# Viscoelastic Properties of the Aorta of Hypertensive Rats[1] (34727)

NEAL BANDICK AND HARVEY SPARKS
(Introduced by D. F. Bohr)
*Department of Physiology, University of Michigan, Ann Arbor, Michigan 48104*

Regardless of the mechanism responsible for initial blood pressure elevation in experimental hypertension, changes occur in the physical properties of the arterial wall (4, 6, 8) which may influence the course of the disease. The following is a report of our studies of the elasticity and viscosity of aortic strips from normal and renal hypertensive rats. Our measurements of both elastic and viscous parameters support the idea that increased content of vascular smooth muscle may be responsible for the increased elastic modulus and viscosity of the aorta.

*Methods.* Albino rats were housed in air-conditioned quarters and had free access to standard rat chow and water. Hypertension was produced by removing the left kidney and placing a 2 × 6-mm clip on the renal artery of the remaining kidney 1 week later (10). No surgery was performed on the control animals. At weekly intervals the systolic blood pressure of each animal was measured, using a tail cuff and microphone.

The rats were divided into four groups, the first of which was sacrificed to provide base line values for the other groups. A second group was composed of three normotensive control animals and three hypertensive animals which were sacrificed 3 weeks post renal artery clamping. The third group consisted of three controls and three hypertensive animals which were sacrificed 9 weeks post-clamping. A fourth group contained three controls and three animals which were not hypertensive in spite of renal artery clamping 7 weeks prior to being studied. The blood pressures of all animals are listed in Table I.

Viscous and elastic constants of the abdominal aorta (od 1.6 to 2.2 mm) were measured by a modification of the method reported by Apter (2). Using a helical pitch of approximately 30°, strips 2.0 to 3.2 mm wide and 1 cm long were cut from the abdominal aorta. One end of the strip was tied by nylon thread to a jeweler's chain which in turn was attached to a force-displacement transducer (Grass model FT .03). The other end was attached to an air pressure driven apparatus which increased the length of the strip 0.05 cm in 0.02 sec. Each strip was quick-stretched four times at 1.2, 1.5, and 2.0 cm so that 12 measurements were made on each strip. All strips were superfused with physiological salt solution (PSS) free of added calcium for 1 hr prior to and throughout the experiment. Calcium-free PSS was used because preliminary experiments demonstrated that in the presence of calcium the vessels contracted spontaneously giving rise to large variations in values for stiffness and viscosity. The temperature of the strip was maintained at 37°. Studies of the aortas of the hypertensive animals and of weight-paired control animals were done simultaneously. At the end of the experiment the strips were cut at their ties and weighed. Cross-sectional area was calculated from the volume (wt) and length. Elastic and viscous parameters were determined using Apter's model for the blood vessel wall (2).

The strip of aorta is represented as an elastic element with a spring constant $E_1$ in parallel with a Maxwell element with a spring constant $E_2$ and a viscosity $\eta$ (Fig. 1). If the force exerted by the Maxwell element is considered to be virtually absent 3 sec after a quick stretch,

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TABLE I. Viscoelastic Properties of Aortae of Normal and Renal Hypertensive Rats.

