Age-Related Changes in Composition and Mechanical Properties of the Tunica Media of the Upper Thoracic Human Aorta

Michele Spina, Spiridione Garbisa, John Hinnie, James C. Hunter, and Augusto Serafini-Fracassini

A cylindrical segment, free of complex atherosclerotic lesions, was resected at autopsy from each of 59 descending human thoracic aortas by cutting just below the level of the first pair of intercostal arteries and 35 mm distal to this incision. Each isolated tunica media was defatted and subjected to successive treatment with EDTA-Tris, 5 M guanidine hydrochloride-Tris, 5 M guanidine hydrochloride-Tris-DTE, collagenase and either trypsin or hot alkali. After each extraction or digestion, the dimensions and weight of the segments were measured and the extracted materials were analyzed and quantitated. This allowed the total content of the various components of the tunica media to be assessed by both gravimetric and analytical means. An age-related rise was observed in the total content of the following components: proteins and glycoproteins soluble in chaotropic solvents (ranging from 24 mg/cm in the youngest samples to 46 mg/cm in the oldest) and collagen (38 mg/cm to 69 mg/cm).

In contrast, the total content of elastin remained constant at 70 mg/cm at all ages, but its concentration decreased due to the rise in the concentration of the other tissue components as the tunica media thickened with age. It was also noted that with increasing age there was an accumulation of protein(s) which could not be solubilized by extraction with chaotropic agents or with collagenase, but which could be removed by treatment with either trypsin or hot alkali. Mechanical measurements conducted before and after trypsin digestion on samples previously subjected to purification with the first four agents used suggest that this accumulated protein(s) influenced the elastic response of the tissue to the applied stress by increasing the incremental modulus, the breaking stress, and the hysteresis. After the removal of this additional protein(s), the mechanical behavior of the elastin component was found to be identical in all samples, irrespective of age. It is therefore proposed that the morphological changes and the stiffening observed in the aging aortic wall are not due to degradation of its elastin network but to variations in the supramolecular organization of connective tissue components. (Arteriosclerosis 3:64–76, January/February 1983)

With advancing age, the human aorta dilates and becomes stiffer at physiological pressures. To date, investigations aimed at the identification of compositional or structural changes of the wall components responsible for these alterations have produced rather conflicting results. This probably reflects differences in sampling or in the methodologies adopted for the isolation and quantitation of matrix components, complicated by the use of relative, rather than absolute, concentrations in the expression of the results. In addition, it is not yet known whether the mechanical properties of the aortic collagen fibers change with aging, while data relating to the dynamics of the elastin component, which is responsible for the long-range reversible extensibility of the vessel, are largely unreliable due to the degradative purification procedures adopted so far and to the lack of information concerning sample purity. Despite these uncertainties, several hypotheses have been proposed which explain, mainly on the grounds of morphological evidence, the age-related hardening of arteries in terms of a rise in collagen content and a concomitant degradation of the elastin network.

The aims of the present investigation were: 1) to quantitate age-related variations in the major matrix

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components of the tunica media from human thoracic aorta and 2) to evaluate the degree of integrity of the elastin network by chemical and mechanical analyses.

Methods

Materials

Collagenase (EC 3.4.24.3, from Clostridium histolyticum, Type I), DPC-trypsin (EC 3.4.21.4, from bovine pancreas, diphenyl carbamyl chloride treated, Type XI), dithioerythritol (DTE; 2,3-dihydroxy-1,4-dithiolbutane), amino acid standard solutions, d-glucosamine hydrochloride, d-galactosamine hydrochloride and d-glucuronolactone were purchased from Sigma (St. Louis, Missouri). Guanidine hydrochloride (analytical grade) was obtained from Carlo Erba, Milan. All the other reagents were analytical grade and were obtained from Merck (Darmstadt, West Germany) and used in these studies without further purification.

Sampling

Thoracic aortas (58 samples) were isolated from humans of both sexes, who were from 20 to 78 years of age and were divided into six age groups, each spanning one decade. Each group comprised 10 aortas with the exception of the 30- to 39-year-old group which comprised only eight. An equal number of samples from males and females were included in each group. In addition, one aorta was taken from a 19-year-old subject.

To avoid uncertainties arising from nonreproducibility of sampling, a cylindrical segment approximately 3.5 cm long was isolated from each aorta, the upper plane of the section lying immediately below the opening of the first pair of intercostal arteries. None of these segments exhibited complex atherosclerotic lesions. One segment from each age group was chosen at random, labelled, and its age recorded. These will be referred to as the R-samples. The remaining segments were labelled with the average age of the group to which they belonged (23.6, 33.9, 45.1, 54.7, 63.2, and 73.3 years).

All segments were freed from adhering tissue and washed for a few hours in physiological saline at 4°C. The intima and adventitia were removed16,17 and the segments were subjected to successive 24-hour extractions in acetic acid, chloroform:methanol (2:1, vol/vol), chloroform:methanol (3:1, vol/vol), ethanol, and ether. The segments were then dried under vacuum for 48 hours, weighed, suspended from a glass frame, and rehydrated overnight at 4°C. Excess water was removed by blotting with filter paper, and the weight, external circumferences (proximal and distal), and length of each segment were recorded.

Sequential Extraction of Aortic Segments

The segments were resuspended from the glass frame and extracted, with continuous stirring, for three 24-hour periods at 4°C in 600 ml of 0.2 M EDTA-0.4 M Tris (pH 7.4). Further extraction was carried out for six 24-hour periods in 600 ml of 5 M guanidine hydrochloride-0.4% (wt/wt) EDTA-0.1 M Tris (pH 7.4) and finally for two 24-hour periods under N₂ at 37°C in 300 ml of 0.05 M dithioerythritol (DTE)-5 M guanidine hydrochloride-0.4% (wt/wt) EDTA-0.1 M Tris (pH 7.6).18,19 Proteins contained in the guanidine-DTE extract were carboxymethylated.20 All extracts were collected, dialyzed exhaustively against distilled water at 4°C, lyophilized, and weighed. At the end of each group of extractions, the segments were washed with 2 M NaCl followed by water and dried under vacuum before weighing.

