Effect of Salt on the Vascular Lesions of Spontaneously Hypertensive Rats

Catherine Limas, M.D., Barbara Westrum, M.S., Constantin J. Limas, M.D., and Jay N. Cohn, M.D.

SUMMARY High salt intake accelerates hypertension in humans and increases cardiovascular morbidity and mortality. The temporal relation between blood pressure (BP) elevation and appearance of vascular lesions during salt-loading was studied in the spontaneously hypertensive rat (SHR). Starting at 5 weeks of age, SHRs and normotensive Wistar-Kyoto rats (WKYs) were given 1% NaCl in their drinking water; SHRs and WKYs on tap water served as controls. Animals from each group were sacrificed at 10 and 20 weeks of age, and the aorta and intrarenal vessels were studied by light and electron microscopy.

Neither BP nor vascular morphology of WKYs were affected by 1% NaCl. In SHRs, the course of BP was not affected by the addition of salt for at least 11 weeks, but vascular changes were significantly aggravated within 5 weeks. Thus, aortic intimal lesions progressed more rapidly so that, by 20 weeks of age, 50% of the animals had 3+ lesions while, in control SHRs, they did not exceed the 2+ grade. Salt-loading resulted in significant thickening of the aortic media between the 10th and 20th week of age while control SHRs showed no increment within the same time interval. Also, small intrarenal arterial vessels of salt-treated SHRs had significantly narrower lumina and greater wall thickness at both 10 and 20 weeks of age. In addition, they showed intimal proliferations and necrotizing lesions which were absent from control SHRs at these ages.

These results show that, in this experimental model, the aggravation of vascular changes is not merely a sequel of further elevation of BP. Since the adverse effect of salt on the vessels was not seen in WKYs, it is likely that this effect is related to genetic factors or to higher susceptibility of hypertensive vessels.

KEY WORDS • endothelium • vascular smooth muscle • genetic hypertension • vascular pathology

VASCULAR lesions that develop in the course of human and experimental hypertension are the major cause of morbidity and mortality from this disease. Increased intraluminal pressure as well as humoral and neurogenic influences that accompany the hypertensive state contribute to the pathogenesis of vascular lesions.1,2 Structural changes in resistance vessels may maintain the BP elevation even after removal of the initiating hypertensive stimulus.3,4

The Aoki-Okamoto strain of spontaneously hypertensive rats (SHRs), a model of human essential hypertension,5 offers the opportunity to study the pathogenesis of hypertensive vascular lesions. In these animals, BP increases gradually during postnatal growth and is associated with the development of morphologic changes in arterial vessels.5,6 These changes consist of increased wall thickness noted in vessels of all sizes and an expansion of the subendothelial space predominantly in the aorta. Vascular lesions follow the course of hypertension in young SHRs and continue to progress even after stabilization of BP.7

Hypertension in SHRs is genetically determined, but its course can be modified by environmental influences, such as high salt intake.9,10 Administration of excess salt to SHRs results, after a lag period of several weeks, in accelerated BP increase secondary to elevated systemic vascular resistance.8,9 The role of structural vascular changes in the salt-induced modification of the hypertensive process has not been studied and is the focus of this report. The results indicate that vascular lesions precede, and may be partly responsible for, the acceleration of hypertension in salt-loaded SHRs.
Materials and Methods

Experiments were carried out on male spontaneously hypertensive rats (SHRs) of the Aoki-Okamoto strain and age-matched Wistar-Kyoto (WKY) controls. The animals were obtained from Taconic Farms, Inc., Germantown, N.Y. Starting at 5 weeks of age, groups of SHRs and WKYs (12 animals each) were given 1% NaCl in their drinking water; SHRs and WKYs (10 animals in each group) on regular tap water served as controls. The amount of water consumed was measured daily, and body weight was recorded weekly. Systolic blood pressure (SBP) was measured at 2-week intervals between 9 and 10 a.m. The animals were prewarmed to 37°C, lightly anesthetized with methoxyflurane, and their BP obtained with a tail cuff sphygmomanometric apparatus.

