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Age-Associated Alterations in Viscoelastic Properties of Canine Aortic Strips

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SUMMARY. Many studies have delineated the changes in the elastic properties of arterial tissue as a function of age. Despite the fact that viscoelasticity is a prominent feature of these tissues, there is little information or characterization of age-associated changes in viscoelastic properties, over a wide range of smooth muscle activation, particularly in nonhuman tissue where atherosclerosis is not a confounding factor. In the present study, using small sinusoidal length perturbations, we determined the dynamic stiffness properties across a wide range of lengths (stretch ratios from 100 to 135%) and frequencies (from 0.25 to 35 Hz) in strips excised from ascending and descending aortas from six young (2 to 4-year-old) and 12 senescent (10- to 13-year-old) beagles. Studies were performed with the smooth muscle fully activated with calcium and norepinephrine, as well as fully inactivated with cyanide, iodoacetate, and dinitrophenol. There was a cubic nonlinear dependence of stiffness modulus on length only in senescent tissue and, surprisingly, little frequency dependence in tissue of either age. Compared to the young aortas, the three-dimensional surface representing the dependence of stiffness modulus on length and frequency from both the ascending and descending regions of aged aortas was displaced higher on the stiffness axis both with the muscle fully activated and inactivated. This age difference was accentuated at longer lengths. The phase lag between force and length was greater in the young vs. the old strips only in the activated, ascending aortic tissue. We found no age differences in the content of elastin, collagen, or in the collagen/elastin ratio, to account for these mechanical property differences. Thus, circumferential dynamic stiffness is greater in senescent, compared with young, aortas across a wide range of lengths, both with smooth muscle fully activated, and inactivated. (Circ Res 53: 464-472, 1983)

AGE-ASSOCIATED alterations in hemodynamic parameters, such as pulse-wave velocity and amplitude, have long been recognized. Because these hemodynamic parameters are affected by the mechanical properties of the aorta, there has been continuing interest in ascertaining the age-associated changes in the mechanical properties of the vascular system (reviewed by Yin, 1980). With regard to the static elastic properties, most studies demonstrate that there are age-associated decreases in distensibility or, equivalently, increases in elastic moduli in intact arterial segments (Roach and Burton, 1959; Learoyd and Taylor, 1966; Cox, 1974, 1977; Mirsky and Janz, 1976; Dieren, 1976; Cox, 1980; Langewouters et al., 1980). However, generalization is difficult, not only because there are species and regional variations (Hume 1939; Saxton, 1942; Frohne and Fung, 1980), but also because nonlinearity of the stress-strain relationship renders the interpretation of data, obtained at only one pressure or stress level, incomplete.

Because the arteries are subjected to time-varying loads, a more complete description of mechanical properties also requires determination of the viscoelastic properties of these vessels. Even though it is well-known that vascular tissue exhibits viscoelasticity (Remington, 1955; Zaitzman et al., 1954; Bergel, 1961; Apter et al., 1966, 1968; Gow and Taylor, 1968; Cox, 1974, 1976; Tanaka and Fung, 1974; Band et al., 1972; Learoyd and Taylor, 1966; Langewouters et al., 1978), only the last three studies investigated the changes with age in the viscoelastic behavior of arterial tissue. Like the studies of elastic properties, these studies of viscoelastic properties have demonstrated variable results—again related, in part, to species and regional differences—but, what is perhaps more important, to variability of the degree of smooth muscle activation, which was not well-characterized. The mechanical properties of a vessel are dependent not only on the degree of smooth muscle activation but, also, on how this contractile behavior is transduced via nonmuscle constituents (Dobrin, 1978; Cox, 1976). Since the degree of activation could vary with age, any investigation of age-associated changes in mechanical properties should take into account the degree of smooth muscle activation.

