Ultrastructure of Hypertensive Rat Aorta
Increased Basement Membrane–like Material

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To determine the effect of elevated blood pressure on the ultrastructure of rat aorta, hypertension (average mean pressure 163±17 mm Hg) was produced by suprarenal aortic coarctation. After 3 weeks, the subendothelium of the hypertensive thoracic aorta showed significantly increased volume measurements for mononuclear leukocytes and basement membrane–like material compared with the sham-operated control group. Focal areas of rarefaction of the subendothelial extracellular material were associated with the nearby presence of mononuclear leukocytes. None of these alterations were found in the normotensive abdominal aorta. The tunica media of hypertensive thoracic aorta also contained significantly increased basement membrane–like material. This new finding in an animal hypertension model is the direct result of the quantitative morphological approach employed in this study. In some rats, the partially constricting aortic ligature compromised the right renal artery leading to ischemic atrophy of the right kidney and hyperreninemia in addition to hypertension. In this group, excluded from the previous analysis and evaluated separately, subendothelial thickening and accumulation of basement membrane–like material in the thoracic aorta were greatly increased compared with the control group and other hypertensive rats. This result could not be attributed to an effect of blood pressure alone and might have been caused in part by humoral factors. Basement membrane accumulation appears to be an important early response of the arterial wall to hypertension or other factors in this rat model. (Hypertension 1990;15:56–67)

The effects of hypertension on the arterial wall include infiltration of the intima with mononuclear blood leukocytes, widening of the subendothelial space, and increase in collagen and elastin content of the tunica media.5–10 Of these effects, leukocytic infiltration has been quantified by light microscopy,5,6 but only subendothelial widening has been quantified by ultrastructural morphology.4 Furthermore, no quantitative estimates are available for the fractional contributions of a variety of component structures to widening of the subendothelial space. Within this space may be found mononuclear blood leukocytes, smooth muscle herniae and cysts, basement membrane–like material, and flocculent material possibly representing insudated plasma protein.1–8 A recently developed, microcomputer-based technique for locational stereology11,12 has the ability to quantify common and rare arterial intimal structures, as well as structures at various depth ranges beneath the innermost elastic lamina. We report the application of this technique to hypertensive and normotensive rat aorta.

Experimental coarctation of the upper abdominal aorta induces hypertension reliably in the rat and offers the advantage of hypertensive and normotensive arterial sites in the same animal, exposed to the same circulating mediators. A completely occlusive aortic ligature placed between the renal arteries, which induces hypertension with transiently high plasma renin levels, has been used in studies of thoracic intimal structure2–4 and endothelial function in the thoracic and distal abdominal aorta.13 Partial ligation of the aorta above both renal arteries also induces hypertension and gives somewhat less stimulation of the renin-angiotensin system.13–17 We chose to employ a technique of partial ligation developed by Murphy and Coleman.14 Early morphological changes in hypertensive rats have generally been demonstrated between 1 and 4 weeks after the onset of hypertension.1–4,6–8 Because pilot data revealed less pronounced hypertension with our model compared with previous ones, we chose a time period of 3 weeks for morphological studies.

Among the experimental rats with coarctation hypertension, right renal ischemia and atrophy devel-
oped in four rats, due to impingement of the aortic ligature on the right renal artery. These four rats were excluded from the primary analysis and were treated as a separate group. The morphological alterations in this small group were nevertheless of considerable interest because in several respects they appeared to represent extreme forms of the same pathobiologic responses of the arterial wall seen in hypertensive rats with normal kidneys. This was especially true for accumulation of basement membrane–like material. To determine whether rats with ischemic kidneys might have altered blood pressures or plasma hormone levels (renin and catecholamines), an additional set of control and experimental rats were catheterized chronically for physiological studies.

The results of this study confirmed quantitatively a significant accumulation of subendothelial leukocytes and basement membrane–like material in the hypertensive thoracic aorta, but not in the normotensive abdominal aorta. A possible association of subendothelial leukocytes with extracellular matrix alteration was evident in the form of focal rarefaction of the subendothelial matrix near the cells. A previously unreported finding with implications for smooth muscle biology was a significant increase in basement membrane–like material in the tunica media of the thoracic aorta. Subendothelial and medial basement membrane–like material was greatly increased in the thoracic aorta of rats with ischemic kidneys. This effect could not be ascribed to extreme blood pressure elevation but was associated with elevated plasma renin levels.