| | Av blood pressure (mm Hg) | Increase in resting length (%) | Young's modulus ($10^6$ dyn/cm$^2$; $N = 12$) | | Viscous force ($10^6$ dyn/cm$^3$; $N = 12$) | | Viscosity ($10^4$ p/cm; $N = 12$) | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean | $S\bar{x}$ | Mean | $S\bar{x}$ | Mean | $S\bar{x}$ |
| First week control | 93 | 20 | 0.61 | 0.09 | 0.29 | 0.04 | 1.87 | 0.21 |
| | | 50 | 3.15 | 0.32 | 0.52 | 0.03 | 8.16 | 0.84 |
| | | 100 | 50.37 | 6.31 | 2.39 | 0.32 | 88.12 | 10.37 |
| 3-Week normotensive | 109 | 20 | 1.09 | 0.05 | 0.23 | 0.04 | 2.58 | 0.30 |
| | | 50 | 3.24 | 0.12 | 0.33 | 0.04 | 4.70 | 0.42 |
| | | 100 | 51.45 | 2.61 | 3.63 | 0.84 | 124.73 | 36.11 |
| 3-Week hypertensive | 154 | 20 | 1.51[a] | 0.07 | 0.26 | 0.02 | 4.91[a] | 0.61 |
| | | 50 | 4.96[a] | 0.24 | 0.61[a] | 0.08 | 22.29[a] | 4.09 |
| | | 100 | 105.28[a] | 10.72 | 3.43 | 0.58 | 239.11[a] | 46.25 |
| 9-Week normotensive | 106 | 20 | 1.07 | 0.12 | 0.12 | 0.01 | 1.97 | 0.33 |
| | | 50 | 2.74 | 0.13 | 0.17 | 0.01 | 4.69 | 0.73 |
| | | 100 | 36.15 | 8.01 | 3.65 | 0.89 | 101.96 | 23.03 |
| 9-Week hypertensive | 217 | 20 | 1.47[a] | 0.03 | 0.22[a] | 0.02 | 5.78[a] | 0.94 |
| | | 50 | 7.63[a] | 0.31 | 0.89[a] | 0.06 | 42.43[a] | 4.42 |
| | | 100 | 216.75[a] | 6.74 | 17.60[a] | 0.99 | 1169.90[a] | 143.55 |
| 7-Week normotensive | 117 | 20 | 1.19 | 0.07 | 0.13 | 0.01 | 1.80 | 0.26 |
| | | 50 | 3.84 | 0.40 | 0.30 | 0.05 | 4.33 | 0.92 |
| | | 100 | 82.98 | 9.86 | 9.91 | 1.54 | 364.00 | 101.51 |
| 7-Week "sham" hypertensive | 117 | 20 | 1.24 | 0.06 | 0.15 | 0.01 | 1.69 | 0.19 |
| | | 50 | 3.96 | 0.31 | 0.38 | 0.05 | 5.48 | 1.03 |
| | | 100 | 78.90 | 4.79 | 10.46 | 1.08 | 375.00 | 82.68 |

[a] $p < .05$ when compared to normotensive.

$$E_1 = \frac{\Delta f_{3\ \mathrm{sec}}}{A\, l_s},$$

where $\Delta f_{3\ \mathrm{sec}}$ is the increase in force (dyn) 3 sec after the stretch, $A$ is the cross-sectional area of the strip (cm$^2$) and $l_s$ is the amount of stretch (cm).

If the stretch is sufficiently rapid, the peak force is the sum of the forces developed by the two parallel elements. In this case

$$E_2 = \frac{\Delta f_0 - \Delta f_{3\ \mathrm{sec}}}{A\, l_s},$$

where $\Delta f_0$ is the peak increase in force following the stretch.

Young's modulus ($M$) can be calculated from

$$M = E_1 l_i,$$

where $l_i$ is the length of the strip (cm) before the stretch.

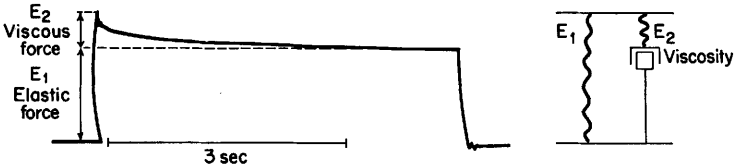


FIG. 1. Tension developed by rat aorta in response to quick stretch. Elastic and viscous constants determined using a model developed by J. T. Apter (2). Stretch interval for all strips <5% of initial strip length. Duration of stretch interval 0.02 sec.