The purpose of this study was to investigate the age-dependence of viscoelastic properties of dog aorta under well-characterized degrees of smooth muscle activation across a wide range of both lengths and frequencies. Circumferential strips of aorta from the proximal ascending aorta just above the coronary sinuses and from the proximal de-
descending thoracic aorta of six mature and 12 senescent beagles were studied. The dynamic stiffness moduli and phase lag of each strip at lengths varying from 100 to 135% of the nearly unstressed length were obtained by subjecting the strips to small sinusoidal length perturbations over a frequency range of 0.25–35 Hz. The measurements were made both with the smooth muscle maximally activated with norepinephrine, and fully inactivated with iodoacetate, cyanide, and dinitrophenol. The circumferential dynamic stiffness modulus could be described by a three-dimensional surface relating stiffness, frequency, and circumferential length. Compared with young aortas, the surface of the old aortas was displaced higher on the stiffness axis, both with the muscle fully activated and with it inactivated. This age difference was more pronounced at longer lengths and with muscle activation. There was little frequency dependence of the stiffness modulus.

Methods

Specimen Preparation

Studies were performed in aortic strips from six young (1- to 4-year-old) and 12 senescent (10- to 13-year-old) purebred, exbreeder female beagle dogs. No dog had evidence by history or physical examination of prior or ongoing cardiovascular disease. The animals were killed by intracardiac injection of a solution to which iodoacetate (240 mg/liter), potassium cyanide (160 mg/liter), and dinitrophenol (36.8 mg/liter) had been added with the muscle held at 115% of Lref. When the force record had stabilized (typically between 45 and 80 minutes), the completeness of the muscle inactivation was verified by returning the muscle to the original solution containing 5 mm calcium and 5 μg/ml norepinephrine. In no case was there any residual smooth muscle response to this challenge. The muscle had been inactivated in this manner, the same frequency and length protocol as before inactivation was repeated.

At the end of the experiment, the specimen was cut from the clamps, blotted dry, and weighed. Portions of aorta immediately adjacent to the test specimens were assayed for both elastin and collagen, as indexed by hydroxyproline content (Jackson and Cleary, 1968).

Test Protocol

After mounting, each strip was equilibrated for 1 hour under a force of approximately 1 g. The specimen was then preconditioned by manually stretching and unstretching three times from 0 to 2.0 g. After preconditioning, a reference length, Lref, defined as the length that produced a stable force of around 250–300 mg, was measured. Next, the bath calcium was increased to 5.0 mm and norepinephrine was added to a final concentration of 5 μg/ml. Pilot studies demonstrated that this procedure produced a stable and maximal degree of force development in both young and old aortas from beagle dogs. When the developed force was stable (typically 15 minutes), the specimen was once more cycled manually three times between 100 and 135% of Lref. The muscle was maintained at 135% Lref after the last cycle and allowed to stabilize for 20 minutes. Then, at each of seven frequencies (0.25, 0.5, 1.0, 2.0, 5, 10, and 35 Hz), 16 cycles of a small sinusoidal length perturbation (0.25–0.50% of Lref) were imposed on the specimen. The strip then was shortened progressively to 130, 125, 120, 115, and 100% of Lref. After a 20-minute stabilization period between length changes, the entire frequency spectrum of sinusoidal length perturbations was imposed at each new length.

The smooth muscle of the strip then was inactivated metabolically (Cox, 1977) by changing the original Krebs-Ringer solution to calcium- and norepinephrine-free solution to which iodoacetate (240 mg/liter), potassium cyanide (160 mg/liter), and dinitrophenol (36.8 mg/liter) had been added with the muscle held at 115% of Lref. When the force record had stabilized (typically between 45 and 80 minutes), the completeness of the muscle inactivation was verified by returning the muscle to the original solution containing 5 mm calcium and 5 μg/ml norepinephrine. In no case was there any residual smooth muscle response to this challenge. After the muscle had been inactivated in this manner, the same frequency and length protocol as before inactivation was repeated.

At the end of the experiment, the specimen was cut from the clamps, blotted dry, and weighed. Portions of aorta immediately adjacent to the test specimens were assayed for both elastin and collagen, as indexed by hydroxyproline content (Jackson and Cleary, 1968).