Methods

Animals

Male Sprague-Dawley rats were purchased from the Holtzman Company, Madison, Wisconsin, and were used at weights of 190–280 g. Except for rats involved in long-term catheterization experiments, all animals were housed in groups in metal cages and maintained on routine laboratory chow (Purina Formula 5008 Rodent Chow,Ralston Purina, St. Louis, Missouri).

Experimental Coarctation

Coarctation technique was adapted from procedures used by Murphy and Coleman.14 Rats were anesthetized with intraperitoneal sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Illinois) (50 mg/kg body wt). In initial work, femoral artery pressure was monitored by direct cannulation with a Statham P23ID pressure transducer (Statham Instr. Division, Gould Inc., Oxnard, California) and Grass polygraph (Grass Instr. Co., Quincy, Massachusetts). The abdominal aorta just proximal to the renal arteries was exposed and dissected free. A blunted 22 or 23 gauge needle for the experimental group or a 2.5 mm diameter glass rod for the control group was placed beside the aorta, and a sterile 2-0 silk ligature was tied snugly around both aorta and tubing or rod. On tightening the aortic ligature, femoral arterial pressure generally dropped from 100–120 mm Hg to the range of 8–15 mm Hg. After the ligature was tied, the tubing or glass rod was removed. Best coarctation results were obtained when the femoral arterial pressure rose over a period of several minutes to a steady level of 60–80 mm Hg. The aortic ligature was initially positioned proximal to the origin of the anterior mesenteric artery, which supplies most of the animal’s small intestine. Because of unexplained late mortality in several rats, the aortic ligature was subsequently positioned between the origins of the anterior mesenteric and right renal arteries. However, the right renal artery often originated at the same level as the anterior mesenteric artery or even proximal to it, and the attempt to constrict the aorta between these vessels resulted in ischemia of the right kidney in about half of the cases. In some rats, ischemia was readily apparent during the operation because blanching of the kidney occurred. The end result was a shrunken, atrophic kidney after 3 weeks. No cases were equivocal; either the right kidney showed atrophy, confirmed by kidney weights, or it did not.

Fixation and Tissue Processing

Three weeks after aortic coarctation, rats were anesthetized with intraperitoneal sodium pentobarbital before blood pressure measurement and fixation. Blood pressures in the carotid and femoral arteries were measured by direct cannulation using a Grass polygraph with Gould-Statham P23 ID transducers. Simultaneous recordings were obtained in the last 10 rats to be fixed, allowing precise determination of the carotid-to-femoral gradient. Rats were fixed immediately thereafter. The carotid catheter (PE-90) was used for infusion, and the femoral catheter was generally removed to facilitate removal of blood during the initial stages of fixation. Perfusion was performed at a pressure equal to the mean carotid arterial pressure with the following solutions: Hank’s balanced salt solution (pH 7.4) for 30 seconds, and 1% paraformaldehyde, 1.25% glutaraldehyde in 0.13 M sodium phosphate (pH 7.3) for 10 minutes. The aorta and other tissues were resected and immersed in 2% paraformaldehyde, 2.5% glutaraldehyde in the same buffer for 2–4 hours at room temperature. After an overnight wash in 0.13 M sodium phosphate, 0.1 M sucrose buffer, the tissues were post-fixed in 2% aqueous OsO4 stained en bloc with 2% uranyl acetate, dehydrated in ethanol, and embedded in Ladd LX-112 resin. The amount of shrinkage occurring in tissue during the processing steps after perfusion fixation has previously been estimated at 0.945±0.012 (SEE) of initial dimensions.11 All dimensions ascertained from the embedded and sectioned tissue were, therefore, corrected by dividing by 0.945; the values presented incorporate this correction.