Before chemical analysis, two small rings of equal weight were removed from the proximal and distal end of all segments except the R-samples and pooled according to age group.

Enzymic and Chemical Purification of R-Rings

Three 5 mm-long rings were dissected from each R-sample and identified as proximal, intermediate, and distal R-rings according to their position in the intact R-sample. After assessment of weight and overall dimensions, the R-rings were digested with collagenase (EC 3.4.24.3) purified by affinity chromatography.21 Digestion was carried out at 37°C for three 24-hour periods in 10 mM CaCl₂-0.1 M Tris (pH 7.5) using an enzyme to substrate ratio of 1 to 1000. After each 24-hour period, the rings were washed with 5 M guanidine hydrochloride-0.1 M Tris (pH 7.5), followed by 2 M NaCl and distilled water. When collagenase digestion was completed, the rings were dried and weighed. The proximal and distal rings from each R-sample were used for mechanical tests and, when these were completed, the resulting broken specimens were bisected longitudinally. One-half was extracted in 0.1 M NaOH for 45 minutes at 100°C,22 before chemical analysis, while the other one-half was analyzed without further extraction. The intermediate rings were further digested with DPC-treated trypsin (EC 3.4.21.4) at an enzyme to substrate ratio of 1 to 10. Rings were suspended in 1% (wt/vol) NH₄HCO₃ (pH 8.0), digested for 24 hours at 37°C, washed in 5 M guanidine hydrochloride (pH 7.4) followed by 2 M NaCl and water, dried, and weighed.23 The trypsin-treated intermediate rings were subjected to mechanical testing and compositional analysis.

Chemical Analysis

Hydrolysis for amino acid analysis was carried out (1 mg/ml) in constant-boiling HCl containing 0.1 M thioglycolic acid. Separation of amino acids was carried out on a Jeol JLC-6AH and a Locarte amino acid analyser. Norleucine was added to the loading buffer as an internal standard. Desmosine, isodesmosine, and lysinosorleucine were estimated as described previously24 while hydroxyproline was quantitated by a colorimetric procedure.24
Total uronate was estimated according to the method of Dische, as modified by Bitter and Muir, and hexoses according to Yemm and Willis. In both procedures the color determined after heating with H₂SO₄ was subtracted from the final color produced. Hexosamines were measured by the method of Cessi and Piliego, after hydrolysis in 4 N HCl for 8 hours at 100°C.

**Dynamometry**

Stress-strain analysis was carried out on proximal and distal R-rings after collagenase digestion, and on the intermediate R-rings after further digestion with trypsin. Following equilibration in water and elimination of excess solvent by blotting, the samples were weighed and their external circumference and length measured. The mid-wall circumference was assessed according to the method of McDonald. The total volume of each hydrated ring was obtained by adding the volume of the water of hydration to the volume of the dry sample. The latter was calculated from the sample density which, in turn, was derived from amino acid compositional data. The reproducibility of measurements was within 3%. In order to compensate for spaces previously occupied by cells and matrix components, the transverse section was evaluated from the ratio of the volume of the dry sample to its mid-wall circumference determined after equilibration with water.

The instrument used for stress-strain analysis has been described elsewhere. The rings were connected using triangular metal hooks and each sample was subjected to three extension-relaxation cycles, not exceeding elongations of 30% to 40%, after which they were extended to the breaking point. The speed adopted was 0.1 mm/second.

Due to the nonlinearity of the stress-strain curves, the incremental modulus (E₀) was calculated according to the methods of McDonald and Dobrin, using stress increments (Δσ) of 1.5 × 10⁵ Nm⁻², according to the formula:

$$E_0 = \frac{\Delta \sigma}{\varepsilon_{inc}} (1 + \Delta L),$$

where $$\varepsilon_{inc} = \frac{L_2 - L_1}{L_1}$$

is the incremental strain. If $$L_0$$ represents the length of the relaxed specimen and $$L_1$$ and $$L_2$$ are the values assumed by this parameter at two successive deformations, then

$$\frac{L_1}{L_0} = (1 + \Delta L).$$

This is the coefficient utilized for the evaluation of the transverse section of samples deformed to $$L_1$$, assuming a Poisson ratio of 0.5.

Hysteresis was calculated from the third extension-relaxation and was expressed as the ratio of the area between the extension and relaxation curves to the area under the extension curve.

**Statistics**

All data are represented as means ± sd, unless otherwise stated. Paired t tests have been used to compare the concentration values, reported in table 6, of the components removed by trypsin digestion or alkali treatment with those of the insoluble polar protein(s) quantitated from chemical analyses. The values in column A in this table have been paired with the corresponding values in columns B and C; values in column B have been paired with those in column C. Unpaired t-tests have been used in all other cases. Least-square linear regression was used to calculate the equation correlating two variables. The correlation coefficient r was used as a measure of association, the significance level of which was tested by comparing the computed value of r with statistical tables that give values of this parameter corresponding to a range of probability levels with n-2 degrees of freedom.

**Results**

As shown in figure 1, in all age groups the rings of defatted aortic tunica media were found to have an average proximal external circumference (Cp) significantly greater than the average distal external circumference (Cd). The mean of the differences within each group was 4.3 ± 0.3 mm ($t = 14.3333; p < 0.001$). Moreover, up to the seventh decade, both Cp and Cd were positively correlated with age ($r = 0.9917$ for Cp and $r = 0.9963$ for Cd with $p < 0.001$ for both).