Data Analysis

The force and length signals were digitized at 0.5-msec intervals, and the data were stored on digital tape for later analysis. The force signals were converted to Lagrangian stress by dividing the instantaneous cross-sectional area obtained, assuming tissue incompressibility and a specific gravity of 1.06. The instantaneous length was normalized to a strain component (stretch ratio) by dividing by Lref. The dynamic stiffness modulus at the prescribed length and frequency was calculated by dividing the sinusoidal peak-to-peak stress response by the peak-to-peak stretch ratio: stiffness = Δstress/Δstretch ratio. In this calculation, the cross-sectional area of the specimen at the unperturbed study length was used to calculate the stress induced by the length perturbation. Tests with a soft steel spring in place of the tissue in the apparatus across the same range of frequencies used in this study revealed that there was negligible phase lag between the length and force signals. Consequently, the phase lag between the force and length
Analysis of Stiffness Phase Lag

Since there is a range of both lengths and frequencies for each experimental condition, the dynamic stiffness modulus can be graphically depicted as a surface in three dimensions, with the modulus a function of both frequency and length. Our analytic approach to analysis of stiffness data depicted in this form utilized three steps. (1) The three-dimensional relationships among modulus, frequency, and length defined by the eight combinations of the three experimental conditions (maximally activated/inactivated, ascending/descending, and young/old) were derived. (2) The young and old surfaces then were compared for differences in shape for each combination of experimental conditions. (3) Since vessel size differences are one of the usual concomitants of aging, we further wished to determine whether, in addition to shape changes, there was a significant difference in the mean slope of the stiffness-length relationship accounting for the nonlinearity in length. The detailed method of analysis of these three steps is described below.

Step 1. The surface for each combination of old/young, active/inactive, and ascending/descending was generated by a multiple covariance model. For each surface, coefficients accounting for dog-to-dog variability, length, frequency, length-frequency interaction, length^2, frequency^2, length^3, and frequency^3 were derived. The coefficients were tested sequentially for significance over lower order terms. If a coefficient was significant in the depiction of any one of the eight surfaces, it was included in the covariance model. Of the terms examined, all but frequency^2 were found to be important. This variable was therefore omitted from the final covariance model. Using the remaining variables, the data were fitted to a separate surface for each of the eight conditions. Qualitative examination of residuals for each condition revealed no systematic trend, indicating that the errors were roughly normally distributed and that the data appropriately fit the model.

Step 2. To test for shape differences between the young and old surfaces, the coefficients of the length cubed and frequency squared terms were compared. This comparison was performed by calculating t defined as

\[ t = \frac{b_y - b_o}{SE_b_y - b_o} \]

where b_y and b_o are the coefficients of the terms being tested for the young and old aortas, respectively, and SE_{b_y - b_o} is (SE_{b_y}^2 + SE_{b_o}^2)^{1/2}.

Step 3. To derive estimates of the average slope along the length and frequency axes, the same covariance model was fitted, but was limited to linear terms in length and frequency. The linear coefficients of length and frequency were then compared as in the higher order model, but with the standard errors of the coefficients being adjusted for the known nonlinearity in length and frequency.

Analysis of Stiffness Phase Lag

For the phase lag, there was not an easily quantifiable dependence on frequency because of marked nonlinearity. In addition, we found that, unlike the modulus, there was little length dependence. Consequently, rather than using the same approach as for the modulus, we treated the length and frequency as discrete variables and employed repeated-measures analysis of variance techniques. Frequency effects were found for all conditions across the entire frequency range. However, the only age effect we found was for the ascending/activated condition in the frequency range 0.5–2 Hz (see Fig. 5). Therefore, separate detailed analyses restricted to these frequencies were then performed to delineate age effects.

Results

The ages of the young and old dogs were 2.6 ± 0.3 and 11.6 ± 0.5 years, (P < 0.0001), respectively. The body weights of the two groups did not differ significantly 11.0 ± 1.0 vs. 10.2 ± 1.1 kg. At 135% of L_ref, the aortic strips did not differ in cross-sectional area (2.06 ± 0.2 vs. 2.42 ± 0.2 mm^2, P = NS) or length (14.4 ± 0.9 vs. 13.8 ± 1.4 mm, P = NS).

Stiffness Modulus

Step 1

The surfaces representing the dynamic stiffness moduli of the aortic strips as a function of both length and frequency are illustrated in Figures 1–4.* In each figure, the stiffness is plotted on the vertical axis, and the frequency and length axes are as shown. All these figures are drawn to the same scale. It should be pointed out that the origin of the frequency axis is 0.05 and not 0 Hz. Figures 1 and 2 illustrate the data for the proximal ascending and

*For interested readers, the coefficients describing the model for each of the experimental conditions can be obtained by written request to FCPY.

