In Vitro Demonstration of Vascular Hyper-responsiveness in Experimental Hypertension

By Joseph A. M. Hinke, M.D.

With the technical assistance of Patricia A. Simpson, B. Sc.

Generalized and persistent narrowing of small arteries is primarily responsible for the increased peripheral resistance characteristic of hypertension. This narrowing could be produced by thickening the arterial wall at the expense of the lumen, or by increasing the activity of smooth muscle in the arteriolar wall. Pathological thickening could be due to muscle hypertrophy, to increased content of water or to infiltration of the wall by foreign material. Increased muscular activity could be due to excessive sympathetic nerve impulses, elevated concentrations of circulating pressor substances or increased contractile sensitivity of the muscle cells.

Good evidence is available to support each one of these possible mechanisms but no conclusive evidence excludes any.

In experimental hypertension, in vitro isolation of the diseased artery is one way to separate arterial from extra-arterial mechanisms. It is certainly the best method to study vascular hyper-responsiveness without nervous and humoral interference. Obviously, the method does not separate hyper-responsiveness from the structural changes in the artery.

The in vitro approach in vascular hypertension is most useful when the following criteria are met. First, the test artery must represent that part of the vascular tree known to be affected in the hypertensive disease. At best, it should be an arteriole; at least, it must be a small muscular artery. Second, the natural geometry of the artery should be preserved so that the structural and functional changes in the wall can be assessed in terms of their increased ability to narrow the lumen. This implies that the isolated artery must be perfused under pressure. These criteria are satisfied to some extent by the recently developed technique of isolating and perfusing the rat caudal artery. This artery is far from being an arteriole but it is certainly a small muscular artery (500 μ O.D.).

The main purpose of this study was to establish the relative importance of wall thickening and vascular hyper-responsiveness as mechanisms which reduce the lumen of an artery in a hypertensive animal. All experiments were made on isolated perfused ventral caudal arteries of rats, some of which were made hypertensive by means of desoxycorticosterone acetate (DCA) implantation and 1% saline ingestion.

Methods

The experimental assembly has already been described. Briefly, it consists of three chambers: a mounting chamber containing two glass cannulas to which the artery segment is ligated; a pressurized chamber containing fluid for intraluminal perfusion; and a nonpressurized chamber containing fluid for extra-arterial perfusion. The outlet of the pressure chamber is connected to the proximal cannula in the mounting chamber by means of thick walled Tygon tubing. The distal cannula remains open and is oriented over a drop counter. Thus, fluid leaves the pressure chamber under a preset pressure and passes through the lumen of the artery segment to reach the open end of the distal cannula where it leaves to fall through the drop counter. Driving pressure is measured with a transducer (Statham P23G) which is connected via a sidearm to the proximal cannula. The artery segment is mounted...
in the horizontal plane, illuminated from below and visualized by a binocular microscope. All chambers are surrounded by water jackets for temperature regulation. These are interconnected in a common circulating system which is driven by a thermostat unit (Haake F).

SOLUTIONS

The perfusing fluid was Krebs-Henseleit solution with the following composition, in millimoles per liter: NaCl 115.0, KCl 5.0, NaHCO₃ 25.0, NaH₂PO₄ 1.2, CaCl₂ 2.1, MgSO₄ 1.2, dextrose 11.0, CaNa₂ Versenate 0.026. This solution was aerated continuously with 95% O₂ and 5% CO₂ while in the two perfusing chambers.

DRUGS

Norepinephrine (levarterenol bitartrate), angiotensin II (Hypertensin, Ciba Company Ltd.), and vasopressin (Pitressin) were used, always in freshly prepared solutions and with distilled water as solvent. The dilution factor was such that never more than 1.0 ml of drug solution was added to 50 ml of perfusate. Drugs were always perfused through the lumen of the artery.

In some experiments, rat plasma was used as a pressor agent. It was obtained from normotensive or hypertensive donor rats one day before the experiment. The plasma was stored overnight at −10°C then warmed to +4°C until used. The perfusion dose of plasma was 0.5 ml per 40 ml perfusate.

