Quantitative Structural Changes of the Rat Thoracic Aorta in Early Spontaneous Hypertension
Tissue Composition, and Hypertrophy and Hyperplasia of Smooth Muscle Cells

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SUMMARY. The thoracic aorta of 21-, 28-, 35-, and 45-day-old spontaneously hypertensive (SH) and Wistar Kyoto (WK) rats was analyzed morphometrically to evaluate the cellular hypertrophy and proliferation of medial smooth muscle cells during the development of genetically determined hypertension. The absolute increase in volume of collagen and ground substance, and elastic tissue was also measured. In SH animals, cellular hypertrophy was found to be the dominant mechanism of muscle growth in the 21- to 28-day and 35- to 45-day intervals, resulting in an overall 68% enlargement of the mean cell volume from 21 to 45 days. The total number of smooth muscle cells increased only 21% (not statistically significant) and the tendency toward hyperplasia was restricted to the 28- to 35-day period. Normotensive controls showed cell proliferation mainly from 21 to 28 days and cellular hypertrophy from 35 to 45 days with an absolute 33% increase in the number of cells and a 26% larger volume of the mean smooth muscle cell at 45 days of age. From 21 to 45 days, the above changes in cell size and number provoked an overall 104% and 67% growth of the muscle mass in SH and WK rats, respectively. The initial response phase of spontaneous hypertension was also characterized by an increase of collagen and ground substance, 184%, which was slightly greater than that of the elastic component, 168%. Changes in mural concentration of fibrous proteins that were similar to but of less magnitude than those of controls, were seen. These results demonstrate that short-term spontaneous hypertension determines a simultaneous growth adaptation in every component structure of the media of the thoracic aorta leading to a disproportionate accumulation of scleroproteins that markedly exceeds that of the contractile component of the vessel wall. At a cellular level, smooth muscle cell hypertrophy is the prevailing process that underlies the tissue response of the aorta in early hypertension. (Circ Res 51: 19-26, 1982)
stance, and elastic tissue, were measured. These are the morphometric results that relate most nearly to mechanical parameters measured in the intact thoracic aortic segment.

Methods

The thoracic aorta of 23 male spontaneously hypertensive (SH) rats of the Okamoto strain in the seventh generation, was studied at 21, 28, 35, and 45 days of age. Beginning at 21 days of age, systolic blood pressure was measured in conscious rats twice a week, and again before they were killed, using the tail cuff method validated by Pfeffer et al. (1971). At sacrifice, the hearts were arrested in diastole by an intravenous injection of KCl (1 mEq/ml), the right atrium opened, and the thoracic aorta perfused at a constant pressure of 60 mm Hg. Perfusion with phosphate buffer (0.2 M, pH 7.2) for 3 minutes was followed by 15 minutes of perfusion with formaldehyde-glutaraldehyde mixture diluted 1:1 with phosphate buffer. After fixation, the length of the aorta was measured, 15 rings, approximately 1 mm thick, were sampled and the collected tissue processed for electron microscopy, as previously described (Olivetti et al., 1980b). The volume of the media in each animal was obtained from the product of the length of the thoracic segment and its corresponding mean cross-sectional area. The amount of sampling used and the sequential steps involved in these measurements were identical to those employed for previous work performed in this laboratory (Olivetti et al., 1980a, 1980b).

Morphometric sampling by electron microscopy utilized four random micrographs of the media taken at 1,900 X in transverse sections of 2 blocks from each animal and printed at exactly 5,200 X. A grid containing 266 sampling points and 617.5-cm sampling line was superimposed on each print of the media and the volume fractions of smooth muscle cells, smooth muscle cell nuclei, collagen and ground substance, and elastic laminae were determined by the fraction of points overlying each of these components. The surface density of smooth muscle cells and their nuclei were evaluated from the frequency of profile intersections with the test line (Anversa et al., 1979). Nuclear profiles in the sampled area also were counted. Measurements of both cell surface and nuclear density were corrected for the effects of compression artifact (Olivetti et al., 1980a, 1980b).