Ultrathin transverse sections from each thoracic aorta (T4–T10 and from two blocks in the abdominal aorta (3–5 mm below the renal arter-
Aortas from six controls rats, seven rats with coarctation hypertension, and four rats with coarctation hypertension and ischemic kidneys were analyzed morphometrically. We used one thin section per block: three blocks from the thoracic aorta and two blocks from the abdominal aorta in each rat.

The technique of locational stereology, as described earlier, was employed to ascertain in detail the structure of the intima and the intimal-medial interface. To avoid bias, sections for electron microscopy were cut without prior observation of light microscopic sections. During the electron microscopy session, the first encounter with two adjacent grid squares displaying a continuous arc of arterial intima was selected for study. For each section, two micrographs per block: three blocks from the thoracic aorta and two blocks from the abdominal aorta in each rat.

The adequacy of perfusion fixation was confirmed by the elimination of corrugations from the elastic laminae in the thoracic aorta and the presence of a generally circumferential orientation of medial smooth muscle cells. In one coarcted rat with an ischemic kidney, relatively low blood pressure (124 mm Hg mean pressure), and poor weight gain (+1 g), corrugations of the elastic laminae were present in the thoracic aorta, such that circumferential contraction of the vessel of 20–30% may have occurred. However, the degree of thoracic aortic intimal change in this rat, including subendothelial basement membrane thickness fourfold greater than control, was far too great to be explained by fixation artifact and was similar to other rats with ischemic kidneys. Data from this rat were analyzed with the rest, uncorrected for circumferential contraction. In the abdominal aorta, corrugations of the internal elastic lamina were present in several rats from the two hypertensive groups. Data from these abdominal aortas were also included and were left uncorrected for circumferential contraction. These uncommon instances of apparent circumferential contraction, whether present in vivo or because of inadequate local perfusion pressure, had no bearing on the conclusions drawn from the study.

Stereology

Stereologic measurements were also made on micrographs positioned in the tunica media midway between the internal and external elastic laminae. Two micrographs per section were taken, one section per block, and two blocks each from thoracic and abdominal aorta. A routine point-counting method was used, with 120–170 points counted/micrograph.

Chronic Catheterization and Blood Pressure Monitoring

When the results of morphometry on rats in which ischemic kidneys developed became apparent, an
additional experiment was performed to follow carotid arterial pressure chronically and to determine plasma hormone levels. Coarctation and sham procedures were performed exactly as before. At various times 2–7 days after coarctation or sham procedure, a chronic indwelling carotid catheter was implanted. Polyethylene tubing (PE-50 or PE-90) was preshaped by gentle heating, then sterilized with Zephiran (Winthrop-Breon, New York). The catheter was implanted under anesthesia. It exited the skin at the back of the rat's neck beneath a collar fashioned from silastic sheet and aluminum wire screen, which was fixed to the rat's skin with 1-0 silk sutures. The catheter then passed through a stainless steel wire spring to a fluid-conducting swivel connector (Alice King Chatham Medical Arts, Los Angeles) mounted 10 inches above the rat's head. These rats were housed singly in four round transparent cages in view of each other. Ringer's lactate with added sodium heparin (30 units/ml) was infused continuously at a rate of 0.25 ml/hr except during blood pressure measurements.

The first and second weeks after coarctation were chosen for physiological studies, because of the hypothesis that differences between experimental rats with and without renal ischemia would be most evident soon after the onset of hypertension. Periods of chronic arterial catheterization ranged up to 9 days. Each rat had blood pressures recorded in morning and afternoon, and the average of the two readings was used for analysis. Hemorrhage developed in some rats at the catheter site; they were disqualified from future pressure or hormone determinations. The catheterizations generally were terminated by drawing arterial blood samples. Arterial blood for the determination of plasma renin activity and catecholamine levels was drawn at approximately noon 7–10 days after coarctation. Because blood drawing followed several days of hemodynamic monitoring, the rats by this time were accustomed to the presence of a human operator and exhibited only mild behavioral responses to the preparations for drawing blood.

