Albumin Transport Characteristics of Rat Aorta in Early Phase of Hypertension

Alain Tedgui, Régine Merval, and Bruno Esposito

The effects of early-stage hypertension on the macromolecular transport characteristics of the aorta have been investigated in rats 1 week after the ligature of the abdominal aorta between the two renal arteries. The animals were left untreated or treated for 1 week with an angiotensin converting enzyme inhibitor (enalapril, 6 mg/kg per day). Blood pressure of a subgroup of hypertensive rats was acutely lowered to a normal level by injection of enalaprilat (1.5 mg/kg) at the time of the experiment. 125I-Albumin and 131I-albumin were injected 90 minutes and 5 minutes, respectively, before the rats were killed. The transmural distribution of the relative tissue concentrations across the wall was obtained using a serial frozen-section technique. Short-term albumin uptake permitted calculation of apparent endothelial permeability coefficients, and 90-minute uptake was used to estimate the steady-state albumin distribution within the media. The effect of early-stage hypertension on the characteristics of the arterial macromolecular transport depended on the aortic site; the ascending aortic arch appeared not to be affected. In the thoracic and abdominal aorta, the endothelial permeability coefficients increased significantly in hypertensive rats. This increase was not a direct effect of the arterial pressure, since the values were not significantly different when the pressure was acutely normalized. The 90-minute albumin concentration in the media was enhanced in hypertensive rats and returned to the normal value by acutely lowering the blood pressure, indicating that the increase observed in hypertensive rats resulted from a direct effect of pressure, possibly increased pressure-driven convection and/or pressure-induced stretching of the wall. Treatment by angiotensin converting enzyme inhibitor prevented hypertension and protected against its effects in hypertensive animals. (Circulation Research 1992;71:932–942)

Key Words • endothelial permeability • aorta • albumin concentration • hypertension • rats

Even though the accelerating effect of hypertension on atherosclerosis is well documented,1–4 the actual mechanism by which it occurs has not been established. Pressure-induced structural and functional alterations of the arterial wall are believed to contribute to this phenomenon. Among the accelerating factors, changes in the transmural mass transport might play a major role.5 The remodeling of the vessel wall that occurs in hypertension is a time-dependent process, and the transport characteristics of the arterial wall might thus be expected to change during the development of hypertension.6–10 Earlier studies have shown that the aortic intima reacts differently to different types of hypertension, depending on both the model and the duration of hypertension,11,12 and we recently reported a decrease of the albumin distribution in the media of long-term hypertensive rats.13 However, the changes in macromolecular transport in the media in the early stage of hypertension are not known. Moreover, the modifications of arterial transport characteristics seen in chronic hypertension can be a direct consequence of hyperpressure14 or can result from humoral factors associated with hypertension, such as those due to the activation of the renin-angiotensin system. This has not yet been clearly elucidated.

The present study was performed specifically to examine the effects of early-stage hypertension on transendothelial and transmedial labeled albumin transport in the rat aorta. Hypertension was induced in rats by complete ligature of the abdominal aorta between the two renal arteries, and experiments were carried out after 1 week. Acutely normalizing the arterial blood pressure in hypertensive animals allowed us to examine the issue of direct and indirect effects of the arterial blood pressure. Protection against hypertension-induced modifications by treatment with enalapril, an angiotensin converting enzyme inhibitor, was also tested in this model of hypertension.

Materials and Methods

Experimental Protocol

Male Wistar rats weighing 330–360 g were used. Their systolic blood pressures, measured before surgery using the tail-cuff method (W&H Electronic, Varese, Italy), ranged from 115 to 155 mm Hg (mean±SEM, 137±2 mm Hg). Hypertension was induced by complete ligature of the abdominal aorta between the two renal arteries and below the superior mesenteric artery.15 Approximately 15% of the animals did not survive the surgical procedure. Animals with aortic ligature were randomly distributed into two groups. Fifteen rats were kept untreated for 1 week, and six were treated with an

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angiotensin converting enzyme inhibitor (enalapril). Antihypertensive therapy was administered twice a day by gavage at a dose of 3 mg/kg in 1 ml distilled water over a 1-week period starting from the day after surgery. These animals constituted the CEI group.

In nine of the 15 untreated hypertensive animals, blood pressure was brought back to a normal level at the time of experiment by an intravenous injection of enalapril (1.5 mg/kg). With this procedure, blood pressure remained normal throughout the experiment. These hypertensive animals studied under normal blood pressure conditions constituted the NP-HR group. The six other untreated hypertensive animals studied under high blood pressure conditions constituted the HR group. Eight male Wistar rats (330–360 g) were sham-operated and served as the control (normotensive) group. All animals received a normal diet and were given water ad libitum.

To determine the time course of increase in blood pressure, in one additional rat with aortic ligature, a catheter connected to a telemetry device (Data Sciences, Inc., St. Paul, Minn.) was chronically placed in the abdominal aorta above the ligature. An average value from a 20-second pressure recording was obtained every 30 minutes, and the blood pressure was thus continuously recorded up to 3 weeks after surgery.