Two additional blocks from each animal were utilized for light microscopic determinations. Cross-sections of the entire aorta were cut at nominal thicknesses of 0.5, 2.5, and 4.0 μm. The measured thickness of these sections, also corrected for compression artifact, was found to be 0.653, 2.507, and 4.849 μm, respectively (Olivetti et al., 1980a, 1980b). The plastic embedding matrix was dissolved (Mayor et al., 1961), and the sections stained with Harris' hematoxylin. Morphometric sampling at a magnification of 2,000 X consisted of counting the total number of smooth muscle nuclear profiles in a square area of aortic media equal to 2,025 μm². Such area was defined by ocular reticle (no. 105844, Wild Heerbrugg Instruments, Inc.), and four fields located at 0°, 90°, 180°, and 270°, were examined in each section. Alternate fields bordered on the internal elastic lamina and adventitia.

The light microscopic counts of smooth muscle nuclei and the previous electron microscopic evaluation of the volume fraction of smooth muscle cells in the media of the aortic wall were combined to calculate the number of nuclei per unit volume of cells, Nv, and the mean cell volume, V, as previously described (Anversa et al., 1979, 1980; Olivetti et al., 1980a, 1980b). All the results in each table show the mean ±SD of values determined for the individual animals in each group. For the comparison of two groups of data, levels of significance were evaluated by Student’s t-test. Statistical significance in multiple comparisons among independent groups of data, in which analysis of variance and the F-test indicated the presence of significant differences, were determined by the Bonferroni method (Wallenstein et al., 1980). Changes in parameters are shown as a percent increase. The ratio, R, of the same parameter in older animals to that in younger animals is presented in the tables as a percent increase, 100 (R-1). Relative growth rates between SH and WK rats are derived from Rsh/Rwk.

Results

Table 1 shows the body weights and the values of systolic blood pressure of SH and WK rats at 21, 28, 35, and 45 days of age. The rate of increase of body weight was approximately identical in hypertensive and normotensive animals. Systolic pressure was similar at 21 days in both groups of rats, but an average 28% greater value in SH animals was observed at each of the three following age intervals examined. Throughout the entire experimental period, an overall 75% and 41% rise in pressure was seen in SH and WK animals, respectively.

The tissue area sampled and the number of smooth muscle cell nuclear profiles counted in electron micrographs taken from the media of the thoracic aorta of spontaneously hypertensive and control animals are presented in Table 2. The volume composition of the media, which was nearly identical in both groups of rats at 21 days after birth, was found to become significantly different by 28 days. At this age, SH rats contained a greater concentration of smooth muscle cells (13%) and elastic laminae (17%) but significantly less collagen and ground substance (−30%) than WK rats. Elastin and muscle decreased markedly (−14%) from 28 to 35 days in SH rats due to the corresponding increase in the relative volume of collagen and ground substance. During the following time interval, from 35 to 45 days, only a moderate but significant increase of elastin (7%) was seen. Similar changes in the volume fractions of fibrous proteins and contractile mass of the media were observed in controls, but, with respect to the experimental group, a lag of a week in such age related alterations was detected throughout the 21- to 35-day period. At 45 days, the proportion of the different component structures of the aorta was again very much alike in the two groups of animals. However, an overall decrease of the muscle component associated with a proportional expansion of the scleroproteins fractions was noted from 21 to 45 days. This change was greater in hypertensive animals.

The indicated sampling of intersection counts used to measure the surface membrane of smooth muscle cells (Table 3) showed an increase of the mean cell surface-to-volume ratio which, together with the increased nuclear-to-cytoplasmic ratio (Table 2), suggested a significant hyperplasia of this cell population with the development of hypertension. This suggestion was strengthened by the finding of a progressive...
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Table 1

<table>
<thead>
<tr>
<th>Changes in Systolic Blood Pressure with Age in Spontaneously Hypertensive (SH) and Wistar Kyoto (WK) Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of rats</strong></td>
</tr>
<tr>
<td>SH</td>
</tr>
<tr>
<td>WK</td>
</tr>
<tr>
<td><strong>Body wt (g)</strong></td>
</tr>
<tr>
<td>21 days</td>
</tr>
<tr>
<td>28 days</td>
</tr>
<tr>
<td>35 days</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. % Indicates the percent difference between SH and WK rats. * Indicates a percent change that is statistically significant, P < 0.05. † Indicates a statistically significant difference between SH and WK rats, P < 0.05.