FIGURE 1. Three-dimensional representation of the relationship among dynamic stiffness moduli (stress perturbation: length perturbation), normalized length expressed as stretch ratio (study length: reference length), and frequency for maximally activated strips from the ascending aortas of senescent (upper surface) and young (lower surface) beagle dogs. The origin of the frequency axis is 0.05 Hz. The vertical bar represents one unit on the stiffness axis.
FIGURE 2. Stiffness modulus surfaces for maximally activated strips from the thoracic descending aortas of senescent (upper surface) and young (lower surface) beagles. Nomenclature is identical to that of Figure 1.

FIGURE 3. Stiffness modulus surfaces for metabolically inactivated strips from the ascending aortas of senescent (upper surface) and young (lower surface) beagles. Nomenclature is identical to that of Figure 1.

FIGURE 4. Stiffness modulus surfaces for metabolically inactivated strips from the thoracic descending aortas of senescent (upper surface) and young (lower surface) beagles. Nomenclature is identical to Figure 1.

descending aortic strips, respectively, under maximal muscle activation. Figures 3 and 4 show the corresponding data after metabolic inactivation. The stiffness modulus surface from the old aorta was displaced higher on the stiffness axis for the strips from each site during maximum muscle activation, as well as after muscle inactivation. The stiffness of the old aorta had a much more nonlinear dependence on length than the young aorta, and the surfaces became more widely displaced at increasing lengths. The cubic nonlinearity in length in the old vessel was only partially attenuated after muscle inactivation. After muscle inactivation, the moduli of both the old and young strips decreased, but the separation between the surfaces persisted. By comparing the appropriate figures, it can be seen that—within an age group—the ascending aorta was stiffer than the descending aorta, both during muscle activation and after inactivation.

Step 2

From these three-dimensional plots, one can make only qualitative assessments of the aortic properties. The data that enable one to make quantitative comparisons are listed in Table 1, in which only the significant coefficients in the covariance model for length cubed and frequency squared are shown. These data demonstrate a significant age difference, in that there is a consistent and highly significant cubic dependence on length of the old aortas under all conditions, whereas there is only a marginally significant cubic dependence of length of the young aortas. There is a frequency effect only in the activated, ascending aortas, and there is no age difference in this dependence on frequency.

Step 3

Table 2 lists the length and frequency coefficients for the linearized surfaces. The significant age difference between the length coefficients implies that, with increasing length, the old aorta becomes stiffer than the young. This age difference is present in both regions, whether the muscle is fully activated or is inactivated. As was the case in the nonlinear model, there was only a weak frequency dependence that did not demonstrate any age difference.

Stiffness Phase Lag

In contrast to the stiffness moduli, the phase lag demonstrated very little length dependence but
TABLE 1  
Coefficients of the Length Cubed and Frequency Squared Terms in the Covariance Model of the Surfaces Representing the Three-Dimensional Relationship among Circumferential Dynamic Stiffness Modulus Length and Frequency

<table>
<thead>
<tr>
<th></th>
<th>Length$^3$</th>
<th>Frequency$^2$</th>
<th>Young vs old</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
<td>t</td>
<td>P</td>
<td>Young</td>
</tr>
<tr>
<td>Activated</td>
<td>0.000064</td>
<td>0.00022</td>
<td>-3.04</td>
<td>0.001</td>
<td>-0.0017</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.0001)</td>
<td></td>
<td></td>
<td>(0.001)</td>
</tr>
<tr>
<td>Activated</td>
<td>0.000027</td>
<td>0.00016</td>
<td>-2.25</td>
<td>0.05</td>
<td>-0.0011</td>
</tr>
<tr>
<td></td>
<td>(0.20)</td>
<td>(0.0001)</td>
<td></td>
<td></td>
<td>(0.001)</td>
</tr>
<tr>
<td>Deactivated</td>
<td>0.000019</td>
<td>0.000081</td>
<td>-1.68</td>
<td>0.01</td>
<td>-0.00015</td>
</tr>
<tr>
<td></td>
<td>(0.17)</td>
<td>(0.0014)</td>
<td></td>
<td></td>
<td>(0.30)</td>
</tr>
<tr>
<td>Deactivated</td>
<td>0.000030</td>
<td>0.000014</td>
<td>-3.26</td>
<td>0.001</td>
<td>-0.00018</td>
</tr>
<tr>
<td></td>
<td>(0.06)</td>
<td>(0.0001)</td>
<td></td>
<td></td>
<td>(0.30)</td>
</tr>
</tbody>
</table>