**Hormonal Assays**

Because of the volume of blood drawn, only one set of hormone determinations was performed for each catheterized rat. A volume of 2.2 ml blood was drawn within 30 seconds from the carotid catheter into a heparinized syringe and placed immediately on ice. The rat was then killed quickly by an overdose of intra-arterial pentobarbital. The blood was divided into two parts and placed in microcentrifuge tubes containing either EGTA/glutathione (for catecholamine assay) or EDTA (1.5 mg/ml). Samples were collected in chilled tubes and centrifuged to separate plasma, which was kept at −20°C (renin) or −80°C (catecholamines) until analyzed. Plasma renin activity is reported as nanograms antitensin I per milliliter plasma per hour of incubation after a 3-hour incubation. Plasma catecholamines were assayed by the radioenzymatic method of Da Prada and Yurcher and reported as picograms per milliliter plasma.

**Statistics**

Analysis of variance was used to analyze the morphometric data, with multiple comparisons based on the Bonferroni inequality. Because leukocyte infiltration of subendothelium deviated highly from a normally distributed process and resembled instead a Poisson distribution (i.e., many sections having no leukocytes at all, large interanimal variances), a square root transformation was performed in this instance before statistical analysis. The use of the multiple pairwise comparisons test with $n=3$ (grouped as control rats, coarcted rats, and coarcted rats with ischemic kidneys) may be too conservative with respect to the comparison between control and coarcted rats because the original intent of the study was to compare these two groups. A priori comparison by means of a $t$ test was performed in some instances in addition to the multiple comparisons procedure.

**Results**

**Condition of Rats in Morphometric Study**

The rats recovered well from the initial coarctation procedure. However, in the two experimental groups, 27% of the rats died between 2 and 13 days postoperatively. The placement of the aortic ligature proximal or distal to the anterior mesenteric artery origin had no apparent effect on mortality. Platelet-fibrin embolism from the stenotic site was suspected as a cause of this mortality (see Discussion). All rats submitted to fixation showed no signs of functional impairment or acute distress throughout the study. However, the four rats with ischemic kidneys gained only 73±54 g (mean±SD) as compared with weight gains of 144±13 g in the control group and 161±14 g in rats with coarctation and normal kidneys.

**Attainment of Hypertension**

Table 1 shows hemodynamic data obtained in rats just before fixation and heart weights determined after fixation. Mean arterial pressures ranged from 116 to 138 mm Hg in control rats, from 144 to 193 mm Hg in coarcted rats with normal kidneys and from 124 to 180 mm Hg in coarcted rats with ischemic right kidneys. Thus, a moderate elevation of blood pressure was induced regularly. When heart

**TABLE 1. Hemodynamic Data and Heart Weights in Rats Studied Morphologically**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>Coarcted</th>
<th>Coarcted and ischemic kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. in group</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>125 (7)</td>
<td>163 (17)</td>
<td>161 (22)</td>
</tr>
<tr>
<td>Carotid-femoral pressure gradient (mm Hg)</td>
<td>2 (7)</td>
<td>45 (15)</td>
<td>34 (15)</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.62 (0.11)</td>
<td>2.11 (0.27)</td>
<td>1.70 (0.16)</td>
</tr>
<tr>
<td>Heart wt/final body weight (g)</td>
<td>0.0042</td>
<td>0.0055</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

Values are mean±(SD).
weight was expressed as a percentage of final body weight, significant increases in heart weight were seen in both hypertensive groups (p=0.02 for each).

**Thoracic Aorta—Intimal Cellular Responses**

The endothelium of the thoracic aorta appeared similar in all three groups. Rats with ischemic kidneys showed slightly increased endothelial thickness, but this was not statistically significant (p=0.14 vs. control rats, see Table 2). No areas of endothelial desquamation or platelet adherence were seen in any group.

Mononuclear leukocytes occupied significantly increased volume, about twice control levels, in rats with coarctation hypertension (p=0.02) (Table 2 and Figures 1A and 1B). Rats with ischemic kidneys had similarly increased subendothelial leukocyte volumes, but the result was not significant because of the small number of rats in this group.