![Figure 1. Average proximal (a) and distal (b) external circumferences of defatted aortic segments, measured after rehydration and plotted against the average age of each group.](http://atvb.ahajournals.org/)
Figure 2. Dry weight per unit of hydrated length (mg/cm) of aortic segments plotted against the average age of each group and measured after: a) defatting; b) as in a) followed by extraction with EDTA-Tris; c) as in b) followed by further extraction with guanidine hydrochloride-Tris and guanidine hydrochloride-Tris-DTE. Correlation coefficients and significance levels of association are: a) $r = 0.9858$, $p < 0.001$; b) $r = 0.9867$, $p < 0.001$; c) $r = 0.9541$, $p < 0.005$.

The mean value of the dry mass per unit of length was found to be positively correlated with age at all stages during the sequential extraction procedure (figure 2) and showed a 48% increase between the third and eighth decade on completion of the extraction schedule. However, as this increase was matched by a similar rise in the value of the average external circumference (figure 1), the weight per unit of external surface area remained constant (figure 3).

Each soluble fraction isolated during the sequential extraction procedure was analyzed after dialysis and its concentration in the defatted and decalcified tissue is given for each age group in table 1 where compositions are reported only in terms of broad categories because of the heterogeneous nature of the materials extracted. Amino acid analysis revealed in all fractions high relative concentrations of polar and hydroxylated residues. The following observations were made in relation to amino acids indicative of specific matrix components: 1) desmosines were absent in all hydrolyzates, suggesting that the amount of elastin released was nil or negligible; 2) hydroxyproline was found in A, B, and C in quantities corresponding to 0.04%, 0.9%, and 2.1%, respectively, of that originally present in the tissue; 3) hydroxylysine could be quantitated only in C where it was found in a 1-to-2 molar ratio relative to hydroxyproline, which suggested that the small amount of collagen released in guanidine-DTE might be related to Type IV.

The amino acid composition of each pooled insoluble residue analyzed after completion of the sequential extraction procedure is reported in table 2. Comparison of these analyses showed each amino acid fraction.

### Table 1. Undialyzable Fractions Extracted by Chaotropic Solutions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration % (wt/wt) of dry, defatted, decalcified tissue</th>
<th>Composition % (wt/wt) of total protein and carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age group by decade</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3    4  5    6    7    8</td>
<td>Protein</td>
</tr>
<tr>
<td>EDTA-Tris</td>
<td>1.4  2.0 ND  3.2  2.6 ND</td>
<td>81.5±4.1</td>
</tr>
<tr>
<td>Guanidine-Tris</td>
<td>5.8  5.8  5.0  7.0  7.7  8.2</td>
<td>87.3±1.8</td>
</tr>
<tr>
<td>Guanidine-Tris-DTE</td>
<td>4.8  4.4  5.2  4.7  5.5  4.4</td>
<td>89.0±0.7</td>
</tr>
</tbody>
</table>

ND = not determined.
The presence of this third insoluble protein component is also borne out by the amino acid composition of aortic samples after collagenase treatment (table 5). While generally conforming to the pattern of human aortic elastin (see sample 19 in table 5 and mean values in table 4), they nonetheless reveal differences which are significantly correlated with age. These age-related compositional differences are almost completely abolished by the subsequent digestion of the specimens with trypsin (table 4). The concentrations of components removed from each sample by this treatment or by hot alkali are reported in table 6, columns A and B respectively. The same procedure. Therefore, it would appear that the two structural proteins are associated with an additional insoluble protein component, the concentration of which, calculated by analytical difference, is reported for each pool in table 3.

Table 2. Amino Acid Composition of Pooled Residues after Sequential Extraction

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>p*</th>
</tr>
</thead>
<tbody>
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<td>OH-Lysine</td>
<td>1.6</td>
<td>1.2</td>
<td>1.7</td>
<td>3.0</td>
<td>4.1</td>
<td>5.2</td>
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<tr>
<td>Lysine</td>
<td>13.2</td>
<td>13.9</td>
<td>13.5</td>
<td>17.2</td>
<td>18.0</td>
<td>18.1</td>
<td>&lt;0.02</td>
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<tr>
<td>Histidine</td>
<td>2.2</td>
<td>4.7</td>
<td>4.4</td>
<td>4.8</td>
<td>5.1</td>
<td>5.0</td>
<td>NS</td>
</tr>
<tr>
<td>Arginine</td>
<td>19.2</td>
<td>25.9</td>
<td>20.8</td>
<td>30.4</td>
<td>30.0</td>
<td>31.0</td>
<td>&lt;0.05</td>
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<tr>
<td>OH-Proline</td>
<td>34.4</td>
<td>37.1</td>
<td>37.4</td>
<td>51.7</td>
<td>56.3</td>
<td>53.5</td>
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</tr>
<tr>
<td>Aspartic acid</td>
<td>3.8</td>
<td>4.0</td>
<td>3.8</td>
<td>5.1</td>
<td>5.4</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>15.5</td>
<td>19.6</td>
<td>28.3</td>
<td>30.1</td>
<td>32.0</td>
<td>29.9</td>
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</tr>
<tr>
<td>Serine</td>
<td>10.4</td>
<td>12.5</td>
<td>18.8</td>
<td>21.4</td>
<td>20.0</td>
<td>18.9</td>
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</tr>
<tr>
<td>Glutamic acid</td>
<td>13.9</td>
<td>17.9</td>
<td>22.6</td>
<td>22.2</td>
<td>29.0</td>
<td>26.7</td>
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<td>Proline</td>
<td>33.3</td>
<td>39.8</td>
<td>48.8</td>
<td>53.2</td>
<td>57.1</td>
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<tr>
<td>Glycine</td>
<td>120.0</td>
<td>123.8</td>
<td>129.5</td>
<td>113.7</td>
<td>109.2</td>
<td>108.3</td>
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<tr>
<td>Alanine</td>
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<td>334.0</td>
<td>290.1</td>
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<tr>
<td>Half-cystine†</td>
<td>188.2</td>
<td>178.7</td>
<td>170.7</td>
<td>161.0</td>
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<tr>
<td>Valine</td>
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<td>88.5</td>
<td>88.3</td>
<td>82.1</td>
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<tr>
<td>Methionine‡</td>
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<td>16.0</td>
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<td>23.9</td>
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<td>23.0</td>
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<td>Isodesmosine§</td>
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<td>6.2</td>
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<td>Desmosine§</td>
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<tr>
<td>Lysinonorleucine§</td>
<td>1.2</td>
<td>1.2</td>
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<td>ND</td>
<td>ND</td>
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</table>

Values are expressed as residues/1000 amino acid residues. Figures in italics refer to the concentration (g/100 g) of hydroxyproline and desmosines (ID + D) in the samples.

