPlace: tension (dynes/cm) = blood pressure (dynes/cm²) \( \times \) radius (cm); wall stress (dynes/cm²) was calculated by the additional step of dividing the total tension by medial thickness (24). Average systolic blood pressures and internal radii of vessels were used in these calculations. Mean arterial blood pressure would have been a better value to use than mean systolic blood pressure, but only the latter value was determined in these studies.

All micrometric measurements were corrected for tissue shrinkage due to fixation and further processing as determined in previous studies (23).

The Student t-test for differences between means was used for significance of results; significance was considered present at the 5% level.

CHEMICAL

After the animals were killed by exsanguination, the thoracic aortic segment exactly delimited by the left subclavian artery and celiac artery was removed from each animal and blotted free of most blood. All segments were then frozen until chemical analyses were performed, a 1-month interval. The segments were then defrosted and taken through the following steps in two series of six aortas, each series including a control and hypertensive specimen from each group.

When defrosted, the aortic segment was opened longitudinally and the media and intima...
FIGURE 1
Microscopic appearance of a cross section of the rat thoracic aortic media and intima (M) after removal of the adventitia (A). The M preparation was analyzed in the chemical studies reported here. Weigert-van Gieson stain.

of the entire segment was removed from the adventitia. Details of this method will be described elsewhere, but the typical microscopic appearance of a rat aorta prepared in this way is shown in Figure 1. The elastic stain of the media and intima shows the same number of medial lamellae as is usually found in the intact rat thoracic aorta (23), indicating that the media is essentially retained in its entirety in the media-intima preparation and is free of adventitia. The fragments of media-intima strips obtained from each aorta were homogenized and then pooled in separate test tubes and washed once with 2 ml of cold normal saline and twice with 2 ml of distilled water at room temperature. The duration of each washing was 10 minutes, followed by centrifugation of the tubes for 5 minutes at 206 × g. The supernatant fluid of these washings was aspirated with great care to prevent loss of any particulate material and was discarded.

Since this study, these saline and water washings of the aortic fragments have been eliminated from the procedure without effect on results. The washed residues were placed in 4 ml of an ethanol-ether mixture (3 parts ethanol to 1 part ether) for 2 hours at room temperature and agitated frequently. After centrifugation for 10 minutes at 206 × g, the supernatant fluid was discarded and 4 ml of anhydrous ether was added to each residue and was agitated frequently over 30 minutes. It was then centrifuged again for 10 minutes at 206 × g and the supernatant fluid discarded; the remaining ether was driven off the residue by immersing the tubes in water at 50°C for several minutes. The tubes containing the residues were then placed in a vacuum oven at 50 to 60°C for 24 hours to dry to constant weight. The dried material was kept in a desiccator until weighing, when the entire residue of each aorta was transferred to a preweighed hydrolysis ampoule; the total initial fat-free dry weight was thus obtained for each aortic segment.

The subsequent preparation of soluble and residue fractions was carried out using the following modification of the method of Lansing (25). 2 ml of 0.1N NaOH were added to each hydrolysis tube; the tubes were then kept in a 95°C water bath for 50 minutes. After cooling, the solution was neutralized with 2 ml of 0.1N HCl. A yellow amorphous insoluble material and a colorless supernatant fluid were apparent. The former is referred to as the residue fraction. The tubes were centrifuged at 755 × g for 15 minutes and the supernatant fluid was saved. The residue was then washed twice with 1 ml of distilled water each time; these washes added to the supernatant fluid constituted the soluble fraction of each aorta, which consisted of approximately 8 ml.

The soluble fraction and the residue were each hydrolyzed in 2 ml of 6N HCl at 105 to 110°C for 48 hours in evacuated, sealed tubes. Amino acid analyses were performed as described by Spackman, Stein, and Moore (26) on a Beckman analyzer. Measured aliquots of the residue and soluble fractions of each aorta were analyzed and the results corrected to the total absolute amounts of each material. The amount of each amino acid, including isodesmosine and desmosine in the case of elastin, and hydroxyproline and hydroxylysine in the case of collagen, was calculated from the chromatogram in the usual manner and the total content of nitrogen in each fraction was calculated from the amount contributed by each amino acid. The amino acid profile of the soluble fraction suggested the presence of collagen.