PRODUCTION OF HYPERTENSION

Male albino rats (80 to 100 g) of the inbred Wistar strain (W/Fu) were used. One kidney was removed from each animal 5 days before the start of treatment. Four 25 mg DCA pellets were implanted subcutaneously on days 1, 7, 14, and 21. Between days 2 and 30, the DCA treated rats were given 1% saline instead of water to drink. These rats were used for experiments 6 to 13 weeks after the start of the treatment. Blood pressure was determined electromanometrically from the femoral artery using a 22 gauge needle coupled to a Statham transducer.

PROCEDURE

The rat was first given light ether anaesthesia and its blood pressure was recorded. It was then narcotized by an intraperitoneal injection of pentobarbitone (3.5 mg/100 g wt.), for the excision of the caudal artery. Starting at the base of the tail, a 5 cm length of the ventral caudal artery was exposed and freed from surrounding tissues by blunt dissection. A 3 cm length of artery was excised and transferred to the mounting chamber for cannulation and ligation. A 1 cm length of artery was fixed in 10% formol-saline. Except for the brief period of transfer, the arterial segment used for experiments was always bathed in warm (37°C) aerated Krebs solution. The average operating time from skin incision to cannulation was about 40 min.

The mounted segment was perfused on both sides of its wall with aerated Krebs solution (37°C) for two hours. After this rest period, the artery was tested for viability by recording the constriction produced by perfusing 0.025 µg/ml norepinephrine (five times the experimental dose) at 50 mm Hg pressure. If this perfusion did not reduce flow by at least 50%, the artery was discarded. The discard rate (about 10% of dissected arteries) was similar for normotensive and hypertensive arteries.

Before the start of an experiment, the artery was perfused routinely at 150 mm Hg for several minutes. During this perfusion, the artery was inspected for uniform distension and its length was adjusted to eliminate any buckling. If any portion of the artery segment failed to distend at this high pressure, the artery was discarded. About 5% of all dissected arteries behaved in this manner and they were usually from hypertensive animals.

Thus, two arbitrary criteria were used to reject an artery before the start of the experiment. The first criterion rejected arteries which failed to contract moderately to a strong norepinephrine stimulus, and the second criterion rejected arteries which failed to relax uniformly during high pressure perfusion. Since these criteria were designed to reject extremely abnormal behavior of arteries, they tended to favor the normotensive arteries more than the hypertensive arteries.

The main experiment involved systematic perfusion of the artery at increasing pressures from 10 to 150 mm Hg. The perfusate was free of pressor agent during the first and third perfusion series and contained 0.005 µg/ml norepinephrine during the second perfusion series. This second series was preceded by an initial norepinephrine perfusion at 50 mm Hg for 3 min to allow time for maximum vasoconstriction. Pressure and flow rate were recorded continuously and outside diameter was measured at the midpoint of the segment whenever pressure and flow were constant. All other procedures were performed at a constant perfusing pressure of 50 mm Hg. In some arteries, a steady pressure-flow state was maintained for 10 min while inside and outside diameters were both measured carefully every 2 min.

At the end of a successful experiment (3 to 5 hr), the arterial segment was removed from the chamber and fixed in 10% formol-saline (under zero intraluminal pressure). Serial sections were made every 3 mm along a 10 mm length of the perfused and nonperfused artery segments. Slides from each serial group were stained with...
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haematoxylin-eosin, periodic acid-Schiff and Gomori's aldehyde fuchsin.

Results

HISTOLOGICAL APPEARANCE OF PERFUSED AND NONPERFUSED ARTERIES

Figure 1 shows examples of normotensive and hypertensive arteries. The internal elastic lamina showed stages of fragmentation, duplication, and obliteration. The inner half of the tunica media showed degrees of degeneration characterized by hyalinization (H and E stain), increased basophilic staining (periodic acid-Schiff and Gomori's aldehyde fuchsin), vacuolation and clefting. These changes were similar to those described by Skelton in the renal arteries of the DCA hypertensive rat. Comparison of pictures 2 and 4 in figure 1 illustrates how the pathological findings were altered by artificial perfusion. Basophilic staining, as in picture 2, was absent in all the damaged areas found in the perfused artery segments.