The values of the eight coefficients for the length$^3$ and frequency$^2$ terms in the model are listed. Beneath each coefficient (in parentheses) is the $P$ value indicating the significance level of that coefficient from zero.

Discussion

The present study demonstrates that, across a wide range of lengths and frequencies, the circumferential dynamic stiffness modulus of both maximally activated and metabolically inactivated aortic strips from senescent dogs is greater than that from young dogs. This age difference pertains to strips from the proximal ascending as well as the thoracic descending regions of the aorta. Within an age group, the ascending aorta was stiffer than the descending, with the muscle both activated and inactivated. To our knowledge, these are the first data that document, in an age range that encompasses senescence, age-associated alterations in viscoelastic properties in nonhuman arterial tissue. Band et al. (1972) found no difference in longitudinal dynamic stiffness between aortas of young and senescent rats. Use of a different species, measurement of longitudinal rather than circumferential stiffness, and the shorter absolute duration of aging in the rat could explain why their results differed from ours.

Before discussing the implications of these findings, some possible limitations of the methodology

TABLE 2  
Coefficients of the Linearized Covariance Model Representing Dynamic Stiffness Modulus as a Function of Length and Frequency

<table>
<thead>
<tr>
<th></th>
<th>Length</th>
<th>Frequency</th>
<th>Young vs old</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
<td>t</td>
<td>P</td>
<td>Young</td>
</tr>
<tr>
<td>Activated</td>
<td>0.0740</td>
<td>0.01083</td>
<td>-7.27</td>
<td>0.0001</td>
<td>-0.0728</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
<td></td>
<td>(0.02)</td>
</tr>
<tr>
<td>Activated</td>
<td>0.0612</td>
<td>0.0836</td>
<td>-4.06</td>
<td>0.0001</td>
<td>-0.0220</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
<td></td>
<td>(0.03)</td>
</tr>
<tr>
<td>Inactivated</td>
<td>0.0458</td>
<td>0.0822</td>
<td>-11.08</td>
<td>0.0001</td>
<td>-0.0074</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
<td></td>
<td>(0.4)</td>
</tr>
<tr>
<td>Deactivated</td>
<td>0.0464</td>
<td>0.0588</td>
<td>-3.99</td>
<td>0.0001</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>0.0001</td>
<td></td>
<td></td>
<td>(0.9)</td>
</tr>
</tbody>
</table>

The format for this table is identical to that for Table 1.
should be considered. As with all isolated tissue studies, there is a possibility that damage at the ends of the specimen introduced stray compliance into the system. To take any stray compliance into account requires some method to measure and control the lengths in the central, presumably undamaged portion. Our apparatus did not allow for this. Therefore, our results may be affected to some degree by uncertainties associated with this type of artifact. However, since the age differences were found across a wide range of lengths, both with the smooth muscle fully activated (which should exaggerate the effects of the damaged ends, since they presumably no longer retain contractile activity), and with the muscle inactivated (which should minimize effects from the ends), we feel that the directional differences we found are probably real. The absolute magnitudes of the stiffness moduli in both age groups are, however, open to some question.

There may also be some effects from the longitudinal curvature of the aorta, as well as from the natural tendency of a circumferential ring to curl. The former effects were minimized by using very narrow specimens from regions of the aorta that are relatively straight. As for the tendency to curl, the specimen certainly demonstrated this. However, using strips that were roughly one-third of the circumference minimized some of this tendency to curl. The 300-mg force used to define our reference length was chosen to help ensure that we started the study with a straight, flat specimen. Finally, with the passage of time in a single experiment, all specimens underwent some creep; that is, the unloaded length became longer. However, the absolute amount of creep was always quite small (about 1–2% or so of the original L_{ref}). Since we studied a length range much wider than this, and since the final data analysis treated length as a continuous variable, we feel that neglecting this creep effect in our calculations did not significantly affect our interpretations.