Ampoules, 10 cc, Owens-Illinois, Inc., Toledo, Ohio.
Model MS, Amino Acid Analyzer, Beckman Instruments, Inc., Palo Alto, California.

Circulation Research, Vol. XXVI, April 1970
“diluted” by other soluble proteins. The actual amounts of collagen and the other soluble proteins were obtained from each chromatogram by calculating a factor which would correct the key amino acids of collagen, including glycine, proline, alanine, hydroxylsine and hydroxyproline, to the typical amino acid content of collagen expressed as number of residues per 1000 residues. Invariably, these manipulations resulted in an amino acid pattern pathognomonic for collagen (27, 28). In this manner, the relative contributions of collagen and other soluble proteins to the total nitrogen of the soluble fraction could be obtained. The total weights of collagen and soluble proteins were computed from their nitrogen contents (collagen, 18.9% nitrogen; soluble proteins, 16% nitrogen).

In every instance, the amino acid pattern obtained from the residue fraction was identical to the known amino acid composition of pure elastin (27, 28). In this case the weight of elastin in the aliquot was computed from the total nitrogen contributions of its constituent amino acids (a nitrogen content of 18.9% was assumed). The weight of elastin and collagen in an entire thoracic aortic segment was then calculated from the values obtained for the known aliquots used for amino acid analyses.

Since vessel segments were used in their entirety for chemical studies, mean values for cross-sectional area of the media and total wall tension obtained for each group in the morphological studies were related to the amounts of elastin and collagen. Correlation coefficients (Pearson’s) were calculated and regression slopes were determined by the method of least squares. Significance levels were the same as those used for the morphological studies.

Results

MORPHOLOGICAL STUDIES

Gross Appearance

When removed from the body, the aortic segments of all hypertensive animals seemed to retract less than those of normotensive animals. In addition, the walls seemed to be thicker and stiffer than normal. Dilation of the hypertensive segments compared to normotensive ones was also noted; this was most prominent in group I and least prominent in group III.

Microscopic Appearance

Figure 2 shows the typical microscopic appearance of a cross section of the thoracic aorta of each of the three groups of normotensive and hypertensive animals. In each of the three groups the greater overall wall thickness of hypertensive segments is obvious, with the differences from normotensive vessels being most marked in group I and least marked in group III. Note also the tendency for the
spaces between adjacent medial elastin lamellae (thick black lines) to be greater in hypertensive vessels; in each of the hypertensive segments a striking increase in fine fibrils with the staining properties of elastin is also seen between the heavier elastin plates.

Vessel diameter at the exact midpoint of each thoracic aortic segment is shown for the three groups in Figure 3. The difference between mean diameter of the control and hypertensive animals is significant in groups II ($P<0.001$) and I ($P<0.001$); that between controls and hypertensives of group III is not ($0.1<P<0.2$). Significant intergroup differences are also present; the mean value for aortic diameter of the heaviest control animals (I, C) is significantly greater ($P<0.01$) than the diameter of the intermediate group (II, C). Due to the scatter of points for the control group of lightest body weight (III, C), a significant difference of the mean value from that of other groups was not achieved ($0.1<P<0.2$). With regard to the hypertensive animals, mean diameter was significantly greater in each successively heavier and older group ($P<0.01$ between groups II and III and $<0.001$ between groups I and II). In summary, the diameter of the normal rat thoracic aorta is significantly greater at age 26 weeks than at age 17 weeks, but is not significantly greater at age 17 weeks than at age 14 weeks. The increase in aortic diameter associated with 8 weeks of previous hypertension was significantly greater than normal at ages 17 and 26 weeks, but not at age 14 weeks and was significantly greater with each increment in age and body weight studied.