*Significance level of the correlation between age and amino acid concentration.
†Comprises cysteic acid.
‡Comprises methionine sulfoxide.
§Expressed as lysine equivalents.
NS = not significant.
ND = not determined.
Figure 4. Stress-strain diagrams of collagenase-treated proximal R-rings. Age of samples tested: 1 = 29 years; 2 = 39 years; 3 = 44 years; 4 = 56 years; 5 = 65 years; and 6 = 76 years.

Figure 5. Stress-strain diagrams of collagenase-treated distal R-rings. Age of samples tested: 1 = 29 years; 2 = 39 years; 3 = 44 years; 4 = 56 years; 5 = 65 years; and 6 = 76 years.

reported for each sample in columns A, B, and C do not differ significantly and are positively correlated with age ($p < 0.001$).

The stress-strain diagrams produced during the fourth extension by the proximal and distal collagenase-treated R-rings are shown in figures 4 and 5, respectively. All curves are biphasic with a flex point located in the strain range 0.45–0.55, for the proximal rings, and 0.30–0.60, for the distal rings. With increasing age, both the flex and the breaking point occur at progressively lower strain values while the slope of the linear part of the curve becomes steeper. The mean value of the breaking stress of the six collagenase-treated R-rings isolated from the 29-, 39- and 44-year old aortas was $2.02 \pm 0.06 \times 10^6$ Nm$^{-2}$, while the corresponding value for the six older rings was $1.80 \pm 0.31 \times 10^6$ Nm$^{-2}$.

Incremental modulus vs stress plots are shown in figures 6 and 7. At a stress of about $1.4 \times 10^6$ Nm$^{-2}$, in all samples the relationship between the two variables becomes linear and the curves exhibit almost identical slopes. The average incremental modulus calculated at this stress value for the six younger rings was $8.83 \pm 1.03 \times 10^6$ Nm$^{-2}$, while that of the

Figure 6. Incremental modulus versus stress diagrams of enzymically-treated R-rings. Collagenase-treated proximal R-rings. Age of sample tested: $\square = 29$ years; $\Delta = 39$ years; $\bullet = 44$ years; $\mathbf{m} = 56$ years; $\circ = 65$ years; and $\Delta = 76$ years. Collagenase and trypsin-treated Intermediate rings. $\circ$ indicates results obtained for all samples (from figure 8).
Table 3. Relative and Absolute Concentrations of Soluble and Insoluble Components of the Tunica Media of Human Upper Thoracic Aorta

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<tr>
<td>OH-Lysine</td>
<td>17.1 ± 1</td>
<td>15.6 ± 2</td>
<td>17.2</td>
<td>22.0</td>
<td>19.9</td>
<td>18.4</td>
<td>19.8</td>
</tr>
<tr>
<td>OH-Lysine (mg/cm²)</td>
<td>23.6 ± 1</td>
<td>24.0 ± 2</td>
<td>28.2</td>
<td>37.6</td>
<td>36.9</td>
<td>33.0</td>
<td>39.7</td>
</tr>
<tr>
<td>Elastin</td>
<td>53.1 ± 2</td>
<td>49.1 ± 2</td>
<td>46.3</td>
<td>37.9</td>
<td>37.7</td>
<td>40.4</td>
<td>37.1</td>
</tr>
<tr>
<td>Elastin (mg/cm²)</td>
<td>73.4 ± 2</td>
<td>75.5 ± 2</td>
<td>75.8</td>
<td>64.7</td>
<td>69.9</td>
<td>72.4</td>
<td>74.4</td>
</tr>
<tr>
<td>Collagen</td>
<td>23.5 ± 3</td>
<td>24.9 ± 3</td>
<td>25.6</td>
<td>25.2</td>
<td>25.0</td>
<td>26.1</td>
<td>33.9</td>
</tr>
<tr>
<td>Collagen (mg/cm²)</td>
<td>32.5 ± 3</td>
<td>38.3 ± 3</td>
<td>41.9</td>
<td>43.0</td>
<td>46.3</td>
<td>46.7</td>
<td>68.0</td>
</tr>
<tr>
<td>Insoluble polar-protein</td>
<td>6.3 ± 4</td>
<td>10.4 ± 4</td>
<td>10.9</td>
<td>14.9</td>
<td>17.4</td>
<td>15.1</td>
<td>9.2</td>
</tr>
<tr>
<td>Insoluble polar-protein (mg/cm²)</td>
<td>8.7 ± 4</td>
<td>16.0 ± 4</td>
<td>17.8</td>
<td>25.4</td>
<td>32.2</td>
<td>27.0</td>
<td>18.5</td>
</tr>
</tbody>
</table>

Values are expressed as: % (wt/wt) with reference to dry, defatted, decalcified tissue weight, and as mg/cm with reference to the length of the defatted, rehydrated segments.

*Significance level of the correlation between age and concentration values.
†Pool.
‡Single. The footnote symbols below are inserted only in the first two columns but apply in the same order throughout, as follows.
§Gravimetric difference of residues before and after treatment with chaotropic solutions.
¶From desmosine + isodesmosine concentration.
*From total protein-elastin after collagenase treatment.
††From protein-(collagen + elastin) before collagenase treatment.
‡‡Gravimetric difference of residues before and after treatment with chaotropic solutions.
†††Weight of residue recovered after all treatments.
‡‡‡Gravimetric difference of residues before and after collagenase treatment.
#Total protein-elastin after collagenase treatment.
NS = not significant.