**Albumin Transport Measurement**

**Tracer.** Rat serum albumin (fraction V, Sigma Chemical Co., St. Louis, Mo.) was iodinated with 125I-sodium and 131I-sodium (Amersham, England) using the iodine monochloride method of McFarlane as modified by Bilheimer et al.16 Non–protein-bound radioactivity was removed by multiple dialysis against 0.9% NaCl for 24 hours at 4°C before use. The efficacy of the dialysis was verified using 10% (wt/vol) trichloroacetic acid (TCA) to precipitate the protein-bound label. The free/protein-bound iodine ratio before injection was approximately 0.3% and 0.5% for 125I and 131I, respectively. The specific activities of 125I- and 131I-albumin were 100 μCi/mg and 50 μCi/mg, respectively.

**Animal procedure.** On the day before the experiment, animals were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). A catheter was inserted into the carotid artery and another into the jugular vein, and they were tunneled under the skin to the neck. The rats were housed individually. The arterial catheter was connected to a Statham P23 1D pressure transducer (Gould, Cleveland, Ohio) for measuring blood pressure.

On the day of the experiment, the animals were conscious, and blood pressures were measured again directly through the carotid artery catheter. In animals injected with enalapril, tracers were injected after the arterial blood pressure reached a stable normal value. In a series of three animals not used for tracer uptake studies, we ensured that arterial blood pressure was normalized for more than the duration of the 90-minute experiment. Thus, at the time of the 125I-albumin injection (5 minutes before killing the animal), we ascertained that the blood pressure was still normalized.

The venous catheter served for labeled albumin and drug injection, and the arterial catheter was used for blood sampling.

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**Figure 1. Schematic diagram of aortic segments from rats used in this study.** AA, ascending aortic arch; DA, descending aortic arch; T1–T3, segments from descending thoracic aorta; A1 and A2, abdominal aorta above and below ligature, respectively.

The rats were injected with 500 μl 131I-albumin (≈100 μCi, 2 mg) and with 200 μl 125I-albumin (≈100 μCi, 1 mg) 85 minutes later. Animals were then killed with a lethal dose of sodium pentobarbital 5 minutes after this second tracer injection (i.e., 90 minutes after the first tracer injection). Ninety-minute uptake studies were used to estimate the steady-state distribution of labeled albumin within the media, and 5-minute uptake permitted assessment of the endothelial permeability. Blood samples were taken for plasma radioactivity determination at 5 and 90 minutes after injection. In two hypertensive rats, 131I- and 125I-albumin were injected at 3 hours and at 90 minutes, respectively, before killing to ascertain that a steady state had indeed been achieved after 90 minutes.

After the rats were killed, the whole aorta from the heart to iliac bifurcation was immediately excised and quickly rinsed in cold saline buffer. This took approximately 3 minutes, during which labeled albumin may have continued to enter the wall but probably at a lower rate because the vessels were no longer distended and the blood pressure fell to zero. The aorta was then divided into several segments (Figure 1): one segment from the ascending part of the arch, one segment from the descending part of the arch, three segments from the descending thoracic aorta, two segments from the abdominal aorta, one segment from above the ligature between the celiac trunk and the left renal artery, and one from below the left renal artery. Each segment was cleaned of adventitial debris, and collateral branches were removed. The segments from the ascending and descending parts of the arch were further divided into
superior (outer wall) and inferior (inner wall) portions. The rest of the aorta was slit open longitudinally. Each of the arterial segments was briefly rinsed in saline buffer to remove traces of blood, laid flat on a lightly greased microscope slide with the intimal surface down, and rapidly frozen at \(-20^\circ\text{C}\). The elapsed time between killing and freezing was approximately 10 minutes. During this period, tracers may have continued to diffuse within the wall, yet no more tracer may have entered the tissue since it was no longer exposed to plasma.

Determination of radioactivity within the wall. The spatial distribution of tracers within the wall was obtained using a serial frozen-section technique on a refrigerated microtome (SLEE TE, London), previously described in detail.\textsuperscript{13} A sample of frozen aorta was gently thawed, and a small quantity of mounting medium was spread on the uppermost adventitial surface. The microscope slide, with the tissue sample facing down, was quickly lowered onto the frozen-specimen holder by using a spring device that held the microscope slide parallel to the cut face. The entire tissue was easily detached from the greased microscope slide and fixed to the specimen holder. No visible trace of tissue remained on the slide. The sample edges were trimmed to remove overhanging material. The dimensions of the segment were measured using Vernier calipers, and the surface area (\(=0.2\text{ cm}^2\)) was calculated. En face serial sections (10 \(\mu\text{m}\) thick) were then cut parallel to the luminal surface. The first slice was incomplete and was discarded when it was visually excessively thin (likely <5 \(\mu\text{m}\) thick). Therefore, the thickness of the first section kept for analysis ranged between 5 and 10 \(\mu\text{m}\). The boundary between the media and the adventitia was defined by an alteration in the appearance of the sections. Flat, milky slices were obtained in the media, whereas adventitial slices were curled and less opaque. The sections were placed in tubes for radioactivity assay.

To correct for contamination by free labeled iodide in plasma, 500 \(\mu\text{l}\) of 1% unlabeled albumin solution and 500 \(\mu\text{l}\) of 20% TCA were added to the slices of the descending thoracic aorta of each animal. The mixture was centrifuged at 2,500 \(\text{g}\) for 15 minutes, and TCA-precipitable and TCA-soluble radioactivities were measured. The same procedure was used to measure TCA-precipitable labeled albumin in duplicate 20-\(\mu\text{l}\) aliquots of plasma. The TCA-soluble radioactivity fraction in aorta was previously found to be similar in the different parts of the aorta.\textsuperscript{13} Therefore, the TCA-soluble fraction obtained in the thoracic aorta was used to calculate protein-bound radioactivity in the other parts of the aorta.