Calculated wall tension is largely dependent on values for radius, since differences in blood pressure are not significantly different among control animals of the three groups and among hypertensive animals of the three groups (Table 1). As a result, calculated tension shows approximately the same relations among groups as does diameter. It has been established that a medial lamellar unit, consisting of an elastin lamella and an adjacent interlamellar space, normally corresponds to a relatively constant tension in the adult mammalian aorta, regardless of species, body weight, or segment (23, 29). The total number of medial lamellar units in any aorta, therefore, normally corresponds to calculated wall tension of that vessel. In Figure 4, it is seen that over the range of ages and body weights studied, no significant increase above normal in the number of medial lamellar units of the rat thoracic aorta accompanied hypertension in any group (group I: $0.2<P<0.3$; group II: $0.3<P<0.4$; group III: $0.4<P<0.5$); intergroup differences were also not significant ($P>0.4$).

Calculated total medial tension must be greater than normal in hypertensive animals because of increased blood pressure (Table 1) and greater aortic radius (Fig. 3). Since the total number of medial lamellar units is similar in normotensive and hypertensive aortas, calculated values of tension per lamellar unit are strikingly greater than normal in hypertensive animals ($P<0.001$) (Fig. 5). Control values of approximately 2000
among groups \((P > 0.3)\). In hypertensive animals, calculated tension per lamellar unit ranges from approximately 2 to 2.5 times greater than the corresponding value in normotensive animals and also is significantly greater in groups II and I than in group III \((P < 0.01)\).

In Figure 6, values for medial thickness of the thoracic aortas of the three groups are shown. The medias of the hypertensive segments in each group are significantly thicker than the normotensive ones \((P < 0.05\) for groups II and III, <0.01 for group I). None of the intergroup differences among hypertensive vessels or normotensive vessels were significant \((P > 0.2)\).

Peterson and associates \((24)\) have suggested that the concept of wall stress, which relates calculated total tension to wall thickness is more satisfactory on a theoretical basis when applied to blood vessel walls which have finite thickness. Accordingly, the values for this variable are shown in Figure 7. Wall stress is significantly greater in hypertensive

**Figure 4**

Medial lamellar units of the thoracic aortic media in control (C) and hypertensive (H) animals.

**Figure 5**

Calculated tension per aortic medial lamellar unit in normotensive (C) and hypertensive (H) animals. See text for discussion.

**Figure 6**

Medial thickness of the thoracic aorta in normotensive (C) and hypertensive (H) animals. See text for discussion.

dynes/cm per lamellar unit correspond well to previously obtained data for mammalian aortas \((23)\) and do not differ significantly

\[ \text{Circulation Research, Vol. XXVI, April 1970} \]
vessels than in normotensive vessels of each group \((P < 0.01\) for groups III and II, \(< 0.05\) for group I). Of intergroup differences, only group I versus group II and group I versus group III of normotensive animals are significant \((P < 0.05)\). The intragroup differences are less significant statistically than those found for tension per lamellar unit (Fig. 5). This would be expected from the previous findings of no increase in medial lamellar units but a significant increase in medial thickness of hypertensive vessels. The elevation in wall stress on hypertensive vessels indicates that their increase in thickness does not fully parallel the increase in tension.

Calculated cross-sectional area of the media represents a synthesis of values for diameter and wall thickness in a given vessel. This represents the most accurate quantitative indication of the amount of aortic tissue that can be obtained from histological sections; it should therefore be the value that can most meaningfully be related to calculated total wall tension in assessing the morphological response to tension. In addition, cross-sectional area of the wall is used in calculating the longitudinal elastic modulus of the wall, which is further reason for obtaining accurate calculations of this variable. Calculated values for cross-sectional area of the media are shown in Figure 8. In each group, hypertensive vessels have significantly greater medial area than normotensive ones \((P<0.05\) for groups III and I, \(< 0.01\) for group II). The differences among hypertensive vessels or among normotensive vessels of different groups are not significant \((P>0.3,\) except for group I versus group III of the normotensive vessels, where \(0.1<P<0.2)\). These findings suggest that the significantly greater diameter of group I compared to group II of normotensive vessels seen in Figure 2 results only from thinning of the wall associated with growth, since no net increase in the cross-sectional area of the media is apparent.

In Figure 9, cross-sectional area of the
The response of aortic media to hypertension is related to total calculated wall tension in the three groups. The regression slope can be expressed by the formula: 

\[ y = 0.32 + 0.21 \times 10^{-4} (x) \]

and the correlation coefficient of the linear relation between \( x \) and \( y \) is significant at 0.79 (\( P < 0.001 \)). The relation can also be expressed as follows: each increment in total tension of 5000 dynes/cm is associated with an increase of approximately 0.1 mm² in cross-sectional area of the media over the range of values included in this study.