Smooth muscle cytoplasm in the subendothelial space was uncommon in all groups and showed no trends in the experimental groups. When observed, it tended to result from protrusion of a portion of cytoplasm through a wide fenestra of the elastic lamina, rather than dislocation of an entire smooth muscle cell into the subendothelium. It should be clearly stated, however, that these negative results may not pertain to intimal versus medial location of smooth muscle cells. The innermost elastic lamina, which marked the outer boundary of the subendothelial space in this study, is not always identical to the internal elastic lamina, which marks the boundary between intima and media. Intimal pads of mature smooth muscle cells, covered by elastic laminae, were found in all groups of rats. These were not quantifiable, because of frequent uncertainty about which elastic lamina represented the internal elastic lamina. In addition to mature smooth muscle in intimal pads, smooth muscle cells of immature appearance were found rarely in the intima in all groups. These immature cells contained increased endoplasmic reticulum and were generally covered by a thin discontinuous layer of elastin.

Intimal cysts were defined as membrane-bounded regions containing generally homogeneous granular material that sometimes contained abnormal cellular organelles (Figure 1B). The aggregate volume of cysts in the subendothelium was increased significantly in rats with ischemic kidneys (Table 2). Smaller cysts were found, though rarely, in the medial layer in all groups.

**Thoracic Aorta—Extracellular Material**

Extracellular structure could be satisfactorily divided into five major categories: basement membrane–like material, banded fibrillar collagen, elastin, dense clumped granular material, and light granular areas. Basement membrane–like material was the predominant component of subendothelium in control and coarcted hypertensive rats with normal kidneys. It had a multilamellar appearance (Figure 1). Thoracic aortas from rats with coarctation hypertension showed an average 67% increase in thickness of the region of basement membrane–like material. In a test comparison against control rats (according to the original intent of the study), the significance of this finding was p=0.02. In a three-way comparison, this effect remained significant (0.04 < p < 0.05). In rats with ischemic kidneys, pronounced subendothelial thickening with a 260% increase in basement membrane–like material (p=0.01) was found (Table 2).

The stereologic technique employed in this study allowed ascertainment of the depth-related volume composition of the first 10–15 μm of the aortic wall lying beneath the innermost elastic lamina (Figure 2). The most striking finding was an increase in volume fractions of basement membrane–like material appearing within the inner thoracic aortic media. Statistical analysis was performed within depth ranges of 0–0.5, 0.5–3, and 3–10 μm. In the last instance,

### Table 2. Selected Morphological Features in Thoracic and Abdominal Aorta Within Innermost Elastic Lamina

<table>
<thead>
<tr>
<th>Morphological feature</th>
<th>Thoracic</th>
<th>Abdominal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Coarct</td>
</tr>
<tr>
<td>Endothelium</td>
<td>0.970 (0.124)*</td>
<td>0.920 (0.086)</td>
</tr>
<tr>
<td>Subendothelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>0.055 (0.051)</td>
<td>0.126 (0.047)†</td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>0.049 (0.148)</td>
<td>0.051 (0.278)</td>
</tr>
<tr>
<td>Cysts</td>
<td>0.049 (0.056)</td>
<td>0.057 (0.074)</td>
</tr>
<tr>
<td>Basement membrane–like material</td>
<td>0.344 (0.125)</td>
<td>0.576 (0.172)†</td>
</tr>
<tr>
<td>Light granular material</td>
<td>0.008 (0.019)</td>
<td>0.059 (0.111)</td>
</tr>
<tr>
<td>Clumped granular material</td>
<td>0.060 (0.093)</td>
<td>0.052 (0.123)</td>
</tr>
</tbody>
</table>

Values given are mean±(SD).
*Average thickness or volume/luminal surface area, in micrometers.
†Significantly different from control (p<0.05).
the increase in basement membrane–like material was statistically significant; this was true for either hypertensive group compared with the control group. Point-counting techniques on separate micrographs, positioned approximately halfway between the internal and external elastic laminae in the thoracic aorta, confirmed that the increase in basement membrane–like material was also found deep within the tunica media (Figures 3 and 4, \( p=0.04 \) for control vs. hypertensive rats with normal kidneys, \( p<0.001 \) for
control rats vs. rats with ischemic kidneys). In the case of rats with ischemic kidneys, the volume fraction of mature elastin was significantly lower in both shallow (3–10 μm) and deep media, making room for the increased fraction of basement membrane–like material.