Table 4. Amino Acid Composition of Elastins Isolated from Human Thoracic Aorta at Various Ages

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>29 yrs</th>
<th>39 yrs</th>
<th>44 yrs</th>
<th>56 yrs</th>
<th>65 yrs</th>
<th>76 yrs</th>
<th>Mean ± sd</th>
<th>Pool 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH-Lysine</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>NR</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.4</td>
<td>3.5</td>
<td>3.7</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9</td>
<td>3.9 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>Histidine</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Arginine</td>
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<td>6.7</td>
<td>6.5</td>
<td>6.5</td>
<td>8.3</td>
<td>7.2</td>
<td>7.2 ± 0.9</td>
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</tr>
<tr>
<td>OH-Proline</td>
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<td>6.6</td>
<td>6.7</td>
<td>6.7</td>
<td>6.7</td>
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<td>6.5 ± 0.3</td>
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<td>Aspartic acid</td>
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<td>5.1</td>
<td>4.9</td>
<td>5.3</td>
<td>7.9</td>
<td>9.3</td>
<td>6.3 ± 0.7</td>
<td>6</td>
</tr>
<tr>
<td>Threonine</td>
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<td>12.2</td>
<td>15.0</td>
<td>12.8</td>
<td>15.0</td>
<td>16.0</td>
<td>13.7 ± 2.0</td>
<td>12</td>
</tr>
<tr>
<td>Serine</td>
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<td>8.5</td>
<td>9.3</td>
<td>9.0</td>
<td>11.0</td>
<td>9.7</td>
<td>9.3 ± 0.9</td>
<td>8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>15.9</td>
<td>18.8</td>
<td>19.4</td>
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<td>21.3</td>
<td>23.5</td>
<td>19.5 ± 2.6</td>
<td>18</td>
</tr>
<tr>
<td>Proline</td>
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<td>142.3</td>
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<td>122.4</td>
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<td>294.5</td>
<td>283.6</td>
<td>283.6</td>
<td>292.1 ± 6.7</td>
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<td>229.2</td>
<td>223.3</td>
<td>231.3</td>
<td>227.4</td>
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<td>154.2</td>
<td>143.5 ± 6.9</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0 ± 0.0</td>
<td>0</td>
</tr>
<tr>
<td>Isoleucine</td>
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<td>24.3</td>
<td>23.8</td>
<td>23.4</td>
<td>24.1</td>
<td>21.7</td>
<td>23.5 ± 0.9</td>
<td>23</td>
</tr>
<tr>
<td>Leucine</td>
<td>60.9</td>
<td>56.2</td>
<td>54.1</td>
<td>54.4</td>
<td>58.0</td>
<td>53.0</td>
<td>56.1 ± 2.9</td>
<td>57</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>22.7</td>
<td>22.9</td>
<td>22.5</td>
<td>22.6</td>
<td>23.9</td>
<td>23.3</td>
<td>23.0 ± 0.5</td>
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</tr>
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<td>Phenylalanine</td>
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<td>20.4</td>
<td>20.9</td>
<td>20.4</td>
<td>19.4</td>
<td>20.3</td>
<td>20.5 ± 0.8</td>
<td>22</td>
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<tr>
<td>Isodesmosine‡</td>
<td>6.3</td>
<td>7.3</td>
<td>6.9</td>
<td>7.0</td>
<td>7.7</td>
<td>7.5</td>
<td>7.1 ± 0.5</td>
<td>8.7</td>
</tr>
<tr>
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<td>9.0</td>
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<td>10.1</td>
<td>10.3</td>
<td>10.1 ± 0.8</td>
<td>11.0</td>
</tr>
</tbody>
</table>

Values are expressed as residues/1000 amino acid residues.
*Modified from reference 45.
†Modified from reference 53. Mean ± so calculated on three different samples, as specified in Discussion.
‡Expressed as lysine equivalents.
NR = not reported.
Table 3. (Continued)

<table>
<thead>
<tr>
<th>Pool</th>
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<th>Pool</th>
<th>Single</th>
<th>Single</th>
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<td>19.5</td>
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<td>46.5</td>
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<td>27.3</td>
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<td>71.7</td>
<td>64.6</td>
<td>76.0</td>
<td>65.1</td>
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<td>31.5</td>
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<td>81.0</td>
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<td>77.3</td>
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<td>21.2</td>
<td>40.4</td>
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</table>

The stress-strain diagrams produced by the trypsin-treated intermediate R-rings are reported in figure 8 together with the corresponding incremental modulus vs stress plots. All tested samples produced superimposable nonlinear curves with the exception of the 76-year-old R-ring that partially failed at a strain of 0.3 and then deviated from the general pattern. The breaking stress and the breaking strain of the 29-year-old R-ring were $1.20 \times 10^6 \text{Nm}^{-2}$ and 0.74, respectively, while the corresponding values of these parameters calculated for the 39-, 44- and 56-year-old samples averaged $0.66 \pm 0.07 \times 10^6 \text{Nm}^{-2}$ and $0.50 \pm 0.05$. The combined incremental modulus vs stress plot of the trypsin-treated R-rings is also included for comparison in figures 6 and 7 where it can be seen to follow closely, albeit at a lower level, those of the youngest collagenase-treated R-rings in the nonlinear part of their diagrams.

The amount of work not recovered during the relaxation phase was positively correlated with age ($p < 0.001$) and with the concentration of the additional insoluble protein component ($p < 0.005$). On the other hand, in the trypsin-treated R-rings, hysteresis values did not show significant age-related changes.