Morphologic studies of salt-loaded and control animals were carried out at 10 and 20 weeks of age. The animals were lightly anesthetized, the abdominal cavity opened, the renal arteries clipped, and the kidneys removed and immediately fixed to be processed for light and electron microscopy. The aortic trifurcation was freed from surrounding tissues, the chest was quickly opened, and perfusion was started through the left ventricle while the aorta was cut open above the trifurcation. Total preparation time did not exceed 30 seconds. Perfusion was performed under 100 mm Hg pressure, and a flow of approximately 20 ml/min, first for 20 seconds with isotonic cacodylate buffer (pH 7.4) and then for 2 minutes with a fixative containing 2.5% glutaraldehyde in cacodylate buffer. At the end of this period, the aorta was clamped just above the diaphragm and equilibrated with the column of fixative fluid. Additional fixative was poured on the aorta, which was left to fix in situ for 3 more minutes. It was then clamped at the arch, removed from the body, cleaned of periadventitial tissues and sectioned transversely into 12–18 segments (approximately 0.3 cm in length) numbered sequentially 1 through 18, which were placed in appropriate fixatives. The preparations obtained by this method of in situ fixation were consistently good; there was no artifact damage to the endothelium, and the vessel wall was well distended as judged by the appearance of the elastic lamellae (fig. 1).

Measurements of wall thickness were made in duplicate independently by two observers (C.L. and B.W.) on epon-embedded sections of aorta cut at 1 μm thickness and stained with methylene blue. A filar micrometer calibrated so that each division corresponded to 0.2 μm was inserted in the eyepiece of a Zeiss microscope. Measurements were taken at a magnification of 400 X. To determine the thickness of aortic media, defined as the distance between the innermost and outermost elastic lamellae, 3–4 transverse segments of the thoracic aorta were used from each animal, and 15 measurements were obtained per section. Segments 1, 5, 9, and sometimes 13 (starting just below the left subclavian artery and at 1.2 cm intervals thereafter) were used for quantitation.

For a semiquantitative assessment of intimal lesions, 1 μm thick sections from all transverse segments from each aorta were examined under oil immersion (1000 X magnification). The changes were classified as follows:

**FIGURE 1.** Transverse section (1μm thick) of thoracic aorta from a 20-week old SHR; note uniform stretching of elastic lamellae (toluidine blue, X 40).
diameters was plotted, and the vessels were classified into three groups with external diameters 10-29, 30-49, and 50-100 \( \mu m \). The external/luminal diameter ratio was then calculated for each vessel. The total number of vessels suitable for quantitation varied from 14-20 per section. The external/luminal diameter ratio was then calculated for each vessel. Because of the heterogeneity of vessel sizes even within the same size class, this ratio is the most meaningful parameter for comparison of experimental groups. The wall thickness of each vessel was obtained within the same size class, this ratio is the most meaningful parameter for comparison in different age groups. As noted above, it was elected not to perfuse the kidneys because, in preliminary experiments, we had found that perfusion is not uniform throughout the kidney, and this may result in uneven distribution of pressures and variable degrees of vascular distention. The kidneys were, instead, removed from the live animal and immediately sectioned and fixed by immersion, a procedure that gave a consistent topographical distribution so that anatomically similar vessels can be identified for comparison in different age groups. As noted above, it was elected not to perfuse the kidneys because, in preliminary experiments, we had found that perfusion is not uniform throughout the kidney, and this may result in uneven distribution of pressures and variable degrees of vascular distention. The kidneys were, instead, removed from the live animal and immediately sectioned and fixed by immersion, a procedure that gave the most reproducible preparations. Four blocks, each representing one-half of a kidney bisected along its long axis, were examined in each animal. In each section, all transversely cut arterial vessels with external diameters 30-100 \( \mu m \) were quantitated by measuring two external and two luminal diameters per vessel using the filar micrometer at a magnification of 400 \( \times \). The total number of vessels suitable for quantitation varied from 14-20 per section. The external/luminal diameter ratio was then calculated for each vessel. Because of the heterogeneity of vessel sizes even within the same size class, this ratio is the most meaningful parameter for comparison of experimental groups. The wall thickness of each vessel was obtained as ED-LD/2. The frequency distribution of external diameters was plotted, and the vessels were classified into three groups with external diameters 10-29, 30-49, and 50-100 \( \mu m \). The means and SE of means for luminal diameters, ED/LD ratios, and wall thickness were then calculated for each size class separately in each animal and the statistical evaluation was based on these values; \( p \) values were obtained by the Student's \( t \) test.