The viscosity of the Maxwell element can be calculated as

$$\eta = E_2 t_e ,$$

where $t_e$ is the time (sec) after the stretch for force to decay to

$$\Delta f_{3\ \mathrm{sec}} + \frac{\Delta f_0 - \Delta f_{3\ \mathrm{sec}}}{e} .$$

Thoracic aortas were removed from control and hypertensive animals for histological study. While the tissue was still *in situ* two loops of suture were bound around the vessel and the distance between them was measured. After the vessel was dissected free from the animal it was fixed at its *in situ* length by attaching the free ends of the suture loops to a bow-shaped glass rod. After fixation in 10% formalin the tissues were paraffin imbedded, sectioned, and stained with hematoxylin and eosin, periodic acid–Schiff, Verhoeff's elastic stain, and Masson's trichrome. Tissues were removed from control and hypertensive animals on the same day and were fixed in the same solutions for the same lengths of time in an effort to assure uniformity of shrinkage. Thickness of the wall was determined by measuring this dimension in each of 4 quadrants of four sections of the aorta using an ocular micrometer. The sections were prepared and photographed in the laboratory of Dr. Gerald Abrams, Department of Pathology, University of Michigan.

*Results.* Figure 1 shows a polygraph tracing of the tension changes which occur following a quick stretch of a helically cut strip of abdominal aorta. The portions of the force development which are used to calculate the spring constant, $E_1$ for the elastic element and the spring constant $E_2$ for the Maxwell element are indicated. When length is increased there is an immediate increase in tension to a peak value and then tension decreases to a relatively stable value within 3 sec after stretch.

The blood pressure of the several groups of hypertensive and control rats are given in Table I. Values for stiffness of the aortas of the hypertensive rats are displayed in Fig. 2. The data shown in Figs. 2 and 3 were obtained with the strip stretched to 150% of the initial length ($\Delta l/l_0 = 0.5$). Young's modulus of aortic strips from control animals does not change appreciably during the 9 weeks of the experiment but in experimental animals increased significantly from an average of 3.1 to 4.96 at 3 weeks after renal artery clamping, and to 7.6 $\times$ $10^{-6}$ dyn/cm$^2$ at 9 weeks after renal artery clamping. Changes in Young's modulus are less dramatic at $\Delta l/l_0 = 0.2$ and more so at $\Delta l/l_0 = 1.0$ (Table I).

After production of renal hypertension the viscous force constant ($E_2$) increases (Fig. 3, left), whereas the viscous force constant of normal aortas decreases over the same time period. Normal aortic strips exhibit no change in viscosity over the 9 weeks, but those from hypertensive animals are more viscous with the increase in duration and magnitude of hypertension. Even though viscosity of the control groups does not change, $E_2$ decreases and the time constant for decay of the viscous force increases.

The viscous and elastic parameters of the aortas of normotensive renal-artery-clamped rats (7 weeks) do not differ from controls (Table I).

Figure 4 is a photomicrograph of a cross

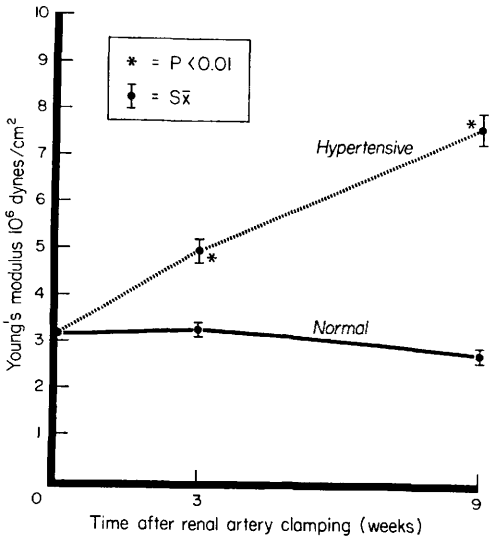


FIG. 2. Young's modulus ($M$) of abdominal aortic strips from normal and hypertensive rats. Resting length of the strips was 1.5 cm. Stretch interval was 0.05 cm.