in the thoracic segment of the aorta is presented in Table 4. The volume of each constituent of the wall was obtained from the volume of the media shown first in Table 4, multiplied by the corresponding volume fraction listed in Table 2. Throughout the 24-day period from 21 to 45 days, the media increased 150% in SH rats and 87% in WK animals. This adaptive response was shared by elastin, collagen, and...
TABLE 3
Surface Density of Smooth Muscle Cells and Smooth Muscle Cell Nuclei

<table>
<thead>
<tr>
<th>Intersection counts</th>
<th>Age: 21 days</th>
<th>Age: 28 days</th>
<th>Percent change 21-28 days</th>
<th>Age: 35 days</th>
<th>Percent change 28-35 days</th>
<th>Age: 45 days</th>
<th>Percent change 35-45 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smooth muscle cell surface</td>
<td>SH</td>
<td>3005</td>
<td>3504</td>
<td></td>
<td>3455</td>
<td></td>
<td>4961</td>
</tr>
<tr>
<td>WK</td>
<td>4438</td>
<td>4277</td>
<td></td>
<td>4996</td>
<td></td>
<td>5034</td>
<td></td>
</tr>
<tr>
<td>Smooth muscle nuclear envelope</td>
<td>SH</td>
<td>502</td>
<td>480</td>
<td></td>
<td>511</td>
<td></td>
<td>298</td>
</tr>
<tr>
<td>WK</td>
<td>582</td>
<td>454</td>
<td></td>
<td>410</td>
<td></td>
<td>410</td>
<td></td>
</tr>
</tbody>
</table>

Surface-to-volume ratio (μm²/μm³)

| Smooth muscle cells | SH | 1.36 ± 0.10 | 2.01 ± 0.28 | 55* | 1.80 ± 0.19 | -10 | 2.98 ± 0.36 | 66* | 129* |
| WK | 1.96 ± 0.26 | 2.24 ± 0.20 | 14 | 2.59 ± 0.29 | 16 | 2.34 ± 0.23 | -10 | 19 |
| % | -31† | -10 | -31† | 27† |
| Smooth muscle cell nuclei | SH | 1.36 ± 0.15 | 1.81 ± 0.27 | 33 | 1.62 ± 0.14 | -10 | 2.36 ± 0.42 | 46* | 74* |
| WK | 1.75 ± 0.20 | 1.97 ± 0.28 | 13 | 1.98 ± 0.32 | 1 | 1.86 ± 0.32 | -6 | 0 |
| % | -22† | -8 | -18† | 27 |

See footnotes to Table 1.