In table 1, the incidence of pathological lesions is related to DCA treatment and to the animals' diastolic blood pressure. The frequency of lesions increased both with DCA treatment and with hypertension. About 77% of the hypertensive arteries had pathological changes in their walls compared to 21% of the untreated normotensive arteries.

To get a rough estimate of wall hypertrophy, radius (r₁ and r₀) and tunica media thickness (TM) were measured on each arterial section. These measurements plus the calculated TM/r₀ ratio and tunica media area*(r₀² − r₁²) are given in table 2. Hypertensive arteries were divided into early and late groups because the TM/r₀ ratio was found to be significantly different in these two groups. Re-examination of the sections revealed that the late hypertensive group (see table 2) contained the arteries with the severe lesions reported in table 1.

Of the four dimensions listed in table 2, only the wall area (r₀² − r₁²) can be used as a measure of wall hypertrophy because it is unaltered by constriction. These calculations indicate that the hypertensive artery, particularly the "early" hypertensive artery, had a thicker muscular wall. Thickening of the muscle coat was found even after the hypertensive artery was artificially perfused for three to five hours. These results do not permit one to say whether or not wall thickening narrowed the arterial lumen.

WALL THICKENING AND LUMEN NARROWING

Arteries from normotensive and hypertensive rats of about equal size (290 to 310 g) were perfused at 50 mm Hg (see Methods). Careful and repeated measurements of inside and outside diameter were made when flow was constant. The average results are given in table 3. The hypertensive arteries not only had a smaller inside radius but, also, flow through them was reduced. Calculation of the total wall area (r₀² − r₁²) again revealed wall thickening in the hypertensive arteries. The average outside radii for normotensive and hypertensive groups were similar. The results obtained by this method permit one to conclude that wall thickening definitely narrowed the lumen.

**TABLE 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lesions*</th>
<th>Diastolic B.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Moderate</td>
</tr>
<tr>
<td>Normotensive control (14)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Normotensive DCA (9)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Hypertensive DCA (22)</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>

*Test of independence 2 x 3 table (rows 1 and 3), χ² = 11.0, P < 0.01. Test of independence 3 x 3 table, χ² = 11.2, P < 0.05.

†Number of rats in group.
TABLE 2

Histometry on Serial Sections of Rat Caudal Arteries

<table>
<thead>
<tr>
<th></th>
<th>Normotensive controls</th>
<th>Hypertensive DCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Time after treatment, wk</td>
<td>6—13</td>
<td>6—8</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>121 ± 1.6*</td>
<td>157 ± 8.5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78 ± 1.7</td>
<td>107 ± 8.6</td>
</tr>
<tr>
<td>Mean</td>
<td>100 ± 1.7</td>
<td>132 ± 8.5</td>
</tr>
</tbody>
</table>

**Perfused arteries**
- Inside radius, \( r (\mu) \): 98 ± 7.5
- Tunica media, \( TM \) (\( \mu \)): 70 ± 5.1
- \( (r_o^2 - r_i^2) \times 10^4 \) (\( \mu^2 \)): 1.80 ± 0.20

**Nonperfused arteries**
- Inside radius, \( r (\mu) \): 37 ± 2.7
- Tunica media, \( TM \) (\( \mu \)): 87 ± 3.5
- \( (r_o^2 - r_i^2) \times 10^4 \) (\( \mu^2 \)): 1.48 ± 0.11

<table>
<thead>
<tr>
<th></th>
<th>Normotensive control (6)†</th>
<th>Hypertensive DCA (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>ml/sec ( \times 10^{-6} )</td>
<td>( r_i^{(\mu)} )</td>
</tr>
<tr>
<td>Normotensive control (6)†</td>
<td>23.5 ± 1.5‡</td>
<td>310 ± 7.9</td>
</tr>
<tr>
<td>Hypertensive DCA (6)</td>
<td>16.6 ± 1.2</td>
<td>269 ± 9.8</td>
</tr>
</tbody>
</table>

*Standard error of mean.
†P: probability that difference from control value is a random one. Probabilities are listed when they were < 0.05.
‡\( r_o \): outside radius.