\textsuperscript{131}I and \textsuperscript{125}I radioactivities were assayed simultaneously in each test tube with a double counting procedure for 3 minutes on a gamma counter (GAMMAmatic, Kontron, Basel, Switzerland). Spillover of \textsuperscript{131}I into the \textsuperscript{125}I channel was corrected by the channel ratio method. The tissue counts ranged from 100 to 1,000 cpm in both isotopes (after subtraction of the background, which was \(\approx 20\) cpm in \textsuperscript{125}I and 0–2 cpm in \textsuperscript{131}I).

Relative tissue concentrations (\(C_T/C_P\)) of both \textsuperscript{125}I-albumin and \textsuperscript{131}I-albumin were calculated for each section as counts per minute per unit volume of wet tissue divided by counts per minute per unit volume of plasma sampled at the time of death. The \(C_T/C_P\) value of the first section was underestimated, because its actual thickness was less than the 10-\( \mu\text{m}\) value used for calculation of the volume of the wet tissue. However, because the unknown actual thickness ranged between 5 and 10 \(\mu\text{m}\) (averaging 7.5 \(\mu\text{m}\)), the error might be estimated to average 25%. The \(C_T/C_P\) values for the multiple sections cut from a single segment were plotted as a function of their distance from the luminal surface to the external surface. For clarity the first value was plotted at 10 \(\mu\text{m}\), because the actual thickness of the first section was not known. The average of the medial \textsuperscript{131}I-albumin \(C_T/C_P\) values was calculated, and the group mean medial values were formed by averaging individual means.

A group average concentration profile was constructed for each arterial region by averaging the \(C_T/C_P\) values at equal intervals across the media. In the thoracic aorta, an individual average concentration profile was first obtained by averaging profiles from the three segments cut in this region. As the number of tissue sections for a given site slightly varied between animals within the same group, the number of 10-\(\mu\text{m}\) intervals was chosen as the mean number of tissue sections obtained in each group, approximated to the closest whole number. The \(C_T/C_P\) values were then calculated by linear interpolation when necessary.

Calculation of endothelial permeability. To estimate the apparent endothelial permeability coefficients, we used the same method as Bratzler et al.\textsuperscript{17} If we assume that the tracer flux per unit surface area (\(J/A\)) is linearly related to the tracer concentration difference across the endothelium (\(\Delta C_p\)), then \(J/A=P \times \Delta C_p\), where \(P\) is the apparent endothelial permeability coefficient. To assess \(P\), we suppose that relatively little tracer has entered the aortic wall for short times. The tracer plasma concentration \(C_p\) can thus be considered as a measure of \(\Delta C_p\). \(J/A\) is the rate of arterial wall uptake (tracer accumulation per unit surface area per unit time). Therefore, \(P\) equals the rate of normalized albumin uptake \(J/(A \times C_p)\). This can be calculated from the 5-minute concentration profiles across the media, except in the abdominal aorta below the ligature in which high medial \(C_T/C_P\) values were obtained. This site was not analyzed for endothelial permeability. In the other aortic regions, the 5-minute \(C_T/C_P\) profiles across the media showed a V shape, indicating that labeled albumin had entered the media from both the lumen and the adventitia via the vasa vasorum. The part of the normalized arterial uptake due to the tracer influx from the lumen was thus calculated by summing the \(C_T/C_P\) values, from the first section to the nadir of the curve, multiplying by the section thickness (\(C_p\) represents the tissue concentration per unit volume instead of unit area), and dividing by the time duration (5 minutes) to obtain the rate of uptake per unit surface area of relaxed tissue. To take into account the actual in vivo luminal surface area, the calculated normalized uptake must be divided by the ratio of the luminal surface area of the artery under in vivo conditions to the luminal surface area of the relaxed excised tissue (\(A/A_0\)). This was estimated in three control and two HRs. After each rat was killed, the carotid catheter was connected to a reservoir containing a buffer solution, which was leveled to a height corresponding to the mean blood pressure of
the animal (=125 mm Hg in control and 160 mm Hg in HRs). Marks 2–3 mm apart were made on the external aspects of the different parts of the aorta (ascending and descending aortic arch, descending thoracic and abdominal aorta). The distances between the marks and the external diameters were measured twice using Vernier calipers with an accuracy of 0.1 mm. In HRs, the reservoir was then lowered to a height corresponding to the mean arterial pressure of NP-HRs (115 mm Hg), and the measurements were repeated. Finally, fresh arterial segments were cut from the animals and opened longitudinally. The distances between the marks and the circumferences were then measured under the relaxed conditions, allowing the calculation of Aφ.

Calculation of Medial Thickness

The medial thickness was calculated in all arterial segments using the number of frozen sections cut from the sample and the thickness of each section (10 μm). We previously showed that a highly significant correlation exists between the medial thickness estimated using this technique and that measured using histomorphometry.13 However, the frozen-section technique systematically underestimated the actual medial thickness as determined by histomorphometry by approximately 30%. Although this affects the absolute values of medial thickness, it does not influence comparisons between groups.