TABLE 4
Absolute Component Volumes of the Thoracic Aorta in SH and WK Rats

<table>
<thead>
<tr>
<th>Age: 21 days</th>
<th>Age: 28 days</th>
<th>Percent change 21-28 days</th>
<th>Age: 35 days</th>
<th>Percent change 28-35 days</th>
<th>Age: 45 days</th>
<th>Percent change 35-45 days</th>
<th>Age: 28 days</th>
<th>Age: 35 days</th>
<th>Percent change 28-35 days</th>
<th>Age: 45 days</th>
<th>Percent change 45-51 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media, mm³</td>
<td>SH</td>
<td>2.95 ± 0.26</td>
<td>4.56 ± 0.23</td>
<td>55*</td>
<td>5.28 ± 0.35</td>
<td>16*</td>
<td>7.37 ± 0.32</td>
<td>40*</td>
<td>150*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK</td>
<td>2.81 ± 0.31</td>
<td>3.61 ± 0.31</td>
<td>28*</td>
<td>4.28 ± 0.27</td>
<td>19*</td>
<td>5.25 ± 0.24</td>
<td>23*</td>
<td>87*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>5</td>
<td>26</td>
<td>24†</td>
<td>40†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>SH</td>
<td>1.01 ± 0.11</td>
<td>1.52 ± 0.13</td>
<td>50*</td>
<td>1.52 ± 0.12</td>
<td>0</td>
<td>2.06 ± 0.14</td>
<td>36*</td>
<td>104*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK</td>
<td>0.94 ± 0.12</td>
<td>1.065 ± 0.102</td>
<td>13</td>
<td>1.176 ± 0.096</td>
<td>10</td>
<td>1.57 ± 0.11</td>
<td>34*</td>
<td>67*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>7</td>
<td>43†</td>
<td>29†</td>
<td>31†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen and ground substance</td>
<td>SH</td>
<td>0.834 ± 0.081</td>
<td>1.059 ± 0.091</td>
<td>27*</td>
<td>1.79 ± 0.13</td>
<td>69*</td>
<td>2.37 ± 0.14</td>
<td>32*</td>
<td>184*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK</td>
<td>0.846 ± 0.096</td>
<td>1.20 ± 0.11</td>
<td>42*</td>
<td>1.274 ± 0.099</td>
<td>6</td>
<td>1.73 ± 0.12</td>
<td>36*</td>
<td>104*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>-1</td>
<td>-12</td>
<td>41†</td>
<td>37†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elastic laminae</td>
<td>SH</td>
<td>1.10 ± 0.11</td>
<td>1.99 ± 0.11</td>
<td>81*</td>
<td>1.97 ± 0.14</td>
<td>-1</td>
<td>2.95 ± 0.17</td>
<td>50*</td>
<td>168*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WK</td>
<td>1.03 ± 0.12</td>
<td>1.35 ± 0.13</td>
<td>31*</td>
<td>1.83 ± 0.12</td>
<td>36*</td>
<td>1.95 ± 0.12</td>
<td>7</td>
<td>89*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>7</td>
<td>47†</td>
<td>8</td>
<td>51†</td>
<td></td>
<td></td>
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</tbody>
</table>

See footnotes to Table 1.
The numbers of smooth muscle cells in the thoracic aorta were derived from their measured mean volumes (Table 5) and the overall volumes of cells (Table 4). The average aorta in SH rats possessed 5.76 million cells at 21 days and 6.97 million at 45 days, indicating a 21% cellular hyperplasia during the interval from 21 to 45 days. This change, however, was not statistically significant. A parallel calculation in controls disclosed a 33% increase in the total number of smooth muscle cells, statistically highly significant.

**Discussion**

The results of this study, in agreement with previous work (Laia et al., 1977; Limas et al., 1980), indicate that mean systolic pressure in SH rats becomes significantly elevated, compared with that of WK controls, during the interval 21-28 days after birth. An average elevation of 28% was maintained from 28 to 45 days of age. These data cannot prove conclusively that variations between strains develop only after 21 days, since a larger number of animals in each group might have shown some significant differences. The media of the thoracic aorta adapts to this increase in intraluminal pressure by progressively expanding its absolute volume, resulting in an overall 150% enlargement from 21 to 45 days. In contrast, control animals undergo only an 87% expansion. The most rapid increase of medial mass (55%) in SH rats occurred from 21 to 28 days, coinciding with the highest percent increment (39%) in blood pressure. Regression analysis between arterial pressures and medial volumes in SH rats shows a strong correlation ($r = 0.952$) indicating that the changes in wall volumes are proportional to the variations in systolic pressure throughout the experimental period studied. Such correlation also exists in control animals ($r = 0.992$).

Determination of mean cell volumes and numbers indicates that the enlargement of the contractile mass of the aortic wall in hypertension results from increases in both the size and number of smooth muscle cells. Cellular hypertrophy is the dominant process from 21 to 28 days after birth, but cell proliferation wholly accounts for the maintenance of the muscle component in the following 7-day period. Cellular hyperplasia during this period is also accompanied by a slight decrease in average cell size. From 35 to 45 days, cellular hypertrophy represents again the only mechanism of tissue growth. Such biphasic adaptive response of smooth muscle cells to the progressive increase in arterial pressure is in agreement with previous studies in rats and rabbits with aortic coarctation-induced hypertension (Bevan, 1976; Olivetti et al., 1980a). Seven days after surgery, aortic medial hypertrophy includes enlargement but no prolifera-
of the muscle component in normotensive controls, it has also been seen that aortic smooth muscle cells are also found in rats with spontaneous hypertension (Jurokova et al., 1976), although cell proliferation seems to play a subordinate role. In addition, it has been shown that aortic smooth muscle cell hypertrophy in SH rats is accompanied by an increase in nuclear ploidy (Owens et al., 1981).