TABLE 3

Measurement of Arterial Radius During Perfusion at 50 mm Hg

<table>
<thead>
<tr>
<th>Groups</th>
<th>Flow rate</th>
<th>Inside radius ( r_i^{(\mu)} )</th>
<th>Wall area ( (r_o^2 - r_i^2) \times 10^4 ) (( \mu^2 ) ( \times 10^{-6} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive control (6)†</td>
<td>23.5 ± 1.5‡</td>
<td>310 ± 7.9</td>
<td>4.13 ± 0.42</td>
</tr>
<tr>
<td>Hypertensive DCA (6)</td>
<td>16.6 ± 1.2</td>
<td>269 ± 9.8</td>
<td>6.12 ± 0.42</td>
</tr>
</tbody>
</table>

*Body weights of all rats: 290 to 310 g.
†Number of animals in group.
‡Standard error of mean.
§P: probability that difference from control value is random.

FIGURE 1

Sample cross sections of two ventral tail arteries (Gomori's aldehyde fuchsin stain), one from a normotensive rat (1,3) and one from a hypertensive rat (2,4). Arteries were fixed in 10% formal saline at zero pressure. Sections 1 and 2 were taken from nonperfused arterial segments and sections 3 and 4 were taken from the artificially perfused segments (Krebs-Henseleit solution for 3 hr). Note: contraction after excision (1,2) and relaxation after perfusion (3,4); degeneration of internal elastic lamina and tunica media of hypertensive artery (2,4); absence of basophilic staining in tunica media of hypertensive artery after perfusion (4).

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PERFUSION OF NOREPINEPHRINE AT VARYING PRESSURES

Figures 2 and 3 summarize the pressure-flow-radius data obtained from the main experimental procedure (see Methods). The curves for normotensive and hypertensive arteries are labelled N and H respectively. Curves N₁ and H₁ are for normal Krebs perfusion and curves N₂ and H₂ are for norepinephrine perfusion.

The average curves in figure 3 illustrate realistically the manner in which a typical artery distended as pressure was elevated from zero to 150 mm Hg. During normal Krebs perfusion, all arteries distended smoothly and at a decreasing rate with each increment of rise in pressure (curve N₁ + H₁). During norepinephrine perfusion, all arteries distended smoothly up to about 90 mm Hg, but at a slightly higher pressure, 80% of the normotensive arteries and 60% of the hypertensive arteries suddenly distended at an accelerated rate (curves N₂ and H₂). This change was sufficiently abrupt, particularly in the normotensive arteries, to be observed under the microscope. This phenomenon is probably related to the strength of contraction of some or all of the muscle fibres in the artery wall because it occurred more frequently during relatively weak contractions and disappeared during strong contractions.

During normal Krebs perfusion, flows through hypertensive arteries were moderately lower than controls at all pressures (fig. 2, N₁ - H₁). This reduction of flow was statistically significant only at low pressures (<90 mm Hg). The average outside radii for normotensive and hypertensive arteries were similar at all pressures (fig. 3, N₁ + H₁).

Norepinephrine perfusion reduced flows through hypertensive arteries more than through normotensive arteries at all pressures (fig. 2, compare H₂ - H₁ to N₁ - N₂). In addition, the outside radius of the hypertensive artery was reduced more than the radius of the normotensive artery (fig. 3). It can be concluded that the hypertensive artery is narrowed more than the normotensive artery by a given dose of norepinephrine. This does not necessarily mean, however, that the muscle fibres in the hypertensive artery are hyper-responsive because the increased narrowing may be due to the thickened wall.

TENSION DEVELOPED AND WORK DONE DURING CONTRACTION

One muscle is more responsive than another muscle of equal size when the former produces a stronger contraction to a given stimulus. Usually, strength of contraction is measured as the amount of shortening when the load is constant (isotonic) or as the increase in tension when length is constant (isometric). When the conditions of contraction are neither isotonic nor isometric, then contraction must be measured as work performed by the muscle. This latter condition exists when the arterial wall constricts, i.e., both muscle tension and length change.

The problem is complicated further by the...
fact that the tension in the artery wall is partly due to stretched elastic components in the wall. Furthermore, this passive elastic tension is a nonlinear function of radius. In an artery at a given radius, both total wall tension and elastic tension must be known before active muscle tension is known.