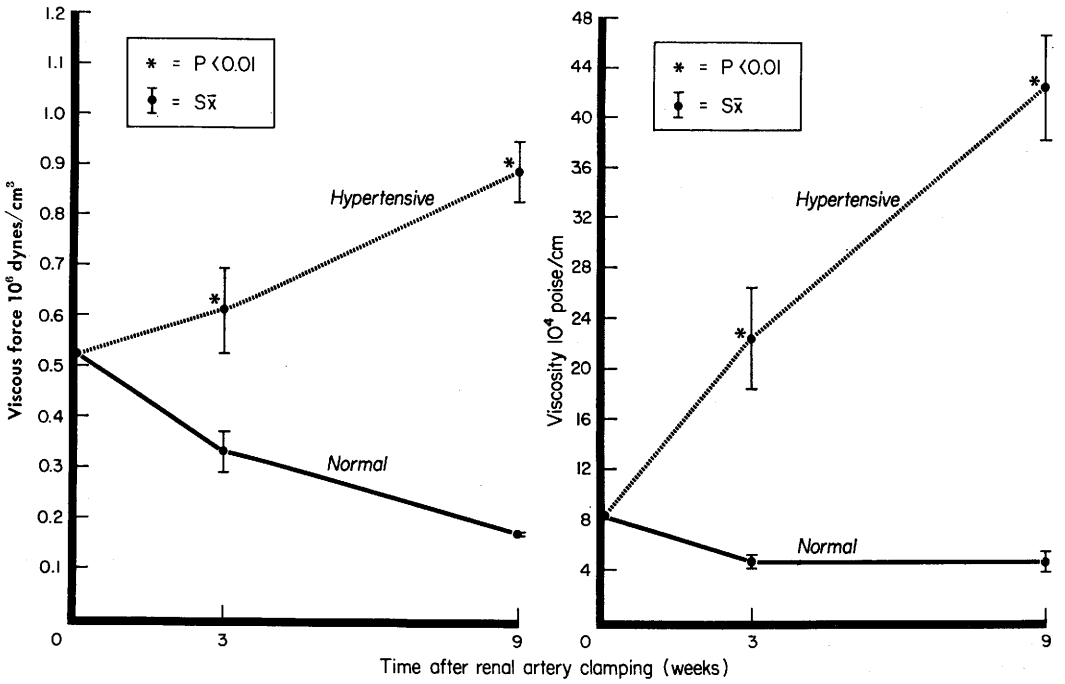


FIG. 3. Viscous force ($E_2$) and viscosity ($\eta$) of abdominal aortic strips from normal and hypertensive rats. Resting length was 1.5 cm while stretch interval was 0.05 cm.

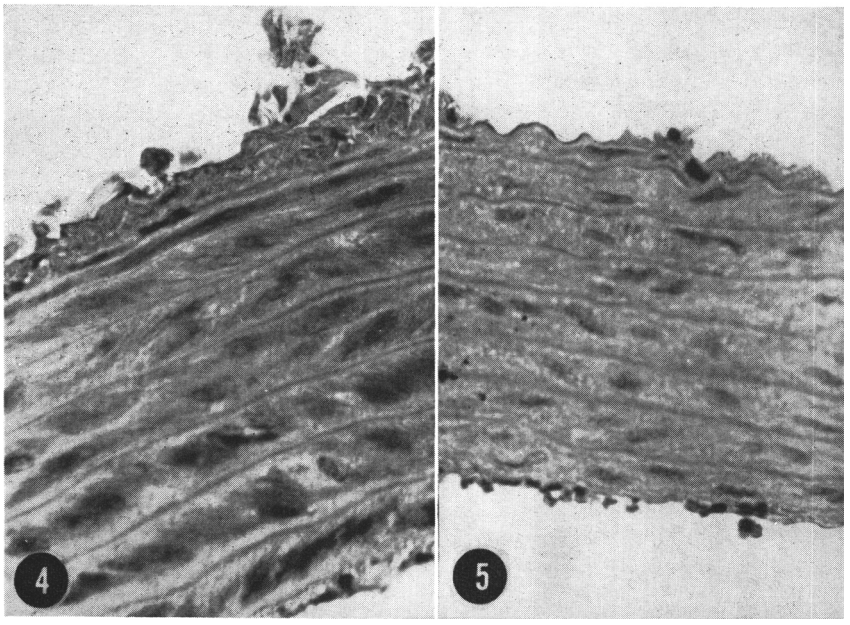


FIG. 4. Photomicrograph of cross section of aorta from hypertensive rat (9 weeks). Dark staining material between elastic laminae stains as does smooth muscle. Masson's trichrome stain, ×320.