Our findings in dog aortas generally agree with the results of two studies in human autopsy specimens (Learoyd and Taylor, 1966; Langewouters et al., 1978) that have demonstrated age-related changes in aortic viscoelastic properties. Learoyd and Taylor (1966) found that the circumferential dynamic incremental modulus over the limited frequency range of 1–10 Hz was higher in old (greater than 35 years) compared with young intact human thoracic aortas, when studied at a common pressure of 100 mm Hg. This modulus was less in the abdominal compared with the thoracic aorta in the older aortas, but there was no such site dependence in the young vessels. However, their results may be somewhat limited, since the same luminal pressure probably resulted in different strain levels in the two groups of vessels. Thus, the two groups of aortas may have been studied at different portions of their nonlinear stress-strain curves, rendering a single measurement of an incremental modulus difficult to interpret. In fact, when they examined the static elastic modulus at a diameter of 1 cm, there was a progressive decrease in the modulus with advancing age. In contrast, when examined at a constant pressure of 100 mm Hg, there was a progressive increase in the modulus with advancing age. These above limitations were obviated in the present study, since we measured the stiffness moduli across a wide and equivalent range of strains. At all strain levels beginning with the nearly undeformed state, the old aortas were stiffer than the young, with the differences being accentuated at higher strain levels.

A greater dynamic stiffness modulus in old com-

**Table 3**

<table>
<thead>
<tr>
<th></th>
<th>Ascending</th>
<th></th>
<th>Descending</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>H</td>
<td>E/H</td>
<td>E</td>
</tr>
<tr>
<td>Young</td>
<td>7.39 ± 1.32</td>
<td>10.68 ± 0.71</td>
<td>0.70 ± 0.12</td>
<td>9.27 ± 3.33</td>
</tr>
<tr>
<td>Old</td>
<td>6.06 ± 1.44</td>
<td>11.61 ± 0.40</td>
<td>0.51 ± 0.11</td>
<td>11.78 ± 4.35</td>
</tr>
<tr>
<td>P (Y vs. O)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are expressed as µg/mg wet weight (mean ± SEM). Abbreviations: E = elastin, H = hydroxyproline.
pared with young aortas can help explain several physiological phenomena. Compared with a young aorta, an aged aorta that is stiffer across the range of physiological strains would be predicted to have an increased pulse wave velocity (Learoyd and Taylor, 1966; Langewouters et al., 1978). Indeed, with advancing age, there are increases in the directly measured pulse wave velocity (Bramwell and Hill, 1922; Hallock 1934; Simonson and Nakagawa, 1960; Schimmel, 1966; O’Rourke et al., 1968). In addition, the input impedance of the aorta is one measure of the load imposed by the vasculature on the heart. The pulsatile components of this load oscillate about the characteristic impedance, whose value is predicted to be directly related to its dynamic stiffness and inversely related to its size (McDonald, 1974; Westerhof and Noordergraaf, 1970; Langewouters et al., 1978). There are, however, few direct experimental measurements of aortic impedance as a function of age. Nichols et al. (1977) demonstrated increased characteristic impedance in patients with coronary artery disease who also happened to be older than the control group. However, these findings may be confounded by the presence of atherosclerosis. Yin et al. (1981) demonstrated an increase in aortic characteristic impedance during exercise in senescent but not in young dogs. At rest, the larger aortas in the old dogs could have compensated for the increased aortic stiffness, or the degree of smooth muscle activation could have been markedly different, producing no net age difference in aortic characteristic impedance. However, during exercise, the increase in adrenergic tone and mean aortic pressure may have combined to move the old dogs to a point on their stiffer stress-strain curve such that the greater stiffness yielded a higher characteristic impedance compared with the less stiff young aorta. This resulted in a greater pulsatile load on the heart which may have played a role in the diminished exercise tolerance in the senescent dogs. The results of the present study are direct supportive evidence that these age-related hemodynamic changes may be attributed, in part, to age-related changes in mechanical properties of the great vessels.