Approximate total wall tension can be calculated from the expression:

$$T = \frac{P \times (r_0 + r_1)}{2}$$  \hspace{1cm} (1)$$

where $P$ is transluminal pressure* in dynes/cm² and $\frac{(r_0 + r_1)}{2}$ is the mean effective radius.

Inside radius ($r_i$) can be estimated by two methods. First, since the cross-sectional area remains constant,\textsuperscript{13} then from table 3, $r_i = \sqrt{r_o^2 - 4.13 \times 10^4} \mu$ for normotensive arteries and $r_i = \sqrt{r_o^2 - 6.12 \times 10^4} \mu$ for hypertensive arteries. Second, $r_i$ can be estimated from the flow curves (fig. 2) using the modified Poiseuille equation:\textsuperscript{12}

$$r_i^1 = \frac{8F}{\Delta P \pi} \left( \frac{dF}{\pi} + \eta l \right)$$  \hspace{1cm} (2)$$

where $\Delta P$ is pressure gradient along the artery of length ($l$) and midpoint radius ($r_i$); $F$ is flow of perfusate with density ($d$) and viscosity ($\eta$). The viscosity and density of Krebs solution were made equal to the viscosity (0.007 poise) and density (1.0 g/cm³) of water at 37°C.

At all pressures and contractile states, the $r_i$ values calculated by the first method were consistently larger than the $r_i$ values calculated by the second method. The range of difference between the two inside radii was 0 to 12% with a mean difference of 6.15% ± 1.5 (SE). This magnitude of disagreement is understandable when one considers the errors in the two methods. The $r_i$ from ($r_o^2 - r_i^2$) is subject to the errors in $r_o$ (fig. 3). For example, $r_o$ may be consistently overestimated because of optical distortions on

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*Since $P$ in this equation must be transluminal pressure at the midpoint of the artery segment, $P = \frac{A}{l} \Delta P$ driving pressure.

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because of swelling of the adventitia. The \( r_t \) from equation 2 may be in error because of the assumption that the mean effective radius (\( r_e \) in equation 2) is located at or near the midpoint of the artery segment (where \( r_e \) is measured).

The values of \( r_t \) from equation 2 are plotted in figure 4. It can be seen that the inside radius of the hypertensive artery (curve \( H_1 \)) was consistently smaller than the inside radius of the normotensive artery (curve \( N_t \)). This agrees with the findings in table 3, and is consistent with the earlier conclusion that the wall of the hypertensive artery thickens at the expense of the arterial lumen.

The inside radii in figure 4 can be used in the expressions, 
\[
(r_o^2 - r_t^2) = 4.13 \times 10^4 \mu \\
(r_o^2 - r_o^2) = 6.12 \times 10^4 \mu,
\]

to calculate the corresponding outside radii. As expected, these calculated \( r_o \) values differed from the measured \( r_o \) values (fig. 3) by about 6%. The calculated \( r_t \) and \( r_o \) values were used in equation 1 to calculate total wall tension. The results produced the tension-radius curves in figure 5. For practical purposes, curves \( N_1 \) and \( H_1 \), without vasoconstriction, define elastic tension in the normotensive and hypertensive arteries. Line \( XY \) defines the variation of wall tension with radius for a given constant pressure (here 120 mm Hg). When the normotensive artery contracted at 120 mm Hg, total wall tension decreased from \( D \) to \( E \), elastic tension decreased from \( D \) to \( F \), and active muscle tension increased from zero to \( (E - F) \). The work done by the muscle fibres is represented by the area \( DEFD \). When the hypertensive artery contracted at 120 mm Hg, total wall tension decreased from \( A \) to \( B \), elastic tension decreased from \( A \) to \( C \) and active muscle tension increased from zero to \( (B - C) \). The work done by the hypertensive muscle fibres is represented by the area \( ABCA \). The numerical values of areas \( DEFD \) and \( ABCA \) are 6.0 and 13.5 ergs/cm length re-

*Actually work is \( 2\pi \) (area).
Elastic diagrams (tension-radius curves) derived from data in figures 2 to 4 and table 3. Line XY defines the variation of wall tension with mean radius when pressure is held constant at 120 mm Hg. Areas DEFD and ABCA are proportional to muscular work performed by normotensive and hypertensive arteries respectively, when they contract against 120 mm Hg pressure during 0.005 µg/ml norepinephrine perfusion.

respectively. Thus, the muscle in the hypertensive artery performed more than twice as much work as the muscle in the normotensive artery. The calculations leave no doubt that the hypertensive artery is hyper-responsive.