A recently recognized morphological finding in the subendothelial space had the form of light granular or reticular areas, which always were surrounded by widened areas of multilamellar basement membrane (Figure 1). These areas were associated with and often adjacent to subendothelial leukocytes or, occasionally, smooth muscle cells. Only one of 18 thoracic aortic sections from control rats showed this appearance. Light granular areas were found in four of 21 sections from hypertensive rats with normal kidneys and in nine of 12 sections from hypertensive rats with ischemic kidneys. These areas typically had distinct outlines with an appearance of being "carved out" of the multilamellar basement membrane in the subendothelium.

Dense clumped granular material could be distinguished from basement membrane–like material by a flocculent appearance and a lack of any regular spacing in the former. This material was located typically just beneath the endothelial layer (Figure 5). It was occasionally associated with attenuation of the overlying endothelium or with the nearby presence of subendothelial leukocytes. Its occurrence was rather variable. Two of 18 control thoracic aortic sections showed dense clumped material. It was found in four of 21 sections from hypertensive rats with normal kidneys and in eight of 12 from rats with hypertension and ischemic kidneys.

Abdominal Aorta

Mean endothelial thickness in the abdominal aorta was slightly increased in the experimental groups compared with the control group (Table 2), but the differences did not approach statistical significance. The subendothelial layer was much simpler in the abdominal aorta (Figure 6) compared with the thoracic. Leukocytes were remarkably absent (Table 2). Of 34 sections from all groups, only one showed a leukocyte. Cystic structures, equally distributed among the control and experimental groups, were likewise much less frequent than in the thoracic aorta. Basement membrane–like material in the subendothelial region had very similar thickness in control and hypertensive rats with normal kidneys. It was modestly increased in rats with ischemic kidneys, but this increase was not statistically significant and did not occur with the proportion or consistency of the effect seen in the thoracic aorta. Dense clumped material was essentially absent, and light granular areas were markedly reduced in abdominal aortic subendothelium in all groups, when compared with thoracic aortic subendothelium.

The volume composition extending 10 μm into the first one or two musculoelastic layers in the abdominal aorta is shown in Figure 2. The increase in basement membrane–like material that marked this region in the thoracic aorta was not found in the abdominal aorta. No statistically significant comparisons were found for any structures. Furthermore, stereologic analysis of the middle tunica media showed no significant differences among the three rat groups.

Results From Catheterized Animals

Because the lesions in the rats with renal ischemia resembled lesions described in the setting of more severe hypertension, it was speculated that these rats early after coarctation may have had blood pressure elevations different from the group with coarctation and normal kidneys. In the rats studied morphometrically, only a single, terminal time point had been used for blood pressure measurement. Therefore, chronic catheterization was performed to determine blood pressures in an additional set of coarcted and control rats with and without renal ischemia, focusing on the period 4–8 days postoperatively, when differences should have been most evident. Figure 7 is a composite record of mean arterial pressure during this time in four control rats, seven rats with coarctation, and six rats with coarctation and right renal ischemia. There is no major difference in the blood pressure responses between the two groups of hypertensive rats. Thus, there is no evidence to suggest that differing blood pressure responses would account for the exaggerated arterial wall changes in rats with ischemic kidneys.

**Figure 2.** Schematic diagrams of depth-related volume composition of inner aortic wall, derived from microcomputer-aided locational stereology in thoracic and abdominal aorta in all three groups. y Axis shows depth within arterial wall; x axis shows fraction of total volume. Endothelium is represented in white. Its detailed composition (see Table 2) is not shown here. Zero depth corresponds to reference surface (i.e., inner surface of innermost elastic lamina). Below reference surface, volume fractions of basement membrane–like material are represented on left with layered, reticular shading. Smooth muscle (black) is next, followed by collagen (dark diagonal shading) and elastin (light diagonal shading) at the far right. Endothelial and subendothelial thickness, depth ranges, and volume fractions are represented to scale as shown.
Results from hormonal determinations, performed between the seventh and 10th postoperative day, are shown in Figure 8 and Table 3. It was considered that hormonal alterations potentially affecting arterial morphology would most likely be evident at this time. Rats with coarctation alone, lacking renal ischemia, had plasma renin activities no different from the control rats. On the other hand, significant elevation of plasma renin activity was apparent in the rats in which right renal atrophy was found at autopsy.
These results are entirely consistent with the physiological concept of renin release in response to decreased renal perfusion. Plasma norepinephrine and epinephrine levels did not differ significantly among the groups of rats (Table 3).