Determination of Renal Function

In a series of experiments, the glomerular filtration rate was monitored on day 7 in eight sham-operated (control) rats and eight HRs not used for albumin transport experiments. Each rat was placed in a metabolic cage, and 24-hour urine outputs were collected. Urine and serum creatinine were measured, and creatinine clearance was determined. Animals were weighed daily.

Statistical Methods

Results are expressed as mean±SEM. Blood pressures, body weights, and medial thicknesses were compared using a one-way analysis of variance (ANOVA) between experimental groups. A two-factor repeated-measures ANOVA was constructed by using the 90-minute mean medial C_t/C_t values and apparent endothelial permeability coefficients to test the effect of experimental group and aortic site (level of significance at p<0.05). The differences between experimental groups were evaluated using Bonferroni’s test. Differences between aortic sites were evaluated using Student’s paired t test.

Results

Body Weight and Renal Function

Body weights at the time of death (Table 1) in HR and NP-HR groups were not significantly different, but they were lower than in the control group (p<0.01) and in the CEI group (p<0.05). Daily determination of weight showed that animals with aortic ligature lost weight up to day 5 or 6; then the weights became unchanged or increased. Thus, it appears that weights in the 7-day postligature group were probably at their lowest values at the time of the experiment.

Examination of the kidneys at the time of death showed that the right kidneys, proximal to the ligature, were markedly hypertrophied compared with the left kidneys, which showed no renal necrosis, as visually estimated.

The creatinine clearance in HRs was approximately 25% lower than that in control rats (0.98±0.11 versus 1.35±0.18 ml/min, n=8), but the difference was not statistically significant.

Blood Pressure and Medial Thickness

In one rat in which blood pressure was continuously recorded by telemetry, beginning before aortic ligature and continuing up to 3 weeks later, the mean arterial pressure increased markedly from 115 to 138 mm Hg as little as 8 hours after surgery and then progressively within the next 6 days. A maximum of 175 mm Hg was achieved by day 6, and pressure fluctuated about this level for the next 2 weeks of recording. Values of carotid mean blood pressure at the time of the experiment are given in Table 1. One week after aortic ligature, hypertension was achieved in all untreated rats. On the day of the experiment, the mean blood pressure was significantly increased in both the HR and NP-HR groups (p<0.01 versus the control group); the difference between these two groups was not statistically significant. In the NP-HR group, the mean blood pressure after enalaprilat injection was normalized to a value that did not differ from that of the control group. The mean blood pressure of the CEI group was similar to that of the control group.

In the ascending and descending aortic arch, no significant difference in medial thickness was found between the outer and inner parts. Therefore, the data from these parts were pooled. The medial thickness values are presented in Table 2. ANOVA showed that
the medial thickness significantly decreased from the arch to the abdominal aorta (p<0.01); the difference between the ascending and descending parts of the arch was not significant. Experimental group significantly affected the medial thickness above but not below the ligature (p<0.01). The medial thickness was increased by 39.8% and 29.3% in HR and NP-HR groups, respectively, as compared with the control group (p<0.01); the difference between these two groups was not significant. In the CEI group, the medial thickness was not significantly different from that in the control group.

Studies on Albumin Distribution

TCA-precipitable plasma $^{131}$I radioactivity decreased by 26.5±1.3% (n=29) between 5 and 90 minutes after tracer injection. This decrease was similar in all groups. The mean medial $^{125}$I- and $^{131}$I-TCA-soluble/TCA-precipitable tissue radioactivity ratios in the thoracic aorta were 0.192±0.006 and 0.233±0.006 (n=29), respectively.

In the ascending and descending aortic arch, the average profiles of the relative $^{131}$I- and $^{125}$I-albumin concentrations in the outer and inner parts were similar, and the repeated-measures ANOVA showed no significant difference in the mean medial C$_T$/C$_P$ values. Therefore, the data from the outer and inner parts were pooled.

5-Minute Experiments

The average profiles of the relative $^{131}$I-albumin concentration across the media of each aortic site, obtained 5 minutes after tracer injection in control animals, are shown in Figure 2. As indicated in “Materials and Methods,” the abdominal aorta below the ligature was not kept for analysis, because the hypothesis of low uptake during the 5-minute experimental duration was not satisfied in that site because of the thinness of the wall. In the other sites, the transmural distribution of C$_T$/C$_P$ values was V shaped, showing steep gradients at the luminal and adventitial sites. The first values were 0.0112±0.0009, 0.0095±0.0004, 0.0081±0.0007, and 0.0081±0.0008 in the ascending arch, descending arch, thoracic aorta, and abdominal aorta, respectively, and the lowest values were 0.0019±0.0001, 0.0031±0.0001, 0.0021±0.0003, and 0.0039±0.0003, respectively. Inasmuch as this lowest value was not exactly zero, it was possibly the result of tracer influx from both the lumen and the adventitia. Therefore, in the calculation of the tracer uptake due to the transendothelial transport, this value was not included. The average profiles of the relative $^{125}$I-albumin concentration across the media of the ascending aortic arch, the descending aortic arch, the descending thoracic aorta, and the abdominal aorta above the ligature are shown in Figure 3. The curves were similar near the luminal surface in the ascending and descending aorta, whereas the luminal gradients were steeper in the thoracic aorta and abdominal aorta above the ligature in HR and NP-HR groups.