**CHEMICAL STUDIES**

The results of amino acid analysis of aortas from normotensive and hypertensive animals of the three groups are shown in Table 3. It can be seen that total initial aortic fat-free dry weight of hypertensive animals of each group is greater than that of normotensive animals (\( P < 0.01 \)). The absolute weights of elastin and collagen are also significantly greater in hypertensive aortas than in normotensive ones (\( P < 0.001 \) for elastin and \( < 0.01 \) for collagen). However, when these values are expressed as percent of total dry weight, no significant differences are found between control and hypertensive segments (\( P \geq 0.2 \) for both elastin and collagen). Similarly, the percent of total aortic dry weight represented by elastin and collagen together (last column, Table 3) is not significantly different in normotensive and hypertensive animals (\( P > 0.8 \)).

Although the increases in absolute amounts of elastin and collagen in hypertensive animals were striking, it was thought worthwhile to go further and attempt to relate the increments in these fibrous proteins to values for cross-sectional area of the media and total wall tension obtained above. For this purpose, the average of the two values obtained for total aortic weight and the weights of elastin and collagen in control and hypertensive animals of each group (Table 3) were related to the mean value for cross-sectional area of the media and mean value for total wall tension obtained for the corresponding control and hypertensive subgroups in the morphological studies above.

The relation between cross-sectional area of
Table 3
Total Aortic Dry Weight and Amounts of Elastin and Collagen in Thoracic Aortic Segments of Normotensive and Hypertensive Animals

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Total aortic dry wt (mg)</th>
<th>Elastin (E)</th>
<th>Collagen (C)</th>
<th>E and C (total wt)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wt (mg)</td>
<td>(% total wt)</td>
<td>Wt (mg)</td>
<td>(% total wt)</td>
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<td>5.25</td>
<td>1.76</td>
<td>33.5</td>
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<tr>
<td></td>
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<td>5.58</td>
<td>2.20</td>
<td>39.0</td>
<td>0.83</td>
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<td>7.00</td>
<td>2.86</td>
<td>41.6</td>
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<td>6.50</td>
<td>2.78</td>
<td>42.8</td>
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<td>4.96</td>
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<td>7.50</td>
<td>2.70</td>
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<td></td>
<td>10.89</td>
<td>3.50</td>
<td>32.1</td>
<td>2.33</td>
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</table>

\( P < 0.01 \) \( P < 0.001 \) \( P > 0.2 \) \( P < 0.01 \) \( P = 0.2 \) \( P > 0.8 \)

N = Number of animals. C = Normotensive. H = Hypertensive.

The relation of total aortic dry weight and the amount of elastin and collagen to calculated total wall tension is shown in Figure 11. All regression slopes shown are highly significant \( (P < 0.001) \). The thoracic aortas of normotensive rats contain approximately twice as much elastin as collagen, as was already apparent in Table 3. However, the increments in the absolute weights of elastin and collagen associated with hypertension are linearly related to increments in wall tension and are similar for these two fibrous proteins. Since the periods of hypertension were essentially the same for all animals, it would appear that the rates of accumulation of elastin and collagen are also similar and are linearly related to the degree of tension elevation.

Discussion

MORPHOLOGICAL STUDIES

In spontaneously occurring and experimental hypertension, generalized thickening of vessels occurs (12, 13). Since the intima of large arteries is only slightly thickened in association with hypertension (9), the media is apparently responsible for most of the increased thickness of these vessels. It is also clear from pressure-volume studies of the brachial artery in vivo in hypertensive and normotensive humans (15), as well as experimental studies of vessel segments in several animal species (14, 16), that vessels from hypertensive subjects are stiffer than those from normotensive subjects in both the hypertensive and physiological pressure levels.
ranges (14). Karsner (11) compared the morphology of undistended human thoracic aortas from normotensive and hypertensive patients ranging in age from the second to eighth decade. In hypertension, additional thickening was superimposed on medial thickening associated with aging; the difference between the thicknesses of normotensive and hypertensive vessels therefore tended to be greatest in younger vessels, and essentially disappeared by the sixth decade. The increase in total medial thickness was attributed to increased space between medial elastin lamellae; fine elastin fibrils between elastin lamellae were prominent in hypertensive segments at all ages.