Figure 7. Incremental modulus versus stress diagrams of enzymically treated R-rings. Collagenase-treated distal R-rings. Age of sample tested: □ = 29 years; ▲ = 39 years; ● = 44 years; ■ = 56 years; ○ = 65 years; and △ = 76 years. Collagenase and trypsin-treated intermediate R-rings. ■ indicates results obtained for all samples (from figure 8).

Figure 8. Strain and incremental modulus vs stress plots of collagenase and trypsin-treated intermediate R-rings. Solid line is the stress-strain diagram obtained for all samples. The breaking point of individual specimens is: □ = 29-year-old; ▲ = 39-year-old; ● = 44-year-old; ■ = 56-year-old. Broken line with △ shows the behavior of the 76-year-old specimen after partial failure. ● indicates incremental modulus vs stress plot for all samples.
Table 5. Amino Acid Composition of Collagenase-Treated Aortic R-Rings at Various Ages

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Proximal ring</th>
<th>Distal ring</th>
<th>Proximal ring</th>
<th>Distal ring</th>
<th>Proximal ring</th>
<th>Distal ring</th>
<th>Proximal ring</th>
<th>Distal ring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19 yrs</td>
<td>29 yrs</td>
<td>39 yrs</td>
<td>44 yrs</td>
<td>56 yrs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH-Lysine</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Lysine</td>
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<td>10.1</td>
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<td>11.7</td>
<td>13.2</td>
<td>10.3</td>
</tr>
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<td>1.1</td>
<td>1.5</td>
<td>2.8</td>
<td>2.7</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>9.3</td>
<td>12.1</td>
<td>11.9</td>
<td>12.8</td>
<td>13.8</td>
<td>14.7</td>
<td>16.4</td>
<td>15.7</td>
</tr>
<tr>
<td>OH-Proline</td>
<td>6.9</td>
<td>7.7</td>
<td>9.0</td>
<td>7.4</td>
<td>7.5</td>
<td>6.3</td>
<td>11.1</td>
<td>12.2</td>
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<tr>
<td>Aspartic acid</td>
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<td>12.5</td>
<td>16.4</td>
<td>12.8</td>
<td>16.8</td>
<td>19.3</td>
<td>22.1</td>
<td>18.9</td>
</tr>
<tr>
<td>Threonine</td>
<td>16.1</td>
<td>17.5</td>
<td>19.6</td>
<td>18.4</td>
<td>23.3</td>
<td>21.6</td>
<td>20.9</td>
<td>20.4</td>
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<tr>
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<td>23.0</td>
<td>27.2</td>
<td>22.1</td>
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<tr>
<td>Glutamic acid</td>
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<tr>
<td>Proline</td>
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<td>116.7</td>
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<tr>
<td>Glycine</td>
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<td>27.0</td>
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<td>64.3</td>
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<td>19.9</td>
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<td>20.6</td>
<td>22.8</td>
<td>21.1</td>
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<td>27.7</td>
<td>22.8</td>
<td>25.7</td>
<td>22.3</td>
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<td>4.4</td>
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<td>7.0</td>
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<td>1.4</td>
<td>0.9</td>
<td>ND</td>
<td>0.9</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Values are expressed as residues/1000 amino acid residues.

*Significance level of the correlation between amino acid concentration and age.
†Comprises methionine sulfoxide.
‡Expressed as lysine equivalents.
NS = p > 0.05.
ND = not determined.

Discussion

In agreement with several other reports,1-3 we have shown here that both the circumference and the mass of the tunica media of human aorta progressively increase with advancing age. As shown in table 3, this process appears to be associated with a considerable rearrangement of all macromolecular matrix constituents. In this respect it should be noted that data reported in this table, as well as those in figures 2 and 3, are not corrected to compensate for the longitudinal contraction that human aorta undergoes after excision. As reported by Hesse,38 this is an age-related phenomenon negligible in old samples but rather conspicuous in the very young. The concentration values expressed as weight per unit of length or per unit of surface area of the vessel are, therefore, increasingly overestimated with decreasing age.

The total content of collagen in the aortic segment investigated appears to rise with age. In the first three decades, the values of this parameter determined gravimetrically on single samples agreed with those calculated using compositional data obtained for pooled specimens. However, in older age groups the two sets of values progressively diverged with
In other tissue components, the elastin content per unit of length of vessel remains constant (70.5 ± 5.1 mg/cm). In the determination of the latter parameter, the scatter of data was reasonably low; for p < 0.05 the confidence limits were within the range ± 2.3 mg/cm. Therefore, if we take Hesse’s findings into account, it would appear that with aging there is an actual increase in the total content of elastin within the tunica media of human thoracic aorta.

It is interesting to note that the concentration of elastin in the youngest sample investigated agreed with that found in a human aorta of corresponding age by Starcher and Galione who used an alternative purification procedure. This increase in the total content of collagen and, to a much lesser extent, of elastin could be partly interpreted as a compensation for the radial dilation of the vessel, which would otherwise result in a decrease in wall thickness, and as an adjustment to the concomitant rise in tension. The two structural proteins, however, are not responsible for the age-related increase in the thickness of the tunica media (figure 3); this phenomenon appears to be related to the gradual accumulation of the material which can be extracted by chaotropic agents. There has been a tendency in the past to consider this material as representing a relatively homogeneous system of structural glycoproteins, a view which has been recently criticized. In the present investigation it appears to contain proteoglycans and collagen as well as glycoproteins, a finding which is consistent with reports that proteoglycans, collagen, and muscle proteins can be isolated from the aorta and other connective tissues by chaotropic agents.