**Discussion**

Two major features of the early response of the arterial wall to hypertension were demonstrated quantitatively in this study: leukocytic infiltration of the intima and accumulation of basement membrane–like material in both intima and media. Abnormal accumulation of basement membrane–like material in the tunica media has not been described previously. These changes were found in the hypertensive thoracic aorta, but not in the normotensive abdominal aorta. A new morphological feature was discerned in the expanded subendothelial space. This was the light granular area in the extracellular matrix, associated with subendothelial cells. An exaggerated arterial tissue response to hypertension was evident in the small number of rats in which right kidney ischemia developed. Whether this represents a pathogenic response of the artery to humoral, as opposed to mechanical, factors will require further study.

**Coarctation Procedure**

The coarctation procedure consistently produced blood pressure elevation of moderate degree. Carotid-
femoral pressure gradients in all groups were quite consistent with the degree of carotid arterial pressure elevation and support the notion that the lower abdominal circulation was normotensive. The relative increases in heart weight, compared with final body weight, in both hypertensive groups indicated chronicity of arterial pressure elevation.

Coarcted rats had substantial mortality (27%) occurring after recovery from the immediate effects of anesthesia and surgery. Autopsies did not reveal the cause of this later mortality, but two rats that subsequently died had fluctuating femoral artery pressure immediately after coarctation. This was suggestive of the cyclic flow variations at a stenosis described by Folts and coworkers and considered to represent platelet-fibrin thromboembolism. Intestinal ischemia due to thrombosis or embolism represents the best hypothesis for cause of death. Despite this mortality, successful experimental animals were considered to give valid results because the morphological changes of interest were demonstrated proximal to the stenosis and could not be due to embolization.

Subendothelial Leukocytes
The presence of subendothelial, blood-derived leukocytes in arteries of hypertensive rats was first noted by Esterly and Glagov. This finding was confirmed by Still as an early response to hypertension induced by an occlusive aortic ligature placed between the origins of the renal arteries. More recently, Kowala and coworkers have used light microscopy to quantify leukocytic infiltration of hypertensive rat aorta. Because endothelial cytoplasm is not resolved by en face light microscopy, the assignment of leukocytes to luminal versus subendothelial locations depended, in that study, on nuclear morphology and plane of focus. The present results confirm the accumulation of leukocytes in a subendothelial location, documented by quantitative electron microscopic techniques.

Basement Membrane–like Material
Many reports have described widening of the subendothelial space in hypertension. Gabbiani et al noted that this space contained in many instances material with the appearance of multilamellar, reticular basement membrane. The present results demonstrate quantitatively for the first time the accumulation of the basement membrane–like material in the intima and media of hypertensive rats.

The most intriguing new result is the increased basement membrane–like material in the tunica media of hypertensive rats. The dramatic response found in the rats with ischemic kidneys was helpful because it

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Epinephrine (pg/ml)</th>
<th>Norepinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>365 (151)</td>
<td>980 (611)</td>
</tr>
<tr>
<td>Coarcted</td>
<td>5</td>
<td>200 (146)</td>
<td>587 (118)</td>
</tr>
<tr>
<td>Coarcted and ischemic kidney</td>
<td>5</td>
<td>573 (506)</td>
<td>962 (562)</td>
</tr>
</tbody>
</table>