The findings in the present study of the rat aorta correspond closely to the alterations described by Karsner. The thickening of the media associated with hypertension was probably due to increased interlamellar space, since the number of medial lamellar units was not increased. We have recently examined a series of thoracic aortas from humans who were hypertensive during adult life and have found no significant change in the total number of medial lamellar units compared to aortas from age- and sex-matched normotensive adults (unpublished data). The apparent inability of the hypertensive growing rat and adult human to elaborate additional lamellar units in the presence of elevated tension is intriguing and possibly important in light of previous findings by us that during growth of most mammals, including man, an increase in the number of lamellar units in the thoracic aortic media normally occurs (30).

It has been considered highly probable that morphological changes in aortic structure of younger hypertensive patients represent pre-
The relation of total aortic dry weight and weights of elastin and collagen to calculated total wall tension. Each symbol represents the average of determinations on two aortas. All linear regression slopes are highly significant.

cocious senile alterations (11, 31) since they are indistinguishable morphologically. These conclusions, based as they are solely on morphological similarities, must await confirmation from comparative chemical studies.

Although considerable clinical and experimental evidence suggests that hypertension alone accelerates arteriosclerosis the mechanism of this effect is not clear (3, 4). Duncan et al. (10) and Wiener et al. (32) showed that the hypertensive vessel was unduly permeable to albumin and cholesterol, which are normally carried in the intravascular plasma. Increased medial thickness associated with hypertension might interfere with "clearance" of increased amounts of intravascular substances entering the media, leading in turn, to medial accumulation of these materials. If a vessel segment were normally avascular, i.e., totally dependent on transmural filtration and diffusion for its nutrition, medial thickening consequent to hypertension could increase wall thickness beyond the critical depth of 0.5 mm (30), thereby rendering the cells of the deeper portions of the media relatively ischemic. The pattern of arteriosclerotic involvement of the aorta and coronary tree of hypertensive humans (1, 3) and other species (4, 5) suggests that hypertension not only accelerates disease in those areas which normally become diseased but also results in involvement of new segments of the vascular tree in a centrifugal direction (3); that is, smaller branches which are not usually affected by hypercholesterolemia alone become diseased when hypertension is introduced. The generalized vascular thickening which accompanies hypertension may serve to put new vascular segments at risk of becoming diseased by the mechanism suggested above.
The human abdominal aorta may represent an analogous condition, as has been discussed recently (29). This unusually susceptible segment has a strikingly elevated value of calculated tension per lamellar unit and is inappropriately avascular and far thicker than any avascular aorta described to date. The similarity in appearance of that segment to the hypertensive vessels described in this study suggests that elevated tension may be a common pathogenic factor. Further studies should clarify whether the responses of the vessel wall to these experimental and clinical conditions are really comparable.

**CHEMICAL STUDIES**

Many different chemical methods for estimating the amounts of elastin and collagen have been utilized in the past; the advantages and disadvantages of each method have been recently reviewed (33). Particular care must be taken when evidence for the synthesis of new fibrous protein is sought, for harsh treatment, such as autoclaving, during the purification of young, relatively uncrosslinked elastin may result in considerable destruction and loss of this material (34). Degradation and loss of proteins is minimized by the milder alkali solubilization of the method as used here, and the retention of all material, in soluble and residue fractions, for amino acid analysis. The close correspondence of the ratios of several key amino acids to pure collagen, as well as the calculation of the weight of the protein from the contribution of each amino acid, appears to make this method very reliable and accurate when the detection of small increases in the net amounts of new, relatively soluble proteins is desired. This conclusion will, of course, require confirmation by others.