In addition to routine processing for epon embedding, 3-4 rings from each aorta were stained with ruthenium red according to the method of Luft as modified by Groniowski et al. Briefly, aortic segments were immersed for 3 hours at 0\°C in a fixative containing 2.5% glutaraldehyde in cacodylate buffer (pH 7.3) to which 3 mg/ml ruthenium red per ml were added. The tissues were then rinsed in cacodylate buffer and fixed for 3 more hours at room temperature in a medium containing 2% OsO\(_4\) and 3 mg/ml ruthenium red in the same buffer. Cationized ferritin was prepared by Miles-Yeda Ltd. according to the method of Danon et al. Tissue segments were immersed in a solution containing 0.2 mg/ml cationized ferritin in cacodylate buffer, fixed in 2.5% glutaraldehyde, and subsequently processed for electron microscopy as usual. Ruthenium red stains the glyocalyx of endothelium and acid mucopolysaccharides in the subendothelium. Cationized ferritin, under the conditions of the experiment, stains the glyocalyx without penetrating through intact endothelium.

**Results**

The SBP course is shown in figure 2. A difference in BP between SHRs and WKYs was first noted at 4 weeks of age, and this difference increased with advancing age. The SHRs on 1% NaCl showed, prior to the 20th week of age, a BP course similar to that of SHRs on a regular diet. There was a statistically significant difference in the average BP between the two groups of SHRs at 11 weeks of age. However, no such differences were noted in subsequent measurements until 20 weeks of age when higher BP (229 ± 3 vs 202 ± 4 mm Hg, \( p < 0.001 \)) was recorded in salt-loaded SHRs. Water consumption was higher in salt-loaded animals (average per animal, 71 ml/day for SHRs and 62 ml/day for WKYs) compared to the controls (52 ml/day for SHRs and 50 mg/day for WKYs). Body weights increased at a similar rate in both groups between 4 and 10 weeks of age; later, SHRs weighed an average of 15 g less than WKYs. The addition of salt did not change the growth pattern.

**Aortic Intimal Changes**

In normotensive 5 and 10 week old WKYs, the aortic intima consisted of a layer of endothelial cells which, on transverse sections, showed a low profile and lay almost directly upon the internal elastic lamina (IEL). There was essentially no subendothelial space and only a thin layer of basement-membrane-like material separating the endothelium from the IEL (fig. 3). In 20-week-old WKYs, a minimal subendothelial space (SES) containing finely granular material appeared focally. The 5-week-old SHRs showed aortic morphology identical to that of age-matched WKYs, despite a 15 mm Hg difference in SBP. At 10 weeks of age, two of the five SHRs on regular salt intake showed 1+ increase in SES with accumulation of finely granular material (fig. 4a). At 20 weeks, these intimal changes were seen in almost all control SHRs and had advanced in severity but did not exceed 2+ grade. In addition to the granular material, mononuclear cells with ultrastructural characteristics of monocytes and lymphocytes were seen in the subendothelium along with focal bundles of collagen and islets of elastin. The endothelial cells of SHRs were irregular, with laterally attenuated...
Increased NaCl intake did not affect the evolution of aortic intimal structure of normotensive animals (WKYs). The aortic intimal changes of SHRs increased in extent and severity so that 2+ lesions appeared at 10 weeks of age (table 1 and figure 4b) and 3+ changes (figure 5) were noted in 50% of the 20 week old animals. The endothelial abnormalities paralleled the severity of subendothelial expansion so that in salt-loaded SHRs there was often marked attenuation of the lateral endothelial cytoplasm which stretched over the excessive subendothelial matrix (fig. 5).