Work performed by individual muscle fibres can also be estimated if one knows the number of fibres per unit length of artery. Histologically, the thickened wall of the hypertensive artery (table 2) seemed to be due to a combination of muscle hypertrophy and increased extracellular material. In both these conditions, the area of the wall is increased without changing the number of fibres per unit length of artery. If this is the case, then it can also be concluded from figure 5 that each muscle fibre in the hypertensive artery must be hyper-responsive.

Hyper-responsiveness is put to a critical test when one makes the unlikely assumption that wall thickening is entirely due to muscle hyperplasia. It follows that the arterial wall should contain a constant number of muscle fibres per unit volume. The muscle work per unit volume is obtained by dividing the work/cm by the appropriate \( (r_1^2 - r_0^2) \) factor in table 3. This becomes \( 1.45 \times 10^4 \) ergs/cm\(^3\) for the normotensive artery and \( 2.20 \times 10^4 \) ergs/cm\(^3\) for the hypertensive artery. Thus, each hypertensive muscle fibre performed 50% more work than each normotensive muscle fibre. These arguments strongly support the
existence of true hyper-responsiveness in hypertensive arteries.

PRESSOR ACTIVITY OF OTHER DRUGS

Similar experiments were performed on 20 normotensive and 20 hypertensive arteries using vasopressin (0.25 µg/ml) and angiotensin II (0.02 µg/ml) as pressor agents. The hypertensive arteries were consistently hyper-responsive to vasopressin but not to angiotensin II. In addition, the contraction to vasopressin was more difficult to abolish (by normal Krebs perfusion) in the hypertensive arteries.

PRESSOR ACTIVITY OF SERUM

The pressor activity of serum from normotensive and hypertensive rats was tested on isolated arteries from normotensive and hypertensive rats to demonstrate the combined effect of an increased pressor titre and vascular hyper-responsiveness (see Methods). The average pressor responses, expressed as per cent reduction in flow rate, are given in table 4. The perfusing pressure in these experiments was 50 mm Hg. Figures 2 to 5 illustrate that the perfusing pressure was poorly chosen for the demonstration of hyper-responsiveness. Nevertheless, the results indicate not only that the hypertensive artery was more responsive to the pressor agent but also that the hypertensive serum contained more pressor activity.

An approximate figure for the potency of serum pressor activity can be obtained by comparing the responses to serum and norepinephrine. In normotensive arteries, 0.0125 ml hypertensive serum per ml perfusate produced a 33% reduction in flow (table 4) and 0.005 µg/ml norepinephrine produced a 58% reduction in flow (fig. 2). Therefore, the pressor activity in the hypertensive serum was approximately equivalent to 0.2 µg/ml norepinephrine.

ELASTICITY OF THE ARTERY

Incremental elastic moduli were calculated at three pressures using the equation:

$$E_{\text{inc}} = \frac{\Delta P r_e}{2 (1 - \sigma^2) r_i^2 \left(r_o^2 - r_i^2\right)}$$

where $\Delta P$ is the change in pressure which produces a $\Delta r_e$ change in outside radius, and $\sigma$ is Poisson’s ratio which equals 0.5 when volume of the tube wall remains constant. The $r_i$ values were taken from figure 4 and the $r_o$ values were calculated from the $(r_o^2 - r_i^2)$ values in table 3. The moduli of normotensive and hypertensive arteries at rest and during contraction are given in table 5.