Values are mean±(SD).
was the same pattern of response found to a lesser degree in the hypertensive rats with normal kidneys. Although intimal accumulation of basement membrane-like material appears to represent an endothelial cell response, the similar effect in the tunica media must represent a response of smooth muscle cells. Although basement membrane accumulation in the tunica media has not been described previously, either qualitatively or quantitatively, several earlier reports are consistent with this result. Olivetti et al reported an absolute and relative increase in collagen in thoracic aorta of rats with aortic coarctation, determined from ultrastructural morphometry. They did not distinguish basement membrane-like material as an ultrastructural entity separate from fibrillar collagen; their term "collagen" may include both. Accumulation of basement membrane is consistent with early work of Crane, who found in hypertensive rat mesenteric arteries an increase in staining by combined periodic acid-Schiff and colloidal iron technique, as well as an increase in $^{35}$SO$_4$ incorporation. Basement membranes stain in this manner and are associated with sulfate-containing glycosaminoglycans. Hollander and colleagues found increased glycosaminoglycans in the thoracic aorta of monkeys made hypertensive by aortic coarctation. Basement membrane could contribute to the increases of both collagenous and noncollagenous proteins found by Wolinsky in the aortic wall of hypertensive rats.

It has been proposed that smooth muscle throughout the body responds to increased passive tensile stress by the synthesis of additional tension-bearing proteins. In the aorta of hypertensive rats, this response includes cellular hypertrophy with increased actomyosin as well as increased collagen and elastin. Basement membrane accumulation may be an early phase in the response, involved in the distribution and transmission of tensile stress between cells and extracellular matrix as the artery wall remodels. This hypothesis requires further study.

Other Structural Features

The light granular area, appearing in a widened subendothelial region containing multilamellar basement membrane, was first mentioned in the recent report of Kowala and colleagues on hypertensive rat aorta. These areas were essentially always in proximity to a subendothelial cell, usually a monocyte-macrophage or lymphocyte. This relation, plus the carved-out appearance of the light granular area, leads us to speculate that it might represent proteolysis of basement membrane due to proteases secreted from the subendothelial cell. Proteolysis of endothelial basement membrane may occur whenever leukocytes migrate into the subendothelial space. Whether our morphological findings are a result of such a process remains unclear, however. An alternative explanation is that migrating leukocytes may tend to squeeze into the loosest spaces available.

Two other structural features, intimal cysts and dense clumped granular material, were variably increased in the subendothelial space. Intimal cysts have been studied previously in detail and are considered to represent degenerating cytoplasm derived mostly from herniation and detachment of portions of medial smooth muscle cells. Dense clumped granular material occurred mostly in those regions of thoracic aortic subendothelium that exhibited the greatest degree of overall morphological change and subendothelial widening. Similar morphological alterations have been described most often in rat models of severe hypertension (near or over 200 mm Hg mean pressure, see References 33 and 34). It has been suggested that this clumped or flocculent material represents precipitated plasma proteins that have penetrated the endothelium in accord with the known increased permeability of arterial endothelium early after the onset of hypertension.

Renal Ischemia and Morphological Alterations

The development of renal ischemia in more than one third of the experimental rats was advantageous for this study because it produced an extended spectrum and a graded response pattern for certain arterial wall features. The cause of the exaggerated tissue response remains obscure because these rats not only had elevated renin levels, but also exhibited poor weight gain. The blood pressure data from catheterized rats failed to reveal major differences associated with renal ischemia, but less obvious changes, such as an altered rate of rise of blood pressure in the first 4 days, cannot be ruled out. Several hormones, including renin, mineralocorticoids, and catecholamines have been suggested to have pathogenic effects on the arterial circulation. The present results are not definitive with regard to a humoral effect on arterial tissue responses but do give impetus to further studies of these putative effects employing quantitative ultrastructural end points. However, the coarctation model for hypertension may not be ideal for such studies because the time course of blood pressure ideally should be monitored in the same rats examined morphologically. Chronic arterial catheterization is necessary to monitor blood pressure in coarcted rats, and in a previous study, catheterization itself or perhaps stress associated with catheterization appeared to induce morphological alterations in distant arteries in control rats.

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References


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Ultrastructure of hypertensive rat aorta. Increased basement membrane-like material.
J R Guyton, D T Dao, K L Lindsay and A A Taylor

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