A prominent feature of the aging process in the aorta appears to be the progressive accumulation, in

### Table 5. (Continued)

<table>
<thead>
<tr>
<th>Proximal ring</th>
<th>Distal ring</th>
<th>Distal ring</th>
<th>p*</th>
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<td>76 yrs</td>
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<td>3.7</td>
<td>17.1</td>
<td>&lt;0.001</td>
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<td>14.5</td>
<td>22.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>27.2</td>
<td>22.2</td>
<td>24.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>24.2</td>
<td>26.1</td>
<td>113.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>38.9</td>
<td>43.4</td>
<td>295.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>200.8</td>
<td>190.0</td>
<td>110.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>139.7</td>
<td>110.2</td>
<td>259.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>200.8</td>
<td>190.0</td>
<td>253.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>113.7</td>
<td>108.4</td>
<td>252.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>113.7</td>
<td>108.2</td>
<td>29.8</td>
<td>NS</td>
</tr>
<tr>
<td>28.0</td>
<td>4.9</td>
<td>26.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>63.3</td>
<td>59.2</td>
<td>29.8</td>
<td>NS</td>
</tr>
<tr>
<td>22.0</td>
<td>23.3</td>
<td>24.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>26.8</td>
<td>22.4</td>
<td>28.7</td>
<td>NS</td>
</tr>
<tr>
<td>5.1</td>
<td>4.0</td>
<td>4.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>6.4</td>
<td>5.8</td>
<td>6.6</td>
<td>NS</td>
</tr>
<tr>
<td>0.6</td>
<td>1.1</td>
<td>1.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Those analytically derived being apparently overestimated. As the opposite trend is seen in the data referring to the insoluble polar-protein component, these discrepancies could be attributed to the presence of hydroxyproline in the latter material. Such a view is supported by the observation that collagenase-treated preparations, which contain, in addition to elastin, increasing amounts of this component, exhibit an age-related increase in both the hydroxyproline-to-glycine ratio and the concentration of polar amino acids (table 5). Contamination by collagenase-resistant collagen fragments appears unlikely as virtually all known mature collagens are reduced to small peptides by the action of bacterial collagenase. The only known exception is Type IV collagen which possesses high molecular weight collagenase-resistant terminal regions. However, this collagen is readily soluble in the reducing and denaturing conditions used here. Moreover, it should be pointed out that the distribution of hydroxyproline is not limited to collagen and elastin. This amino acid has been detected in several proteins, including one which is similar to osteocalcin and is present in the aorta of rats fed an atherogenic diet.

While the elastin concentration per unit of dry weight of aorta decreases with age, due to increases in other tissue components, the elastin content per unit of length of vessel remains constant (70.5 ± 5.1 mg/cm). In the determination of the latter parameter, the scatter of data was reasonably low; for p < 0.05 the confidence limits were within the range ± 2.3 mg/cm. Therefore, if we take Hesse’s findings into account, it would appear that with aging there is an actual increase in the total content of elastin within the tunica media of human thoracic aorta.

It is interesting to note that the concentration of elastin in the youngest sample investigated agreed with that found in a human aorta of corresponding age by Starcher and Galione who used an alternative purification procedure. This increase in the total content of collagen and, to a much lesser extent, of elastin could be partly interpreted as a compensation for the radial dilation of the vessel, which would otherwise result in a decrease in wall thickness, and as an adjustment to the concomitant rise in tension. The two structural proteins, however, are not responsible for the age-related increase in the thickness of the tunica media (figure 3); this phenomenon appears to be related to the gradual accumulation of the material which can be extracted by chaotropic agents. There has been a tendency in the past to consider this material as representing a relatively homogeneous system of structural glycoproteins, a view which has been recently criticized. In the present investigation it appears to contain proteoglycans and collagen as well as glycoproteins, a finding which is consistent with reports that proteoglycans, collagen, and muscle proteins can be isolated from the aorta and other connective tissues by chaotropic agents.

A prominent feature of the aging process in the aorta appears to be the progressive accumulation, in

### Table 6. Concentration in the Collagenase-Treated R-Rings of Trypsin-Soluble Material, Alkali-Soluble Material, and Insoluble Polar-Protein Component

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Trypsin-soluble material*</th>
<th>Alkali-soluble material†</th>
<th>Insoluble polar protein§</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>17.5</td>
<td>19.3</td>
<td>9.9</td>
</tr>
<tr>
<td>39</td>
<td>28.2</td>
<td>26.6</td>
<td>32.7</td>
</tr>
<tr>
<td>44</td>
<td>27.2</td>
<td>30.4</td>
<td>22.5</td>
</tr>
<tr>
<td>56</td>
<td>34.1</td>
<td>39.7</td>
<td>33.8</td>
</tr>
<tr>
<td>66</td>
<td>38.5</td>
<td>36.8</td>
<td>42.6</td>
</tr>
<tr>
<td>76</td>
<td>46.6</td>
<td>47.0</td>
<td>41.9</td>
</tr>
</tbody>
</table>

Values are expressed as % (wt/wt) of dry weight. *From compositional data in table 4 (see Results). †Values are from intermediate R-rings. §Gravimetric difference of residues before and after treatment in caption. §Averaged values are from proximal and distal R-rings.
the tunica media, of a polar-protein component which is insoluble in chaotropic solutions and resistant to collagenase, but is readily degraded, as shown in table 6, by trypsin and hot alkali. Its presumptive amino acid composition is given in table 7 together with standard deviation values. Since this is an average composition calculated using the compositional data of the five oldest R-rings reported in tables 4 and 5, the low standard deviation obtained for all amino acids indicates that it is almost invariant with aging. Moreover, it differs significantly from that of a mixture of proteins, as presented by Reeck and Fisher. These two observations suggest that we are in the presence of either a specific protein or a mixture of a few components exhibiting constant relative concentrations.

The major criterion adopted for the assessment of the purity of the elastin samples subjected to mechanical tests has been the evaluation of the concentration of polar and hydroxylated amino acids which, in this context, are valuable indicators of protein contamination. After digestion with trypsin, an enzyme that, like collagenase, does not degrade elastin under the conditions used here, all preparations exhibited a composition similar to that published by Starcher and Gallone. As in several laboratories, treatment with hot alkali is still considered the best purification procedure presently available, despite its considerable hydrolytic effect on the protein, the composition of our elastin preparations has also been compared in table 5 with the averaged composition of three human aortic elastin samples isolated in this way from normal young tissue as well as from plaque and nonplaque areas of old aortas. When experimentally obtained values of arginine, threonine, and serine (reported in brackets) for the latter preparation were corrected for hydrolytic losses, concentrations of polar and hydroxyalted amino acids in enzymatic- and alkali-treated preparations were found to be comparable.