FIG. 5. Photomicrograph of a cross section of a normal rat aorta. Masson's trichrome stain, ×320.

section of the abdominal aorta of a 9-week hypertensive rat and Fig. 5 is a photomicrograph of a similar section of a normal aorta. Wall thickness of aortas of hypertensive animals averaged 155 $\mu$ with a standard error of $\pm 2$ $\mu$ whereas control aortas averaged 102 $\pm$ 2 $\mu$. This difference is significant at $p < 0.01$ using Student's $t$ test. The number of elastic laminae counted in sections of aortas from hypertensive animals averaged 9.1 $\pm$ 0.3 as compared to controls which averaged 8.9 $\pm$ 0.3. The increased thickness was accounted for by an increased distance between elastic laminae.

Masson trichrome stain reveals an increased amount of material which stains as does smooth muscle (dark staining) between elastic laminae. Similar changes appear in the 3-week experimental aortas, but the changes are not as great.

*Discussion.* Our results confirm those of several investigators demonstrating that arteries of hypertensive animals have a higher elastic modulus than those of normotensive animals (4, 6, 8). The increase in viscosity which occurs simultaneously (Fig. 3) has two implications, one concerning the structure of the wall and the other the behavior of the aortic wall *in vivo*.

A complete understanding of the response of the blood vessel wall to elevated blood pressure must include knowledge of what elements of the wall participate. There are several facts which indicate that the increase in stiffness of the aorta observed early in the course of hypertension is due to hypertrophy or hyperplasia of smooth muscle. Mallov (9) has measured the alkaline extractable protein of the arterial wall as an index of the amount of muscle, and has found that aortas from hypertensive rats have more muscle than aortas from control rats. We have found an increase in material which has the staining characteristics of smooth muscle in the interlaminar spaces of hypertensive aortas (Fig. 5). Gordon's observation that aortas from hypertensives are capable of developing more active tension than those from normals (7) could be explained by increased muscle content. Apter has shown that muscle is the main contributor of viscous force development by the aortic wall of dog (1). The *pari passu* increase in elastic and viscous modulus of the wall of hypertensive aortas indicates (but by no means proves) that relaxed muscle may be an important structural contributor to the increased stiffness.

Aars has measured aortic baroreceptor activity of the rabbit after the development of renal hypertension (3). His studies on the increase in firing rate associated with the pressure pulse indicate that the aorta must have an increase in both elastic modulus and viscosity. Our study demonstrates that both changes do occur, and thereby supports the hypothesis that the chronic stage of renal hypertension could be due to resetting of the baroreceptors and maintenance of higher mean arterial pressure in this way.

*Summary.* The viscoelastic properties of strips of aorta from normal and hypertensive rats have been determined. The aortas of hypertensive animals are stiffer and more viscous than those of normal rats. The *pari passu* increase in stiffness and viscosity is evidence that muscle is the chief structural component resulting in increased stiffness. Photomicrographs of the aorta of normal and hypertensive rats support this idea.

1. Apter, J. T., Bull. Math. Biophys. **26**, 367 (1964).

2. Apter, J. T., Circ. Res. **19**, 104 (1966).

3. Aars, H., Acta Physiol. Scand. **72**, 298 (1968).

4. Aars, H., Acta Physiol. Scand. **73**, 101 (1968).

5. Brown, T. C., David, J. O., Olichney, M. J., and Johnson, C. I., Circ. Res. **18**, 475 (1966).

6. Feigl, E. O., Peterson, L. H., and Jones, A. W., J. Clin. Invest. **42**, 1640 (1963).

7. Gordon, D. B., and Nogueria, A., Circ. Res. **10**, 269 (1962).

8. Greene, M. A., Friedlander, R., Boltax, A. J., Hadjigeorge, C. G., and Lustig, G. A., Proc. Soc. Exp. Biol. Med. **121**, 580 (1966).

9. Mallov, S., Circ. Res. **7**, 196 (1959).

10. Wilson, C., and Byron, F. B., Lancet **1**, 136 (1939).

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