TABLE 5
Incremental Elastic Modulus ($E_{\text{inc}}$) of Rat Caudal Arteries

<table>
<thead>
<tr>
<th>Driving pressure (mm Hg)</th>
<th>Normotensive arteries</th>
<th>Hypertensive arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At rest</td>
<td>During contraction</td>
</tr>
<tr>
<td>45</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>75</td>
<td>2.60</td>
<td>0.63</td>
</tr>
<tr>
<td>105</td>
<td>10.0</td>
<td>0.55</td>
</tr>
</tbody>
</table>

*Calculated from equation 3 (see text) using data in figure 4 and $(r_o^2 - r_i^2)$ values in table 3.
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during contraction are listed in table 5. There
is a calculated elastic modulus for each inter-
section of a dashed line with a pressure-radius
curve in figure 4. Notice that the slopes
\( \Delta P/\Delta r \) of curves \( N_1 \) and \( H_1 \) (at inter-
sections) reflect the moduli better than the
slopes of curves \( N_2 \) and \( H_2 \) do. This is ex-
plained by the variable factor, \( r_ar^2 \), which is
directly proportional to \( E_{\text{inc}} \) in equation 3.
When the artery is relaxed \( r_ar^2 \) is large and
when the artery is constricted \( r_ar^2 \) is small
in magnitude. Clearly, \( E_{\text{inc}} \) will be influ-
enced more by a reduction in \( r_ar^2 \) than by a
simultaneous elevation in \( \Delta P/\Delta r \). Two con-
clusions can be drawn from the moduli in
figure 5. First, in the absence of contraction,
the hypertensive artery appears to be more
elastic (smaller elastic modulus) than the nor-
motensive artery between 65 and 115 mm Hg
pressure. Second, during contraction the elas-
ticity of normotensive and hypertensive ar-
teries appears to be similar between 35 and
115 mm Hg pressure.

Discussion

Both histologically and physiologically,
the arteries of hypertensive animals were
found to be thickened. This thickening nar-
rowed the lumen of the artery slightly but
significantly in the absence of contraction. In
cross section, the hypertensive arteries always
showed increased basophilic staining (peri-
dodic acid-Schiff (PAS) and Gomori’s al-
dehyde fuchsin) of extracellular structures,
often showed wide intercellular spaces, and
sometimes showed increased elastin content
in the tunica media. The basophilic material
was probably acid mucopolysaccharide which
stains with the above dyes and which is
known to be increased in the arterial walls of
DCA hypertensive rats.16 In the sections
stained with haematoxylin and eosin, there
was no evidence of muscle fibre hyperplasia
or foreign cell infiltration in the walls of the
hypertensive arteries. This evidence suggests
that thickening of the tunica media was due
primarily to expansion of the extracellular
space and also due possibly to muscle fibre
hypertrophy.

These experiments demonstrate unequivoc-
ally that the isolated caudal artery from hy-
pertensive rats was hyper-responsive to norepi-
nephrine and vasopressin. These findings
reinforce those of Mendlowitz et al.8–9 who, by
estimating work performance of the digital
artery from perfusion data, demonstrated
that the digital artery of human hypertensive
subjects was hyper-responsive to neurogenic
and norepinephrine stimulation. In contrast,
Redleaf and Tobian17 were unable to find
hyper-responsiveness in aortic strips from hy-
pertensive rats. It is possible that hyper-
responsiveness in the aorta might have been
masked in some way by the large quantities of
elastin and collagen. Also, both stripping and
the use of NaNO2 to relax smooth muscle
might have produced chemical changes which
masked hyper-responsiveness. The curves in
figures 2 to 5 indicate that this hyper-reactive
property of hypertensive arteries may be
more important than wall thickening17,2 in
narrowing the arterial lumen. Although the
experiments provide no evidence as to how
the muscle fibres become hyper-reactive, they
do demonstrate that the changes responsible
for this property were permanent enough to
withstand the stress of excision and five hours
of artificial perfusion.

True hypertrophy of muscle fibres with in-
crease in the contractile proteins would pro-
vide a simple explanation of hyper-responsive-
ness. It is logical to expect enlarged muscle
fibres to be capable of performing more work,
provided such enlargement is due to a mass
increase in the contractile machinery and not
to "waterlogging."24 Unfortunately, the muscle
fibres in the cross sections of hypertensive
arteries (H and E stain) did not appear to
be hypertrophic compared to controls.