Since the enzymatic purification procedure adopted also resulted in a lowering of the hysteresis values of the samples when mechanically tested, it can be concluded that all components not actively contributing to the elastic recoil were effectively removed.

The human aortic elastin isolated here yielded a nonlinear stress-strain curve, while bovine ligamentum nuchae elastin is known to exhibit a linear response to stress. This may reflect differences in the arrangement of elastin in the two tissues. In the ligament, which is formed by fibers arranged parallel to the major axis, all elastic elements can take up the stress uniformly. On the other hand, in the aorta each segment of the elastin network will contribute to the resistance to stretching as a function of its angular displacement relative to the direction of stress. As with increasing tension there is a progressive orientation of the segments, the summation of their contributions could result in the production of an exponential curve of the type observed in the aorta. Despite this difference, the strain at rupture observed in the youngest aortic specimen was almost identical in magnitude to that reported for bovine ligament elastin (0.77).

With increasing age the strain at rupture progressively decreases (figure 8) suggesting some degree of degradation or rearrangement at the supramolecular level of the elastin network. The invariance in composition of the elastin samples purified with collagenase and trypsin, together with their identical mechanical response to the applied stress up to the yield-point are not compatible with the development during aging of a high degree of polypeptide chain cleavage. Therefore, the morphological changes of the elastic lamellae (namely straightening, thinning, and fragmenting) that occur with aging should perhaps be interpreted as the progressive dispersion of a quantitatively constant (table 3) and largely undegraded elastin network throughout the wall of an enlarging vessel. The only biochemical evidence for extensive cleavage of elastin chains in the human aorta comes from studies of old, heavily calcified tissue affected by complex atherosclerotic lesions. Therefore, it is reasonable to propose that degrada-

### Table 7. Comparison of the Mean Amino Acid Composition of 67 Eukaryotic Proteins with that Proposed for the Insoluble Polar-Protein Component

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Trypsin-soluble proteins</th>
<th>67 Eukaryotic proteins*</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>28 ± 6</td>
<td>65 ± 27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Histidine</td>
<td>9 ± 2</td>
<td>22 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Arginine</td>
<td>38 ± 5</td>
<td>44 ± 20</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>OH-Proline</td>
<td>22 ± 2</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>47 ± 7</td>
<td>107 ± 26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Threonine</td>
<td>39 ± 7</td>
<td>57 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serine</td>
<td>51 ± 8</td>
<td>63 ± 25</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>76 ± 9</td>
<td>106 ± 33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proline</td>
<td>90 ± 13</td>
<td>48 ± 21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glycine</td>
<td>225 ± 26</td>
<td>81 ± 31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alanine</td>
<td>140 ± 11</td>
<td>85 ± 27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>ND</td>
<td>23 ± 27</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>54 ± 12</td>
<td>68 ± 19</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Methionine</td>
<td>11 ± 1</td>
<td>19 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>35 ± 5</td>
<td>50 ± 17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leucine</td>
<td>78 ± 11</td>
<td>81 ± 25</td>
<td>NS</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>22 ± 3</td>
<td>33 ± 16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>34 ± 8</td>
<td>37 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>ND</td>
<td>13 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as residues/1000 amino acid residues, and are calculated from the difference in amino acid composition of five samples before (table 5) and after (table 4) treatment with trypsin.

*From Reeck and Fisher, reference 52.

†Significance level of the difference betweenthe concentration of each amino acid in the two sets of proteins.

NS = not significant.

ND = not determined.

NR = not reported.
tion of elastin in the aortic media is not simply a consequence of aging but occurs as a result of plaque formation in the intima. In this respect it is interesting to note that even the 76-year-old enzymically purified specimen showed a stress-strain curve that was normal up to the point of partial failure.

A much greater effect on the dynamic properties of elastin is brought about by the accumulation of the insoluble polar-protein component. This can be readily appreciated by comparing the response of aortic rings to applied stress before and after trypsin digestion. Before this treatment, the breaking stress was found to be double that of pure elastin and the incremental modulus showed a rise which was positively correlated with the concentration of the contaminating protein. Moreover, the stress-strain curves were biphasic with the diagrams becoming orientated in the direction of the applied stress. Some of these modifications are akin to those brought about by the addition of plasticisers to rubber, i.e., the tensile strength and hysteresis values are increased and the strain at rupture is decreased.

From data presented here it is not possible to ascertain whether this protein contaminant immobilized within the elastic fiber is truly insoluble or whether it is covalently bound to the elastin chain network. However, it seems conceivable, and perhaps even likely, that with increasing age a contaminating protein(s) could become linked to elastin via formation of Schiff bases, particularly if new free amino groups were generated in elastin through some degree of peptide bond cleavage.

It is interesting that the modifications in the mechanical properties of elastin brought about by the insoluble polar-protein component would compensate, if they came into play in vivo, for the loss of resistance to stretching caused with aging by the dispersion of a constant amount of elastin in an increasing vessel wall. However, we feel that caution should be used in the extrapolation of these results to the intact vessel dynamics and in the evaluation of the functional properties of the aortic wall from the summation of each component’s mechanical contribution on the basis of its relative concentration. In fact, in a study of bovine ligamentum nuchae we have shown that the mechanical response to stress in that tissue is mainly governed by the interaction and relative orientation of the structural components.

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Index Terms: aging • human aortic elastin • mechanical properties of aortic elastin • aortic tunica media composition
Age-related changes in composition and mechanical properties of the tunica media of the upper thoracic human aorta.

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