In some respects, the sequence of cellular phenomena observed in hypertension is the reverse of those observed during physiological growth. The expansion of the muscle component in normotensive controls, that practically doubles from 21 to 45 days, is achieved by a 33% increase in total cell number and a 26% increase in the average cell size. Hyperplasia, however, is almost completed at 28 days and hypertrophy occurs only at the latest time interval, from 35 to 45 days. These observations correspond to determinations that show a decreasing concentration but an increasing total amount of DNA in the aorta during postnatal growth (Looker and Berry, 1972; Leung et al., 1977). It has also been seen that aortic smooth muscle cells continue DNA synthesis at a diminishing rate up to 1 year of age (Looker and Berry, 1972). The pattern of cellular growth observed in the two groups of animals during the relatively short time of investigation may have been influenced by different cellular growth occurring in the preceding period, from birth to 21 days of age. How the processes of cellular hypertrophy and cellular hyperplasia contribute to the adaptation of the thoracic aorta in long-term spontaneous hypertension remains to be determined.

Quantitative results indicate that the overall 104% increase of muscle mass in the aorta of SH rats is the result of a 50 and 36% expansion of the muscle compartment during the intervals from 21 to 28 days and from 35 to 45 days, respectively. These growth increments are attained by corresponding 54% and 40% mean cellular enlargements with no evident changes in the number of cells. Thus, smooth muscle cell hyperplasia is not associated with absolute expansion of the muscle mass, and it appears to follow cellular hypertrophy. A similar growth pattern also is detectable in normotensive controls in which the principal increment in muscle volume seen from 35 to 45 days is paralleled by a proportional increase in average cell size. The nature of specific stimuli responsible for the onset of either the hypertrophic or hyperplastic response of smooth muscle cells in the arterial wall is at present unknown.

Electron microscopic morphometry showed that the

| TABLE 5 | Mean Cell Volumes, Surface Areas, and Numbers of Aortic Smooth Muscle Cells |
| Age: 21 days | Age: 28 days | Percent change | Age: 28-35 days | Percent change | Age: 35-45 days | Percent change |
| Smooth muscle cell | | |
| SH | 176 ± 27 | 271 ± 46 | 54% | 211 ± 30 | -22% | 209 ± 29 | 40% |
| WK | 191 ± 18 | 174 ± 32 | -9 | 187 ± 13 | 7 | 240 ± 12 | 28%
| | % | 8 | 56† | 13 | 23† |
| Nucleus | | | | | | | |
| SH | 29.2 ± 4.8 | 41.2 ± 7.6 | 41% | 34.8 ± 5.6 | -16 | 31.3 ± 4.0 | 10 |
| WK | 27.9 ± 3.4 | 21.1 ± 4.5 | -24% | 20.0 ± 3.8 | 23 | 24.5 ± 3.8 | 7 |
| % | 5 | 95† | 74† | 28† |
| Cytoplasm | | | | | | | |
| SH | 146.8 ± 22.6 | 229.8 ± 39.1 | 57% | 176.2 ± 25.2 | -23% | 263.7 ± 26.0 | 50% |
| WK | 163.1 ± 15.5 | 152.9 ± 28.2 | -6 | 167.0 ± 12.1 | 9 | 215.5 ± 11.4 | 29%
| | % | -10 | 50† | 6 | 22† |
| Surface area, μm² | | | | | | | |
| Cell membrane | | | | | | | |
| SH | 239 ± 41 | 545 ± 120 | 128% | 380 ± 68 | -30 | 879 ± 137 | 131%
| WK | 374 ± 61 | 390 ± 80 | 4 | 484 ± 64 | 24 | 562 ± 62 | 16%
| | % | -36† | 40† | 21† | 56† |
| Nuclear envelope | | | | | | | |
| SH | 39.7 ± 7.9 | 75 ± 18 | 80% | 56 ± 10 | -25 | 74 ± 16 | 32 |
| WK | 48.8 ± 8.2 | 42 ± 11 | -14 | 40 ± 10 | -5 | 46 ± 11 | 15 |
| | % | -19 | 79† | 40† | 61† |
| Number of smooth muscle cells | | | | | | | |
| SH | 5.76 ± 1.07 | 6.01 ± 1.07 | -3 | 7.18 ± 1.18 | 28 | 6.97 ± 0.84 | -3 |
| WK | 4.92 ± 0.78 | 6.12 ± 1.27 | 24 | 6.29 ± 0.68 | 3 | 6.54 ± 0.56 | 4 |
| (millions) | % | 17 | -8 | 14 | 7 |