In normotensive control rats, the A/A$_0$ ratios were 1.75, 1.7, 1.6, and 1.45 in the ascending arch, descending arch, thoracic aorta, and abdominal aorta above the ligature, respectively. The assumption that the volume of the arterial tissue remains constant during the change from in vivo conditions to the relaxed excised state, the ratio of luminal surface area under these two conditions is equal to the inverse of the ratio of medial thickness under the same two conditions. This has been accurately measured in the rabbit descending thoracic aorta and found to be 1.72,[18] similar to our A/A$_0$ values. In the HR group, they were 1.65, 1.45, 1.55, and 1.55 in the ascending arch, descending arch, thoracic aorta, and abdominal aorta above the ligature, respectively. When the pressure was lowered to a normal value in these hypertensive animals, the luminal surface area decreased by only 10–15% whatever the sites. By using the values of A/A$_0$, the apparent endothelial permeability coefficients (Table 3) were calculated from the values of

![Figure 2](http://circress.ahajournals.org/)

**Figure 2.** Line plot of transmural distribution of mean relative 5-minute ($^{125}$I-albumin) tissue concentration (×10$^5$) across the media for five aortic sites (ascending [Asc] and descending [Desc] parts of the aortic arch, the descending thoracic aorta [Thor A], and the abdominal aorta [A A] above the ligature) in control rats. The last point is at the medial-adventitial boundary. Mean±SEM values were obtained from eight rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Asc arch</th>
<th>Desc arch</th>
<th>Thor A</th>
<th>AA above</th>
<th>AA below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>94±3</td>
<td>94±3</td>
<td>77±2</td>
<td>70±3</td>
<td>50±3</td>
</tr>
<tr>
<td>HR (n=6)</td>
<td>129±5*</td>
<td>131±8*</td>
<td>110±5*</td>
<td>92±6*</td>
<td>51±5</td>
</tr>
<tr>
<td>NP-HR (n=9)</td>
<td>116±4*</td>
<td>127±6*</td>
<td>102±3*</td>
<td>89±3*</td>
<td>47±3</td>
</tr>
<tr>
<td>CEI (n=6)</td>
<td>89±5</td>
<td>102±3</td>
<td>85±2</td>
<td>81±2</td>
<td>52±2</td>
</tr>
</tbody>
</table>

Asc arch, ascending arch; Desc arch, descending arch; Thor A, descending thoracic aorta; AA above, abdominal aorta above the ligature; AA below, abdominal aorta below the ligature; control, normotensive rats; HR, untreated hypertensive rats; NP-HR, untreated hypertensive rats with acutely lowered blood pressure; CEI, hypertensive rats treated with angiotensin converting enzyme inhibitor. Values of medial thickness were calculated using numbers of serial frozen sections for the different aortic sites of each experimental group. Values are mean±SEM.

*p<0.01 vs. control (other statistical comparisons are given in text).
the amount of tracer entering from the lumen (ΣC/I/C₀ values from the first to that preceding the nadir of the curve). The repeated-measures ANOVA showed that both aortic site and experimental group had a significant effect on the endothelial permeability (p<0.01 and p<0.002, respectively); the interaction between these two factors was also significant (p<0.01). In control animals, the endothelial permeability did not vary significantly with sites. In the ascending aortic arch, the experimental group had no significant effect. Conversely, in the descending aortic arch and in the thoracic and abdominal aorta, the endothelial permeability was significantly increased in HR and NP-HR groups (Table 3); the difference between these two groups was not significant. The endothelial permeability in the CEI group did not differ significantly from that in the control group.

90-Minute Experiments

In experiments conducted for 3 hours in two control and two HRs, the 3-hour 125I-albumin C/I/C₀ values were not significantly different from the 90-minute 125I-albumin values, indicating that a steady state was achieved after 90 minutes.

The average profiles of the relative 125I-albumin concentration across the media of each aortic site, obtained

![Graphs showing transmural distribution of mean relative 5-minute 125I-albumin tissue concentration (×10⁹) in each experimental rat group across the media of the ascending aortic arch (top left panel), the descending aortic arch (top right panel), the descending thoracic aorta (bottom left panel), and the abdominal aorta above the ligature (bottom right panel). Control, normotensive rats; HR, untreated hypertensive rats; NP-HR, untreated hypertensive rats with blood pressure acutely lowered to a normal level; CEI, hypertensive rats treated with angiotensin converting enzyme inhibitor. The last point is at the medial-adventitial boundary. The number of rats per group is given in parentheses. Bars represent ±SEM.](image-url)

**Table 3. Apparent Endothelial Permeability Coefficients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Asc arch</th>
<th>Desc arch</th>
<th>Thor A</th>
<th>AA above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>4.6±0.6</td>
<td>3.95±0.5</td>
<td>3.7±0.3</td>
<td>4.5±0.4</td>
</tr>
<tr>
<td>HR (n=6)</td>
<td>4.5±0.6</td>
<td>6.3±0.8†</td>
<td>7.6±1.1†</td>
<td>7.4±1.7†</td>
</tr>
<tr>
<td>NP-HR (n=9)</td>
<td>5.3±0.3</td>
<td>6.4±0.6†</td>
<td>6.6±0.8†</td>
<td>10.1±1.2†</td>
</tr>
<tr>
<td>CEI (n=6)</td>
<td>3.9±0.2</td>
<td>4.9±0.2</td>
<td>4.5±0.3</td>
<td>6.2±0.4</td>
</tr>
</tbody>
</table>

Asc arch, ascending arch; Desc arch, descending arch; Thor A, descending thoracic aorta; AA above, abdominal aorta above the ligature; control, normotensive rats; HR, untreated hypertensive rats; NP-HR, untreated hypertensive rats with acutely lowered blood pressure; CEI, hypertensive rats treated with angiotensin converting enzyme inhibitor. Values are mean±SEM.