| Table 1. Aortic Intimal Changes in WKYs and SHRs: Effect of Age and 1% NaCl |
|---------------------------------|------------------------|------------------------|------------------------|------------------------|
|                                 | 10 weeks old           | 20 weeks old           |
|                                 | Normal 1+ 2+ 3+        | Normal 1+ 2+ 3+        |
| WKY                             |                        |                        |
| Tap water                       | 5 0 0 0                | 5 0 0 0                |
| 1% NaCl                         | 6 0 0 0                | 6 0 0 0                |
| SHR                             |                        |                        |
| Tap water                       | 3 2 0 0                | 1 2 2 0                |
| 1% NaCl                         | 3 1 2 0                | 0 1 2 3                |

cytoplasm which overlay the expanded SES. The intercellular junctions were preserved although their orientation was distorted and their length shortened.

Figure 3. Normal endothelium from a 10-week old WKY rat. There is only a minimal subendothelial space. Ruthenium red stains the luminal surface of the endothelium and the adjacent pinocytotic vesicles (ruthenium red × 11935).

Figure 2. Course of systolic blood pressure in WKYs and SHRs with or without 1% NaCl in their drinking water. Results are expressed as mean ± se for 12 measurements in each group.
In addition to these quantitative differences, focal electro-dense deposits were seen only in the 20-week-old SHRs on 1% NaCl (fig. 6) and were more frequent and more intense around the ostia of aortic branches. The subendothelial matrix of both groups of hypertensive animals stained with ruthenium red in a pattern considered characteristic for acid mucopolysaccharides and was more intense in salt-loaded SHRs (fig. 7).

Aortic medial thickness was essentially unchanged by salt-loading in WKYs (table 2). The SHRs on high salt intake showed a significant increase in aortic medial thickness at 20 weeks of age compared to SHRs on tap water (table 3).

Intrarenal Blood Vessel Changes

In the kidneys, we studied arcuate and interlobular arteries with external diameter ranging from 30–100 µm. The size of these vessels changes between 5 and 10 weeks of age apparently due to the normal growth process, and this is reflected in the classification of vessels according to size (table 2). In WKYs, there was no change in luminal diameters of small (30–49 µm) intrarenal vessels between 10 and 20 weeks of age (table 2). The 50–100 µm arterial vessels showed a small increase in luminal diameters with a concomitant increase in wall thickness, so that the external/luminal diameter (ED/LD) ratio remained stable for all vessels of normotensive animals.
FIGURE 5. Aortic intima of a 20-week old SHR on 1% NaCl. There is a marked (3+) widening of the subendothelium which contains ruthenium-red positive material and mononuclear cells. The endothelium bulges into the lumen and its markedly attenuated cytoplasm stretches over the subendothelial matrix (ruthenium red. × 7500).

TABLE 2. Effect of 1% NaCl on the Vessels of Wistar-Kyoto Normotensive Rats

<table>
<thead>
<tr>
<th>Intrarenal vessel size (μ)</th>
<th>5 weeks</th>
<th>10 weeks No NaCl</th>
<th>1% NaCl</th>
<th>20 weeks No NaCl</th>
<th>1% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD (μ)</td>
<td>11.9 ± 0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20-29 ED/LD</td>
<td>2.25 ± 0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>7.42 ± 0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LD (μ)</td>
<td>16.84 ± 0.8</td>
<td>15.2 ± 0.6</td>
<td>14.3 ± 0.4</td>
<td>14.9 ± 0.7</td>
<td>14.1 ± 0.3</td>
</tr>
<tr>
<td>30-39 ED/LD</td>
<td>2.25 ± 0.1</td>
<td>2.73 ± 0.07</td>
<td>2.82 ± 0.10</td>
<td>2.82 ± 0.10</td>
<td>3.00 ± 0.3</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>10.55 ± 0.3</td>
<td>12.9 ± 0.4</td>
<td>12.49 ± 0.5</td>
<td>13.5 ± 0.5</td>
<td>13.83 ± 0.9</td>
</tr>
<tr>
<td>LD (μ)</td>
<td>—</td>
<td>22.9 ± 1.1</td>
<td>22.5 ± 1.3</td>
<td>24.2 ± 2.7</td>
<td>23.9 ± 1.1</td>
</tr>
<tr>
<td>50-100 ED/LD</td>
<td>—</td>
<td>2.92 ± 0.12</td>
<td>2.90 ± 0.1</td>
<td>2.84 ± 0.31</td>
<td>2.90 ± 0.13</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>—</td>
<td>21.7 ± 0.35</td>
<td>21.3 ± 0.5</td>
<td>22.5 ± 0.76</td>
<td>23.0 ± 1.7</td>
</tr>
<tr>
<td>Medial</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aorta Thickness (μ)</td>
<td>69.67 ± 2.5</td>
<td>81.0 ± 1.6</td>
<td>79.7 ± 0.2</td>
<td>80.4 ± 2.5</td>
<td>81.7 ± 1.4</td>
</tr>
</tbody>
</table>