See footnotes to Table 1.
ratio of smooth muscle cell surface-to-cell volume significantly increased in hypertension, despite the concomitant enlargement of the average smooth muscle cell, implying a marked change in cell shape toward a much more irregular surface configuration. On a cell basis, from 21 to 45 days, mean cell surface increased 3.68-fold from 239 to 879 μm². Plasma membrane growth in aortic smooth muscle cells has been seen to be associated with a proportional formation of cell to cell contacts and peripheral attachment ridges with the elastic fiber matrix (Cliff, 1967; Osborne-Pellegrin, 1978). It has been suggested that such cellular connections could be implicated in assuring a coordinated response of the media preventing cell separations at elevated pressures (Mullins and Guntheroth, 1965; Clark and Glagov, 1979).

The response of the aorta to a work overload involves not only the adaptation of the smooth muscle cell population but also the simultaneous growth occurring in the other major structural components of the vessel wall: collagen and ground substances, and elastin (Wolinsky, 1972; Ooshima et al., 1975; Greenwald and Berry, 1978; Olivetti et al., 1980a). The significant accumulation of fibrous proteins observed in the present study indicates that the synthesis of elastin and collagen by smooth muscle cells is accompanying the early rise in systolic blood pressure. Changes in the volume composition of the aortic wall resulting from hypertension at 45 days of age, collagen and ground substance (+13%), elastin (+7%), and smooth muscle (−19%), seem to provide a structural basis for the decreased compliance of the aorta measured in SH rats, approximately 6 weeks after birth (Greenwald and Berry, 1978). A similar alteration in the static mechanical properties of the aorta, although of less magnitude, has been shown to occur during early postnatal life in normotensive controls (Berry et al., 1975), in agreement with the quantitative changes in the mural content of collagen and elastin observed here.

The changes in the structural properties of the aortic wall of SH rats, seen from 21 to 28 days after birth, consist of a significant 18% reduction in the volume fraction of collagen and ground substance, whereas the elastic component expands by 17%. These morphometric results suggest that passive stiffness may be reduced at this time. However, the following 7-day period is characterized by a subsequent rise in the relative volume of collagen and ground substance (+46%) which is associated with a decreased volume fraction of elastin (−14%), thus, indicating a possible lower distensibility of the aortic wall.

In contrast with the present findings, however, the initial period of experimental renal hypertension is associated with a striking increase in the amount of smooth muscle cell proteins (Wolinsky, 1970), whereas collagen and elastin accumulate only after long-term hypertension (Wolinsky, 1972). A possible meaningful difference between systems involving genetically determined hypertension and provoked hypertension, by renal artery clipping (Wolinsky, 1970, 1972), is the relatively rapid induction of the hemodynamic changes in the latter model. In addition, the morphometric evaluation of amorphous proteins present in the arterial wall is entirely dependent on their morphological identification. Therefore, caution should be taken in interpreting quantitative data and in comparing such results with biochemical assays of concentration of specific vascular proteins.

The inciting stimulus accountable for the accumulation of extracellular substances in the aortic wall following hypertension is unclear. Mechanical stress (Wolinsky, 1970) such as stretching (Leung et al., 1976; Vandenburgh and Kaufman, 1979) may play an important role, in view of the fact that cyclic stretching of isolated elastic lamellae with attached smooth muscle cells can cause an increase production of collagen and glycosaminoglycans (Leung et al., 1976). Tensile forces are also involved in determining the distribution of the interstitial matrix (Weiss, 1959) in which collagen fibrils are oriented perpendicular to the long axis of smooth muscle cells (Clark and Glagov, 1979). An increased concentration of collagen fibrils and ground substance within the muscle layers further separate the thin irregular cytoplasmic projections of the muscle cells, thus decreasing the force-generating ability of the contractile mass in hypertension (Seidel, 1979).

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