The greater age difference during maximal muscle activation and the persistence of the age difference after inactivation indicate that there are age differences in both the passive properties and in the transduction of smooth muscle activation via nonmuscle components into external force. At the longer lengths, as collagen becomes the predominant determinant of passive mechanical properties (Roach and Burton, 1957), the age difference in the inactivated stiffness surface could be attributed to age differences in either the absolute or relative amount of collagen and elastin, in their viscoelastic properties, or in the structural arrangement of these nonmuscle components of the wall. However, our biochemical analysis revealed no increase in the absolute amount of elastin, collagen, or in the collagen:elastin ratio in the old aortas. Our findings are compatible with studies that have found no correlation between either the amount or concentration of these constituents and the mechanical properties in rabbit aortas (Fronke and Fung, 1980) or dog aortas (Cleary, 1963; Cox, 1978, 1980). In contrast, in earlier studies, Cox (1974, 1976) found good correlations between the concentrations of collagen and the collagen:elastin ratio and mechanical properties in maturing rat and dog carotid arteries. These and other studies indicate the difficulty of relating the results of biochemical analyses to directly measured mechanical properties, because the mechanical properties of a tissue are determined not only by the properties and absolute amounts of its constituents but, what is perhaps more important, by the structural relationship between the various constituents (Burton, 1954; Diamont et al., 1972; Dobrin, 1978).

When one considers the additional complexities of transduction of smooth muscle activation via the nonmuscle constituents to the externally measured active tissue properties, the difficulties become even more pronounced.

Viscoelasticity is manifested by stress relaxation, creep, hysteresis between loading and unloading curves, and frequency dependence of the stress-strain relationship. All these effects have been observed to some degree in arterial tissue (Zatzman et al., 1954; Remington, 1955; Bergel, 1961; Learoyd and Taylor, 1966; Apter and Marquez, 1968; Patel et al., 1970; Azuma and Hasegawa, 1970; Band et al., 1970; Tanaka and Fung, 1974). Since all of these responses are manifestations of the same physical property, they should, in theory, be able to be expressed in a unified manner. But, partly because of difficulties in quantification of the viscous effects (Tanaka and Fung, 1974) and partly because of directional and regional differences (Azuma and Hasegawa, 1970; Tanaka and Fung, 1974), a vessel may not appear to manifest viscoelasticity to the same degree when subjected to different types of testing. In this study, we found very little frequency dependence of the dynamic stiffness in the range 0.25–35 Hz. This lack of frequency dependence is probably not due to the fact that we studied circumferential properties, since previous studies have shown that, when viscoelasticity is manifested, the circumferential, as compared to the longitudinal, direction exhibits a response that is as great, if not greater (Bagshaw and Attinger, 1957; Azuma and Hasegawa, 1970). Similar weak frequency dependence of the stiffness coefficient or the area enclosed by the hysteresis loop despite significant stress relaxation has also been observed previously (Remington, 1955; Bergel, 1961; Learoyd and Taylor, 1966; Patel et al., 1970; Band et al., 1972; and Tanaka and Fung, 1974). Fung (1972) suggested that, for a tissue to be strain rate-insensitive, or, equivalently, frequency-independent, and yet demonstrate significant stress relaxation, it must be a quasi-linear viscoelastic material with a broad relaxation spectrum.
In summary, this study demonstrates that the viscoelastic properties of strips from both ascending and thoracic descending aorta differ in senescent, compared with young, dogs. This difference is manifested primarily as a higher dynamic stiffness modulus in the aortas from senescent dogs and pertains across a wide range of lengths (100–135% of unstressed length) and a range of frequencies (0.25–35 Hz). The age differences in stiffness moduli are more apparent with the smooth muscle fully activated, but are also present when the muscle is metabolically inactivated, thereby demonstrating age differences, both in the passive properties and in the interaction between the smooth muscle and passive wall constituents. We found no age differences in the content of elastin, collagen, or in the collagen elastin ratio, to account for these mechanical property differences.

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\[ \text{INDEX TERMS: Aging • Viscoelasticity • Arterial wall properties • Elasticity} \]