Muscular hyper-responsiveness may be due
to a chemical change in the fibre induced
by the DCA treatment. There is good evidence
that adrenal steroids potentiate vascular con-
traction18–21 and there is some evidence that
this adrenal steroid potentiation is greater in
hypertensive arteries.22 One possibility is that
adrenal steroids react with the muscle fibre
membrane in a way which alters permanently
its selective permeability to ions. Normally, the resting membrane is only permeable to $K^+$ and relatively impermeable to $Na^+$ and $Ca^{++}$. In the "excited" membrane, permeability to $Na^+$ and $Ca^{++}$ is increased. The corollary may also be true, i.e., increased permeability to $Na^+$ and $Ca^{++}$ produces a more excitable membrane and a more hyper-reactive fibre to stimulation.

That steroids alter membrane permeability is consistent with observations that cells, treated with these hormones, accumulate $Na^+$ and lose $K^+$. That this mechanism might be operating in hypertension is supported by evidence that hypertensive arteries often contain increased quantities of $Na^+$ which may be intracellular.

The demonstration of increased pressor activity in plasma from DCA hypertensive rats (table 4) illustrates that increased vascular tone in hypertension may be due in part to increased amounts of circulating vasoconstrictor materials. Since 24 hour whole frozen plasma was used, it is unjustified, perhaps, to comment on the chemical nature of the pressor activity. However, it is unlikely to be angiotensin because the hypertensive rat caudal artery responded normally to this agent and responded more vigorously to plasma (table 4).

The observed change in elasticity of the hypertensive artery requires an explanation. If the values in table 5 are assigned to the appropriate curves in figure 4 or 5, it becomes clear that elasticity is increased (modulus decreased) as the radius is reduced, even when the latter is reduced by muscle contraction. In effect, the artery is more distensible during muscle contraction. The low moduli for the artery during contraction support the idea that muscle must be the only structure under tension. Therefore, vasoconstriction must have permitted stiffer wall structures to slacken (e.g., elastin and collagen). The observation that the moduli for a contracting artery at a high pressure and for a noncontracting artery at a low pressure are similar in magnitude, suggests that the elasticity of muscle fibres is not altered greatly by contraction.

The elastic properties of the hypertensive muscle fibres are probably not affected by the disease because elastic moduli for contracting normotensive and hypertensive arteries were similar. In the absence of contraction, the relatively low elastic moduli for the hypertensive artery may indicate elastin degeneration (fig. 1).

**Summary**

The ventral caudal artery from DCA hypertensive and control rats was excised, mounted in a plastic chamber, and perfused with Krebs-Henseleit solution. Diameter of the artery and flow through the artery were measured at perfusion pressures ranging from 0 to 150 mm Hg. During these perfusions, constriction of the arterial segment was produced by adding norepinephrine, vasopressin, angiotensin II or rat serum in concentrations known to produce submaximal contractions.

Samples of each experimental artery before and after artificial perfusion were fixed, sectioned, and stained. The tunica media of hypertensive arteries was thickened. Also, degenerative changes were observed in the internal elastic lamina and adjacent tunica media. Structural thickening of the tunica media was due primarily to expansion of the extracellular space and also due possibly to muscle fibre hypertrophy.

In the absence of contraction, flow through the hypertensive artery was moderately reduced at all pressures because its lumen was narrowed by the thickened wall.

Total wall tension, muscle tension, and elastic tension of the artery were estimated from the pressure-flow-radius data. Strength of muscular contraction was then measured in terms of work performed during contraction at a constant pressure. From this analysis, the muscle fibres in hypertensive arteries were found to be unequivocally hyper-responsive to norepinephrine, vasopressin, and rat serum. The possible cause of this hyper-responsive property is discussed.

Serum was collected both from normo-
tensive and from hypertensive donor rats and its pressor activity tested on the in vitro preparation. The pressor activity in hypertensive serum was found to be significantly elevated.

The elasticity of the hypertensive artery was increased in the absence of contraction and unchanged during contraction. No hypertensive artery was found to be stiffer than normotensive arteries. These changes are probably related to degeneration of elastin.

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In Vitro Demonstration of Vascular Hyper-responsiveness in Experimental Hypertension
JOSEPH A. M. HINKE and Patricia A. Simpson

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