*p<0.05, †p<0.01 vs. control (other statistical comparisons are given in text).
90 minutes after tracer injection in control animals, are shown in Figure 4. The transmural distribution of $C_t/C_p$ values was nearly uniform across the media. The average profiles of the $^{131}$I-albumin concentration across the media of the ascending aortic arch, the descending aortic arch, the descending thoracic aorta, and the abdominal aorta above and below the ligature in each experimental group are shown in Figure 5. No significant change in the profiles was seen in the ascending arch whatever the experimental group. In the other aortic sites, with the exception of the abdominal aorta below the ligature, a marked increase in concentration of tracer developed on the half luminal side of the wall in the HR group.

The mean medial $^{131}$I-albumin $C_t/C_p$ values in each experimental group and aortic site are given in Table 4. Analysis of the difference between sites showed that, with the exception of the HR group, the mean medial $^{131}$I-albumin $C_t/C_p$ values were significantly higher in the ascending arch than in the descending arch ($p<0.04$) or in the thoracic aorta ($p<0.002$) and higher in the descending arch than in the thoracic aorta ($p<0.007$).

In the HR group, the mean medial $^{131}$I-albumin $C_t/C_p$ values were significantly increased in each aortic site ($p<0.01$), except in the ascending aorta, in which no significant difference was seen as compared with the control group.

Contrary to what was found for the regions of the aorta above the ligature, the mean medial $^{131}$I-albumin $C_t/C_p$ values for the abdominal aorta below the ligature were significantly enhanced not only in the HR group but also in NP-HR and CEI groups as compared with the control group.

**Discussion**

**Effect of the Early Stage of Hypertension on Endothelial Permeability**

The present studies were designed to determine the effects of the early stage of hypertension on the transport characteristics of the arterial wall. The apparent endothelial permeability coefficient obtained in control animals was similar to that previously reported in the rabbit thoracic aorta ($4 \times 10^{-9}$ cm/sec).

The experimental model of complete ligature of the abdominal aorta between the two renal arteries was used because it offers the possibility of rapid elevation of the blood pressure as compared with other models, such as the Goldblatt model. One week after ligature of the abdominal aorta, the arterial pressure was markedly increased. However, the surgical intervention to cause hypertension had inevitable consequences on the metabolic state of the animals at short term. On day 7, animals from the HR and NP-HR groups had lost 20–30% of their initial weights. Yet, when the time course of weight loss was determined in the HR and NP-HR groups up to day 7, it appeared that weight ceased to decrease after day 5 or 6. In a similar model of aortic constriction between the renal arteries, Owens and Reidy also found a 17% decrease in body weight on day 9, which was temporary, since body weights of their hypertensive rats were no longer significantly different from the control rats 30 days after coarctation.

The reasons for weight loss during the first week are not clear. The renal function was not dramatically impaired; a statistically not significant decrease of approximately 25% in creatinine clearance was seen in hypertensive rats. Even though it cannot be excluded that metabolic consequences associated with the weight loss may have influenced the transport characteristics of the arterial wall, it is most likely that the observed changes in these parameters were due to chronic hypertension and/or stimulation of the renin-angiotensin system. Indeed, changes occurring above the ligature were prevented by continuous treatment with an angiotensin converting enzyme inhibitor. It has been shown that the experimental model of hypertension used in our study is associated with stimulation of the renin-angiotensin system, resulting from the low perfusion of the left kidney. The plasma renin level remains high for the early phase of hypertension and then declines after 2–3 weeks while hypertension persists. Therefore, although we did not measure circulating angiotensin II levels in HR and NP-HR groups, they can be expected to be high. This is supported by the fact that enalaprilat injection the blood pressure returned to a normal value. Because angiotensin II is known to increase the endothelial permeability to macromolecules, the increased permeability seen in HRs and NP-HRs might have been due to elevated plasma angiotensin II. However, using angiotensin converting enzyme inhibitor to acutely reduce the arterial pressure of hypertensive animals to a normal value allowed us to show that the enhanced endothelial permeability observed in HR and NP-HR groups no longer required high plasma angiotensin II levels or elevated pressure, although these factors possibly had inductive effects. This suggests that 1) structural alterations of the endothelium were already established at the time of the experiment and 2) pressure-driven solvent drag played no role in the increased transendothelial albumin permeation, which is in agreement with previous results reported by Bretherton et al. These authors found in rabbit aorta that the rate of intimal entry of low density lipoprotein, increased by hypertension, was not normalized when the blood pressure was acutely lowered.
In the same model of experimental hypertension as that used in the present work, it has been reported that 7 days after ligation of the abdominal aorta the permeability to colloidal iron and horseradish peroxidase was increased, and this appeared not to result from hypertension per se. The increased permeability paralleled structural modifications of the endothelial cells, including an increase in the volume of the endothelial cell layer, an increase in the number of cytoplasmic microfilament bundles, and an increase in the tight