LD = luminal diameter; ED = external diameter.
TABLE 3. Effect of 1% NaCl on the Vessels of SHRs

<table>
<thead>
<tr>
<th>Intrarenal vessel size (μ)</th>
<th>5 weeks</th>
<th>10 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No NaCl</td>
<td>1% NaCl</td>
<td>No NaCl</td>
</tr>
<tr>
<td>LD (μ)</td>
<td>10.98 ± 0.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>20-29 ED/LD</td>
<td>2.34 ± 0.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>7.38 ± 0.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LD (μ)</td>
<td>16.38 ± 0.6</td>
<td>13.6 ± 0.3</td>
<td>10.1 ± 0.4*</td>
</tr>
<tr>
<td>30-49 ED/LD</td>
<td>2.31 ± 0.1</td>
<td>2.96 ± 0.06</td>
<td>3.97 ± 0.22*</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>11.48 ± 0.4</td>
<td>13.50 ± 0.1</td>
<td>14.53 ± 0.2*</td>
</tr>
<tr>
<td>LD</td>
<td>—</td>
<td>22.8 ± 1.3</td>
<td>21.9 ± 1.2†</td>
</tr>
<tr>
<td>50-100 ED/LD</td>
<td>—</td>
<td>3.00 ± 0.12</td>
<td>3.7 ± 0.16*</td>
</tr>
<tr>
<td>Thickness (μ)</td>
<td>—</td>
<td>23.81 ± 0.3</td>
<td>26.33 ± 0.4*</td>
</tr>
<tr>
<td>Aorta thickness (μ)</td>
<td>68.75 ± 1.4</td>
<td>89 ± 1.1</td>
<td>88.2 ± 1.1†</td>
</tr>
</tbody>
</table>

LD = luminal diameter; ED = external diameter.
*p < 0.01 (SHRs on 1% NaCl compared to age matched SHRs on tap water).
†p ≥ 0.1 (SHRs on 1% NaCl compared to age matched SHRs on tap water).
‡p = 0.025 (SHRs on 1% NaCl compared to age matched SHRs on tap water).
In SHRs on regular salt diet, there was a progressive decrease in average luminal diameters associated with increasing wall thickness so that the ED/LD ratio increased with age (table 3). When compared to WKYs, differences in LD and ED/LD ratios were significant for the small (30–49 \( \mu \)m) vessels at 10 and 20 weeks, while for 50–100 \( \mu \)m vessels the differences became statistically significant at 20 weeks of age.

Salt-loading did not affect the intrarenal vessels of WKYs. In both age groups of SHRs, however, 1% NaCl administration resulted in further reduction of luminal diameters and increased ED/LD ratios for both vessel size classes (table 3). The differences between the two experimental groups of SHRs for the 30–49 \( \mu \)m vessels were significant at both 10 and 20 weeks of age. For the 50–100 \( \mu \)m vessels, significant differences were observed in ED/LD ratios at 10 weeks of age; at 20 weeks, the difference was blunted. Salt-loaded SHRs had always smaller mean luminal diameters and greater mean wall thickness, although the differences were not always statistically significant.