Chemical changes in vessel walls which have been associated with hypertension include an absolute increase in their water content (19), an increased ratio of chondroitin sulfate to hyaluronic acid in the ground substance (35), and an increased rate of respiration and degree of cytochrome oxidase activity (17). However, no change in the percent of vascular elastin or collagen has been found previously either in experimental (18) or spontaneously occurring (21) hypertension when compared to normotensive conditions. In the present study, the absolute amounts of both aortic elastin and aortic collagen were increased in hypertension but the percent of total fibrous protein and nonfibrous elements of vessel wall remained relatively constant under normotensive and hypertensive conditions. A precedent for such changes in hypertension can be found in the recent findings of an absolute increase in collagen content of the hypertrophied right ventricle (elastin content was not determined) (36).

Total aortic dry weight and the amounts of collagen and elastin were found to be linearly related to total wall tension over the range of values covered in this study. The regression slopes for these two fibrous proteins are parallel, indicating that similar amounts of each accumulate with a given increment in tension. Since the period of hypertension of all animals was 8 weeks, the slopes of Figure 11 indicate that the rates of accumulation of elastin and collagen were also similar. Admittedly, the calculated values for tension are indirectly derived from other measurements and are based solely on systolic pressures, but it is possible to estimate from Figure 11 that, under the conditions of this study, an increase of 10,000 dynes/cm in wall tension over the range of tension covered, is associated with an increase in total weight of thoracic aorta of 0.19 mg/week and that the accumulation of elastin and collagen occurs at similar rates of approximately 0.05 mg/week for each protein.

The high degree of correlation between the amounts of medial elastin and collagen and calculated levels of tension found in these studies is the first such relation described and is compatible with the possibility that tension, either directly or indirectly, provides the stimulus for elaboration of these fibrous proteins. This association of increases in both medial elastin and collagen with increased tension is in accord with the postulation by us (37) and others (22) and that these two fibrous proteins, which differ greatly in their
physical and mechanical characteristics, act in concert at physiological levels of tension and above. Recent careful studies of aortic media explants in tissue culture (38) leave little question that the medial smooth muscle cell is a multi-potential cell, capable of producing elastin and collagen, as well as ground substance and myosin (39). It is not clear whether these cells are genetically either "collagenoblasts" and "elastoblasts" (40), or whether each cell synthesizes both fibrous proteins in proportions and amounts somehow related to the nature and degree of stresses in the vessel wall. The findings here of similar increments in elastin and collagen are compatible with either alternative.

Although these findings represent the first direct, quantitative evidence for a net accumulation of elastin and collagen in the vessel wall associated with hypertension, previous morphological studies have shown an apparent increase in both these fibrous proteins during postnatal growth of the aorta (41, 42). Variable amounts of elastin and collagen are present in the musculo-elastic layer of vessels (43) and in the fibrogenesis associated with arteriosclerosis (44); smooth muscle cells are the likely sources of these proteins (43, 44). However, aging of the vessel wall (45) and healing of nonspecific local trauma (46) or ischemic necrosis (47) of the media, morphologically consist almost exclusively of collagen fibrogenesis; elastin formation is inconspicuous. If these apparent differences are confirmed by quantitative chemical analyses the implications are that predominantly pulsatile stresses (for example, those associated with hypertension or vessel growth) elicit a different response from the medial cell than nonspecific stresses involving local medial damage. Incidentally, it is noteworthy that the response associated with atherosclerosis appears to be of the former type, while that of aging seems to be of the latter type. However, the mere fact that different patterns of fibrogenesis occur points to flexibility in the response of medial cells to stress in the vessel wall. For example, the presence of twice as much elastin as collagen in the normotensive rat thoracic aorta (34) could not have arisen had the one-to-one "formula" for elastin and collagen increments found in this study been fixed from the very onset of aortic development. Indeed, morphologically, the thoracic aortic media of prenatal and newborn rats contains a paucity of collagen compared to elastin.

The heretofore widespread, almost exclusive use of relative expressions, such as percent of dry weight and collagen-elastin ratios, is useful for comparisons of the composition of different vessels. However, from this study it is clear that the net accumulation or reduction of components of the vessel wall during growth or after experimental alterations in wall stress should be assessed by determining the absolute amounts of those materials in carefully defined, identical segments. Further such analyses should help to elucidate the specific stimuli responsible for the elaboration of elastin and collagen under many types of physiological and pathological conditions. At the same time, it should be possible to determine how mural accumulations of these fibrous proteins influence the initiation or progression of vascular disease.

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