![Graphs showing relative concentration of albumin across different sections of the aorta.](https://example.com/graphs.png)

**Figure 5.** Line plot of transmural distribution of mean relative 90-minute $^{131}$I-albumin tissue concentration ($\times 10^2$) in each experimental rat group across the media of the ascending aortic arch (top left panel), the descending aortic arch (top right panel), the descending thoracic aorta (middle left panel), the abdominal aorta above the ligature (middle right panel), and the abdominal aorta below the ligature (bottom panel). Control, normotensive rats; HR, untreated hypertensive rats; NP-HR, untreated hypertensive rats with blood pressure acutely lowered to a normal level; CEI, hypertensive rats treated with an angiotensin converting enzyme inhibitor. The last point is at the medial-adventitial boundary. The number of rats per group is given in parentheses. Bars represent $\pm$SEM.

**Table 4. Mean Medial 90-Minute $^{131}$I-Albumin Relative Concentration**

<table>
<thead>
<tr>
<th>Group</th>
<th>Asc arch</th>
<th>Desc arch</th>
<th>Thor A</th>
<th>AA above</th>
<th>AA below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=8)</td>
<td>2.31±0.25</td>
<td>2.23±0.18</td>
<td>2.02±0.21</td>
<td>2.41±0.36</td>
<td>1.86±0.19</td>
</tr>
<tr>
<td>HR (n=6)</td>
<td>2.76±0.28</td>
<td>3.35±0.32*</td>
<td>3.84±0.31*</td>
<td>4.39±0.31*</td>
<td>3.37±0.39†</td>
</tr>
<tr>
<td>NP-HR (n=9)</td>
<td>2.65±0.23</td>
<td>2.38±0.24</td>
<td>1.85±0.20</td>
<td>3.02±0.31</td>
<td>5.06±0.51*</td>
</tr>
<tr>
<td>CEI (n=6)</td>
<td>2.51±0.14</td>
<td>2.32±0.13</td>
<td>2.11±0.13</td>
<td>2.52±0.17</td>
<td>4.24±0.17†</td>
</tr>
</tbody>
</table>

Asc arch, ascending arch; Desc arch, descending arch; Thor A, descending thoracic aorta; AA above, abdominal aorta above the ligature; AA below, abdominal aorta below the ligature; control, normotensive rats; HR, untreated hypertensive rats; NP-HR, untreated hypertensive rats with acutely lowered blood pressure; CEI, hypertensive rats treated with angiotensin converting enzyme inhibitor. Values are mean±SEM medial values of relative concentrations of $^{131}$I-albumin.

*p<0.05, †p<0.01 vs. control (other statistical comparisons are given in text).
junction density. According to these latter authors, the increase in tight junction density may represent an adaptive process to hypertension, but as a result of the reduction of the percentage of occluding junctions, the barrier function of the endothelium remains impaired. However, it is unlikely that in our experiments the paracellular pathway was involved in the observed increased endothelial permeability, since this did not appear to depend on the level of the arterial pressure. Gabbiani and colleagues found an enhanced replication rate of aortic endothelial cells 7 days after ligation of the abdominal aorta, and Owens and Reidy reported that the endothelial cell turnover rates were increased 23-fold in thoracic aortas of rats 9 days after coarctation. In other experimental hypertensive models, the turnover of endothelial cells was increased as well. It is noteworthy that a striking association between increased endothelial turnover rate and increased macromolecular permeability of the endothelium has been demonstrated. Wu et al found in spontaneously hypertensive rats a concomitant increased endothelial cell turnover and an enhancement of the permeability to macromolecules. Thus, it is likely that the increased endothelial permeability was associated with endothelial replication. Davies and Ross have shown that endothelial pinocytosis is proportional to endothelial proliferation. Increased endothelial turnover induced by hypertension may therefore cause accelerated pinocytosis. Conversely, Majack and Bhalla found no difference in the endothelial permeability to colloidal carbon between normotensive and spontaneously hypertensive rats, which they attribute to possible adaptive wall changes. Yet the use of carbon, by virtue of its large size (25–50 nm), only allows detection of abnormal interendothelial gaps, and their work thus excludes this possibility. This confirms an earlier study by Hüttner et al who showed that in the early stage of hypertension (2 days after aortic ligation) horseradish peroxidase enters the aortic intima in increased amounts both through cell junctions and by vesicular transport rather than through interendothelial gaps, whereas the larger macromolecule, ferritin, increases only within plasmalemmal vesicles.

Earlier studies reported that albumin moves by diffusion and convection through a system of small (≤12-nm diameter) and large (20–50-nm diameter) pores possibly located in the intercellular junctions of the endothelium. However, subsequent studies using immunocytochemistry indicated that albumin is localized primarily in plasmalemmal vesicles, and Milici et al, using monomeric albumin as a tracer and gold-tagged antibody as a detector, found that in murine myocardial capillaries albumin appears to be exclusively transported through plasmalemmal vesicles. The latter have been proposed to be the structural equivalent of the small pores for macromolecular transport, accounting for the diffusive component of the plasma-interstitial fluid macromolecular efflux. These authors did not rule out the possibility that transient transendothelial channels formed by vesicular fusion could result in a convective transport and could relate to the large-pore system. Our present experiments do not support a role for this convective pathway in the transendothelial transport of albumin, which is in agreement with the findings of Renkin et al, who reported endothelial reflection coefficients for albumin in capillaries of rat skin and muscle close to 0.99, indicating that the convective coupling of albumin flux to volume flux is very low.