These quantitative differences were associated with the following changes in the histologic appearance of the intrarenal vessels. Salt-loaded SHRs showed a
EFFECT OF SALT ON VASCULAR LESIONS/Limas et al.

485

FIGURE 8. a. Small intrarenal artery from a 20-week old, salt-loaded SHR. The ultrastructure of the asymmetrical "pad" formation at the area indicated by the arrow is shown in Figure 9 (H & E, × 400). b. Small intrarenal artery of the same animal shows marked luminal narrowing by an aggregate of intimal cells and matrix (H & E, × 400).

marked accentuation of the “pads” at the branching points of small arterial vessels (fig. 8a). Such “pads” are inconspicuous in WKYs and only focally and moderately accentuated in SHRs on tap water. In SHRs on 1% NaCl, there were frequent asymmetrical narrowings of the lumina by clusters of cells and interstitial matrix located inside the IEL (fig. 8b). Such changes are rarely seen in SHRs before 28 weeks of age. By electron microscopy, the prominent “pads” consisted of conglomerates of smooth muscle cells and basement membrane-like material (fig. 9). The intimal proliferations consisted of irregular aggregates of endothelial cells and basement membranes that protruded into the lumen in a polypoid fashion (fig. 10). Finally, fibrinoid necrosis of intrarenal vessels was seen only in salt-loaded SHRs at the two age groups studied (fig. 11).

Discussion

Epidemiologic studies in humans suggest a positive correlation between habitual salt intake and the prevalence of hypertension.14-17 Populations that consume a low-salt diet do not exhibit an age-related rise in BP and are largely exempt from hypertensive vascular disease.14, 17 In contrast, the prevalence of hypertension is high in populations whose diet is high in salt.15, 18 Although a relationship between salt intake and BP can be convincingly demonstrated by comparing populations at the extremes of salt intake, this is less apparent when individual subjects are placed on diets with various salt content.19 The reasons for this are unclear but may be related to the fact that the BP response to increments in salt intake depends to a large extent on genetic susceptibility. Variations in genetic susceptibility to the hypertensinogenic effect of salt have been observed in experimental animals.19-21

Experimental animal hypertension, similar to human essential hypertension, results from the interaction between genetic and environmental influences, the relative importance of which varies in each hypertensive model. There are two types of interaction: 1) development of hypertension is genetically determined and environmental influences serve only to modify its course and severity; 2) hypertension does not develop unless specific environmental influences are present. These two types of interaction are exemplified by the SHR and Dahl salt-sensitive (S) rat respectively. The SHRs are genetically prone to develop hypertension, the course and severity of which is, however, modified by external factors such as salt intake.9, 10, 22, 23 The Dahl S rat, on the other hand, requires salt-loading to become hypertensive.20, 21 Our present study was designed to examine the first type of interplay between an exogenous factor (salt) and a genetic model of hypertension (SHR).

Administration of salt to SHRs results in accelerated rise of BP, the rate of which depends on the amount of salt ingested, the age at institution of high salt intake, and the sex of the animals.9, 10, 22, 23 There is a lag phase of several weeks between the start of salt-loading and the appearance of steeper rises in BP.9 Therefore, this experimental model provides the opportunity to study vascular pathology prior to acceleration of BP in response to salt-loading. The results of the present study strongly suggest that, in the SHR, salt excess induces vascular changes that are not secondary to accelerated BP increase.

Our previous study on the evolution of vascular pathology in SHRs demonstrated that, at 5 weeks of
In SHRs, the small intrarenal arteries show progressive narrowing of the lumina due to increasing wall thickness, which is reflected in increased ED/LD ratios. This ratio is the most appropriate parameter when vessels with a range of external diameters are compared in different experimental groups. Because of the focal nature of the early hypertensive lesions, a large number of vessels must be evaluated for differences to reach statistical significance, particularly in the younger age group. Statistically significant differences were first demonstrated in the smaller intrarenal vessels between 10-week-old SHRs and WKYs. Ultrastructural studies have shown that thickening of the small vessel walls is due to an increase in smooth muscle mass (hyperplasia and/or hypertrophy of smooth muscle cells) while the subendothelial space does not show the expansion noted in the aorta.