Effect of the Early Stage of Hypertension on Albumin Uptake by the Media

The steady-state albumin concentration in the media was increased in hypertensive animals. Yet, contrary to what occurred for the endothelial permeability, it was reduced by acute normalization of the blood pressure. Thus, it appears that high blood pressure, per se, enhances the albumin influx in the media, suggesting that albumin penetration in the media in hypertensive animals was driven by an increase in the transmural convective transport, in agreement with previous results obtained in vivo and in vitro in rabbit thoracic aorta. Analysis of the shape of the transmural 90-minute relative concentration profiles across the media (middle panels of Figure 5) showing increased $C_f/C_p$ values in the inner half of the media, which decrease in the outer media, supports this view. The increased convective transport might result from enhanced water filtration with increasing transmural pressure and/or from a reduction of the barrier function of the internal elastic lamina due to pressure-induced stretching of the wall. Taken together, our results on the transmural distribution of 5- and 90-minute albumin relative tissue concentrations suggest that albumin can gain entry to the subendothelial space by a pathway not influenced by convection, possibly via transendothelial vesicular transport, but once in the intima, protein molecules both diffuse through the interstitium and are convected toward the adventitia with the transmural volume flow.

Our present results differ from those previously found in long-term hypertension (two-kidney, one clip). In that study, after 8 weeks of hypertension, we showed that the 90-minute mean medial albumin concentration was decreased in hypertensive animals and was further reduced when the blood pressure was lowered to the control level. We proposed that this resulted from decreased distribution volume for albumin that was due to the pressure-induced fibrosis of the media and not to the cell mass increase. Therefore, differential evolution of cellular and fibrous responses to hypertension might account for the difference in medial albumin accumulation in short-term and long-term hypertension. It is known that fibrous proteins, collagen and elastin, progressively accumulate with the duration of hypertension, being practically unchanged after 1 week, whereas hypertrophy of the aorta, associated with increased DNA content, is already detectable at the end of the first week. Indeed, in the present experiments, medial thickening was observed in the aorta of hypertensive animals above the ligature. It is noteworthy that below the ligature the medial thickness was unchanged, indicating that the medial hypertrophy was mainly induced by mechanical rather than humoral factors.

Regional Variations

The transport characteristics in the ascending aortic arch appeared to be unchanged in all experimental groups. This differs from previous findings showing in different species that the aortic arch has a higher permeability and is more affected than other aortic
sites. Duncan et al\textsuperscript{43} found in canine aorta that acute hypertension increased albumin uptake most in the aortic arch and less in the distal aorta, and Bretherton et al\textsuperscript{22} reported in rabbit increased medial low density lipoprotein concentrations by hypertension only in the aortic arch. Moreover, Feig et al\textsuperscript{21} showed in rabbit that angiotensin II enhanced labeled albumin concentrations in the media of the aorta proximal to the first pair of intercostal arteries. The discrepancy between these studies and ours might be due to species differences. Hemodynamics in the curved aortic arch differs in small animals as compared with larger animals. In the present work, the regional variation in medial albumin concentrations might be accounted for by regional differences in the structure of the media, including extracellular matrix components,\textsuperscript{44} possibly associated with regional differences in local hemodynamics and mechanical stresses applied to the aortic wall. Another explanation is the existence of regional differences in the response to hypertension. After 8 weeks of hypertension, the hypertrophy of the arch was exaggerated as compared with that of the descending thoracic aorta,\textsuperscript{13} and phenotypes of smooth muscle cells have been shown to vary with the aortic region.\textsuperscript{45} Accordingly, the only slight increase in albumin distribution in the media of hypertensive animals might reflect an early fibrous response of this site to hypertension. Interestingly, a previous study showed that after 40 days of hypertension the endothelial permeability of the descending thoracic aorta was brought back to normal levels,\textsuperscript{2} and in the present work the endothelial permeability of the arch was unchanged. It cannot be ruled out that the evolution of structural and transport characteristics in the arch was accelerated to the point that after 1 week they resembled those in the rest of the aorta after a longer duration of hypertension.

Treatment with enalapril starting at the time of aortic ligature prevented hypertension, medial thickening, and alteration of transport characteristics in the aorta, with the exception of the region below the ligature, in which the mean medial albumin concentration was as high as that in hypertensive animals. Further studies are required to elucidate the mechanisms responsible for this. However, we can speculate that this might be due to major changes in blood flow that occurred below the ligature, causing some kind of injury to the endothelium, whatever the level of pressure above the ligature.

In conclusion, our results suggested differential evolution of the arterial transport characteristics during hypertension. We found that both macromolecular endothelial permeability and medial distribution are increased at the early phase of hypertension, whereas previous studies showed that in the late phase of hypertension the endothelial permeability returns to a normal value\textsuperscript{12} and albumin distribution is decreased.\textsuperscript{13} Whereas the evolution of wall structure during hypertension possibly corresponds to an adaptive process, the functional changes in macromolecular wall transport related to hypertension might be deleterious in atherosclerosis.

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