The SHRs on high salt intake show the vascular changes described above as well as a time-dependent progression. The frequency and severity of these changes are, however, significantly accentuated so that, at 10 and 20 weeks of age, after 5 and 15 weeks on salt, the overall vascular pathology of salt-loaded SHRs is significantly more advanced than in untreated animals. A marked difference in the pathology of small intrarenal vessels is already noted at 10 weeks of age when the average BP has not changed in comparison to SHRs on regular salt intake. The rate of further increases in ED/LD ratios and wall thickness in salt-loaded SHRs slows so that the difference from control SHRs has not widened. It appears, therefore, that the differences are predominantly due to the
FIGURE 10. a. Ultrastructural detail from intimal lesion of a small intrarenal artery of salt-loaded, 20-week old SHR. The lesion consists of endothelial cells and irregularly arranged basement membranes.

b. The intima of the small intrarenal artery from a 20-week old WKY is shown for comparison (both, uranyl acetate-lead citrate, X 8700).
acceleration of vascular damage very early after the institution of a high salt diet. It is possible that morphometric differences between salt-loaded and control SHRs will be less evident or even nonexistent if comparisons are made only in advanced stages. However, destructive necrotizing lesions are evident at an earlier age and become more frequent with time so that acceleration of vascular lesions by salt are expected to result in excess morbidity and mortality, as was observed by Dahl and Tuthill in salt-loaded SHRs.

It must be noted that the differences in the renal vessels between the two age groups (10 and 20 weeks) of SHRs on normal salt diet were not as pronounced as those between age-mated SHRs on normal and high salt intake. The marked and sustained differences in the BP between 10- and 20-week-old SHRs were not sufficient to bring about progression of vascular pathology of the same magnitude as that observed at each age between SHRs on regular and high salt diet. It is therefore unlikely that the effect of dietary salt on vascular pathology is mediated solely through BP increase.

The same argument applies to the progression of aortic medial thickness. No change occurred between 10 and 20 weeks of age in SHRs on tap water, but hypertensive animals on 1% NaCl showed a significant increment (11.3 μm) in medial thickness during the same period. Again, it is unlikely that this marked difference is due to a 20 mm Hg difference of, at the most, 4 weeks' duration when an average of 32 mm Hg difference sustained for 10 weeks did not result in any change of aortic wall thickness.

We also emphasize that, in addition to the quantitative differences, there were morphologic changes in both the renal vessels and the aorta of SHRs on 1% NaCl that are rarely seen in SHRs at these ages. These changes consist of intimal proliferations and foci of fibrinoid necrosis in the intrarenal vessels and the appearance of electron dense deposits, probably fibrin, in the aortic subendothelium.

The susceptibility to the structural alterations caused by salt-loading is probably related to the genetically-determined proneness to hypertension, since WKYs were largely unaffected. It is known that prolonged ingestion of excess salt by unselected, normotensive animals leads to the development of hypertension in a variable proportion, which reflects genetic differences. Whether this variability in BP responses to salt-loading depends on structural vascular changes antedating the hypertensive state is not known. The demonstration of such a relationship in random samples of animal populations requires a larger number of observations than was available in this study.

The two structural components of the vascular changes in SHRs, i.e., intimal lesions and increased medial thickness, have different functional implications. Expansion of the subendothelial space is commonly seen during the induction of atherogenesis and may provide the structural basis for the well-known acceleration of atherosclerosis by hypertension in susceptible species. Medial thickening, on the other hand, is important in sustaining and accelerating the hypertensive state since, as Folkow and his colleagues indicated, the increased wall/lumen ratios in resistance vessels amplifies the responses to vasoconstrictive stimuli. Induction of vascular wall restructuring by salt-loading may, thus, amplify subliminal vasoconstrictive influences and promote or aggravate hypertension.

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